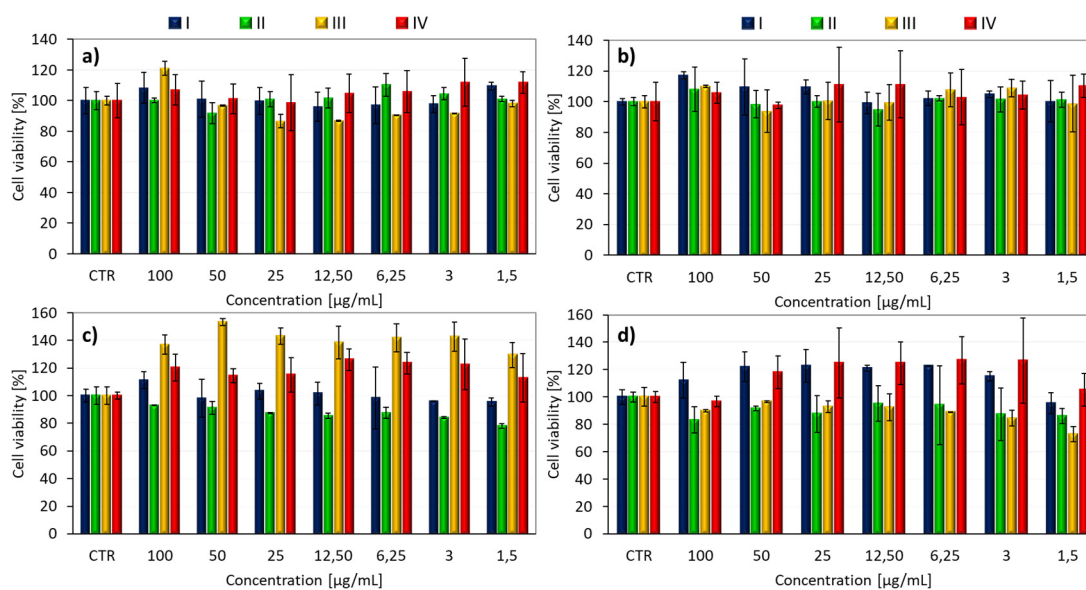


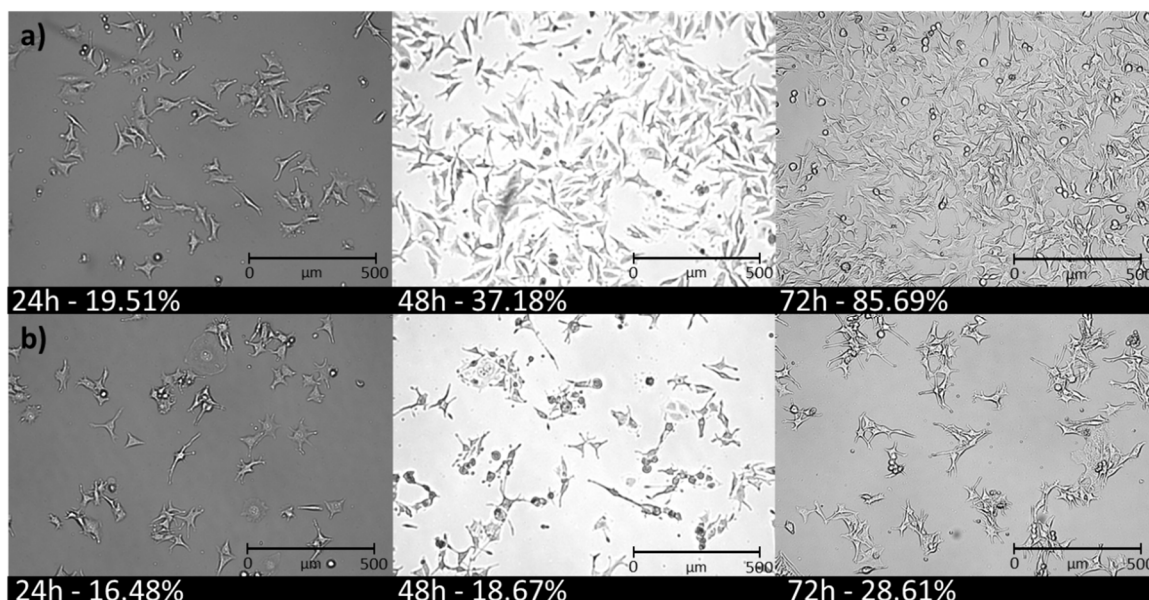
Article

# Supplementary Materials: PEG Graft Polymer Carriers of Antioxidants: In Vitro Evaluation for Transdermal Delivery

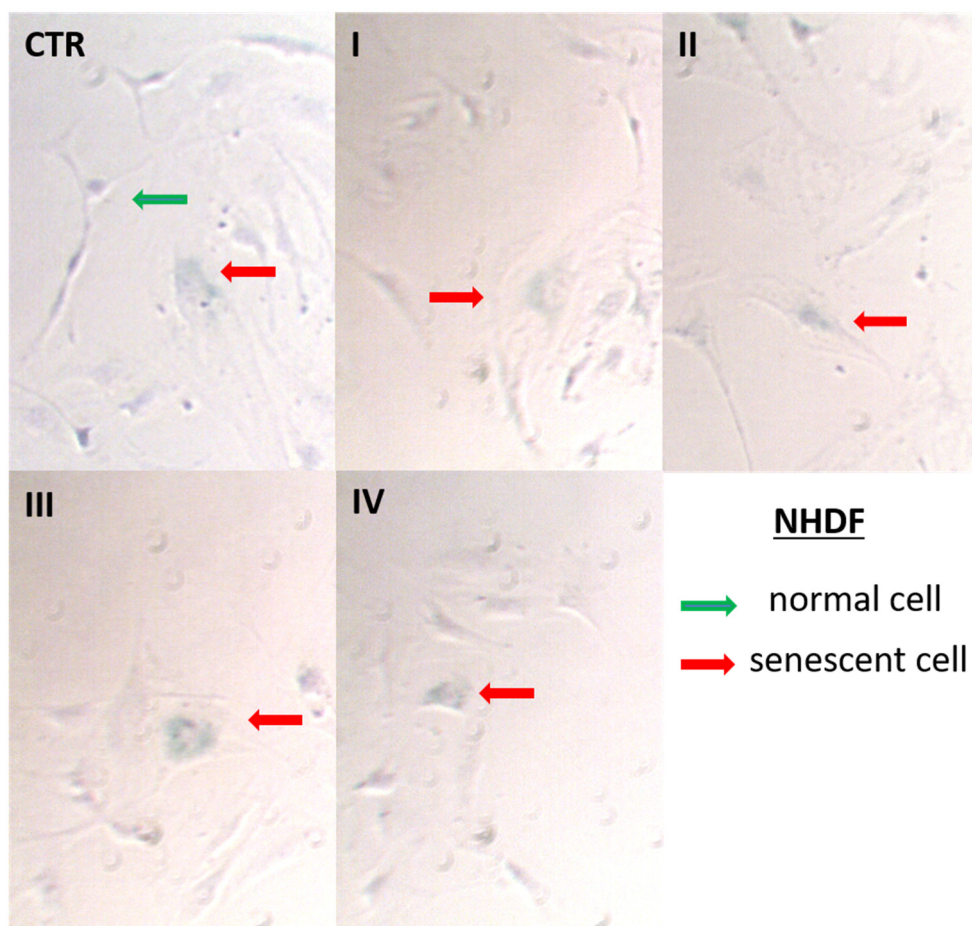
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**Figure S1.** In vitro cytotoxicity effect of carriers on (a) NHDF, (b) HaCaT, (c) Me45, (d) 451-Lu.



**Figure S2.** Increase in confluency of (a) Me45, (b) 451-Lu cells treated with compound III (c = 100 µg/mL).



**Figure S3.** Microscopic visualization of NHDF cell line after senescence test.

**Table S1.** Average cell confluence after 72h.

conc. [ $\mu\text{g/mL}$ ]	Me45 (%)		451-Lu (%)	
	0	100	0	100
CTR	53.26	-	25.94	-
I		86.70		32.48
II		85.28		37.95
III		85.96		26.04
IV		81.98		33.87

