Supplementary Information (SI) for Nanoscale.

Graphene-based materials are not skin sensitizers: adoption of the *in chemico/in vitro* OECD test guidelines

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Supplementary materials

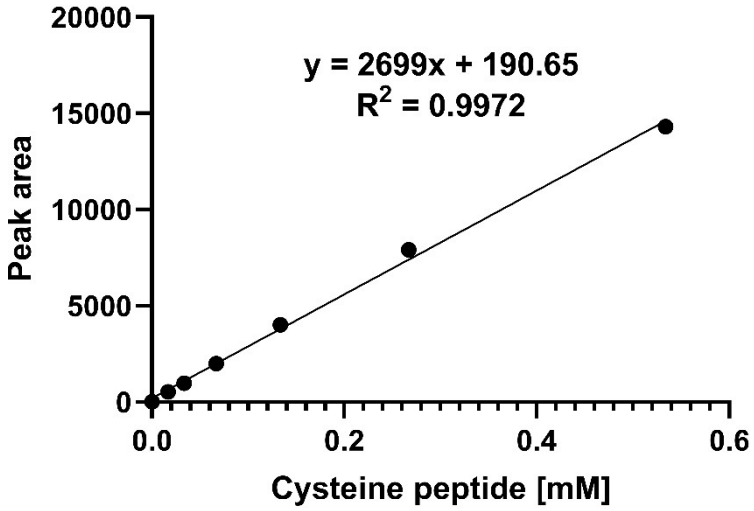

Figure S1. Representative linear calibration standard curve of L-cysteine peptide (0.0167 – 0.0334 – 0.0667 – 0.1335 – 0.267 – 0.534 mM) calculated plotting peptide concentrations versus areas of each corresponding peak obtained by HPLC analysis.