**Table S2.** NHDF and HaCaT cell cycle after carrier addition followed by 72 h of incubation in comparison to untreated controls (CTR).

	NHDF (%)				HaCaT (%)			
	subG1	G0/G1	S	G2/M	subG1	G0/G1	S	G2/M
CTR	17.9	62.3	6.7	13.0	9.5	60.2	18.7	11.6
I - 3 $\mu\text{g/mL}$	19.5	65.7	4.8	9.9	10.7	56.2	17.3	15.8
I - 100 $\mu\text{g/mL}$	19.4	67.7	4.2	8.7	13.8	54.7	19.4	12.2
II - 3 $\mu\text{g/mL}$	21.4	60.5	6.5	11.6	8.7	57.7	18.5	15.1
II - 100 $\mu\text{g/mL}$	22.6	61.0	5.6	10.7	12.6	54.7	20.0	12.6
III - 3 $\mu\text{g/mL}$	22.2	62.6	5.0	10.2	8.4	58.0	19.0	14.6
III - 100 $\mu\text{g/mL}$	25.9	53.2	7.6	13.3	8.1	32.2	26.1	33.6
IV - 3 $\mu\text{g/mL}$	26.0	60.9	4.7	8.4	8.0	58.7	19.2	14.2
IV - 100 $\mu\text{g/mL}$	26.3	50.2	9.5	14.0	9.9	27.4	24.5	38.2

**Table S3.** Me45 cell cycle after carrier addition followed by 72 h of incubation in comparison to untreated controls (CTR).

	Me45 (%)			
	subG1	G0/G1	S	G2/M
CTR	2.9	86.7	2.8	7.6
I - 3 µg/mL	2.5	90.5	2.3	4.7
I - 100 µg/mL	2.4	91.1	2.2	4.4
II - 3 µg/mL	2.9	90.9	2.7	3.6
II - 100 µg/mL	2.6	91.2	2.7	3.5
III - 3 µg/mL	3.5	86.1	3.8	6.5
III - 100 µg/mL	4.1	88.9	2.4	4.7
IV - 3 µg/mL	2.9	87.5	3.0	6.5
IV - 100 µg/mL	3.1	90.0	2.3	4.6

**Table S4.** Results of Annexin V/PI double staining apoptosis assay in NHDF cells.

NHDF	Carrier concentration [µg/mL]	% of cells ± S.D.			
		A-/PI-	A-/PI+	A+/PI-	A+/PI+
CTR	0	95.82 ± 0.24	1.36 ± 0.18	1.64 ± 0.15	1.17 ± 0.13
I	3	95.34 ± 0.85	2.29 ± 0.50	1.37 ± 0.16	0.99 ± 0.37
II		94.45 ± 0.49	2.55 ± 0.12	1.47 ± 0.30	1.52 ± 0.21
III		92.26 ± 0.79	2.41 ± 0.39	2.99 ± 0.33	2.34 ± 0.14
IV		93.77 ± 0.65	2.36 ± 0.53	2.48 ± 0.38	1.39 ± 0.20
I	100	95.42 ± 0.24	1.62 ± 0.14	1.45 ± 0.17	1.52 ± 0.23
II		95.27 ± 0.16	2.25 ± 0.29	1.24 ± 0.16	1.24 ± 0.20
III		82.95 ± 0.74	6.19 ± 0.45	5.26 ± 0.49	5.59 ± 0.27
IV		81.78 ± 0.62	6.18 ± 0.39	6.49 ± 0.75	5.55 ± 0.29

A-/PI-: Live cells; A-/PI+: Necrosis; A+/PI-: Early apoptosis; A+/PI+: Late apoptosis.

**Table S5.** Results of Annexin V/PI double staining apoptosis assay in HaCaT cells.

HaCaT	Carrier concentration [µg/mL]	% of cells ± S.D.			
		A-/PI-	A-/PI+	A+/PI-	A+/PI+
CTR	0	85.07 ± 0.67	14.58 ± 0.72	0.19 ± 0.06	0.17 ± 0.04
I	3	84.34 ± 1.28	15.05 ± 1.30	0.23 ± 0.07	0.39 ± 0.10
II		79.91 ± 0.32	18.70 ± 0.18	0.42 ± 0.04	0.98 ± 0.14
III		81.74 ± 0.70	17.55 ± 0.71	0.25 ± 0.04	0.45 ± 0.09
IV		78.77 ± 0.79	20.62 ± 0.78	0.19 ± 0.04	0.42 ± 0.10
I	100	88.50 ± 0.83	11.26 ± 0.80	0.05 ± 0.02	0.19 ± 0.05
II		90.66 ± 1.19	9.12 ± 1.13	0.08 ± 0.03	0.14 ± 0.08
III		83.40 ± 0.80	14.94 ± 0.44	0.34 ± 0.13	1.32 ± 0.42
IV		87.67 ± 1.12	11.64 ± 1.05	0.21 ± 0.06	0.48 ± 0.16

A-/PI-: Live cells; A-/PI+: Necrosis; A+/PI-: Early apoptosis; A+/PI+: Late apoptosis.

**Table S6.** Results of Annexin V/PI double staining apoptosis assay in Me45 cells.

Me45	Carrier concentration [ $\mu\text{g/mL}$ ]	% of cells $\pm$ S.D.			
		A-/PI-	A-/PI+	A+/PI-	A+/PI+
<b>CTR</b>	0	90.22 $\pm$ 1.63	9.68 $\pm$ 1.60	0.03 $\pm$ 0.02	0.07 $\pm$ 0.03
<b>I</b>	3	91.72 $\pm$ 1.42	8.20 $\pm$ 1.39	0.01 $\pm$ 0.01	0.07 $\pm$ 0.04
<b>II</b>		92.10 $\pm$ 1.51	7.80 $\pm$ 1.50	0.01 $\pm$ 0.01	0.09 $\pm$ 0.03
<b>III</b>		91.04 $\pm$ 1.61	8.71 $\pm$ 1.52	0.05 $\pm$ 0.04	0.19 $\pm$ 0.07
<b>IV</b>		90.90 $\pm$ 1.54	8.94 $\pm$ 1.51	0.02 $\pm$ 0.01	0.14 $\pm$ 0.05
<b>I</b>	100	86.05 $\pm$ 1.49	13.86 $\pm$ 1.45	0.01 $\pm$ 0.01	0.08 $\pm$ 0.04
<b>II</b>		87.60 $\pm$ 2.50	12.30 $\pm$ 2.48	0.01 $\pm$ 0.01	0.10 $\pm$ 0.04
<b>III</b>		85.53 $\pm$ 2.21	14.23 $\pm$ 2.20	0.04 $\pm$ 0.03	0.19 $\pm$ 0.06
<b>IV</b>		89.68 $\pm$ 1.79	10.12 $\pm$ 1.78	0.04 $\pm$ 0.01	0.15 $\pm$ 0.05

A-/PI-: Live cells; A-/PI+: Necrosis; A+/PI-: Early apoptosis; A+/PI+: Late apoptosis.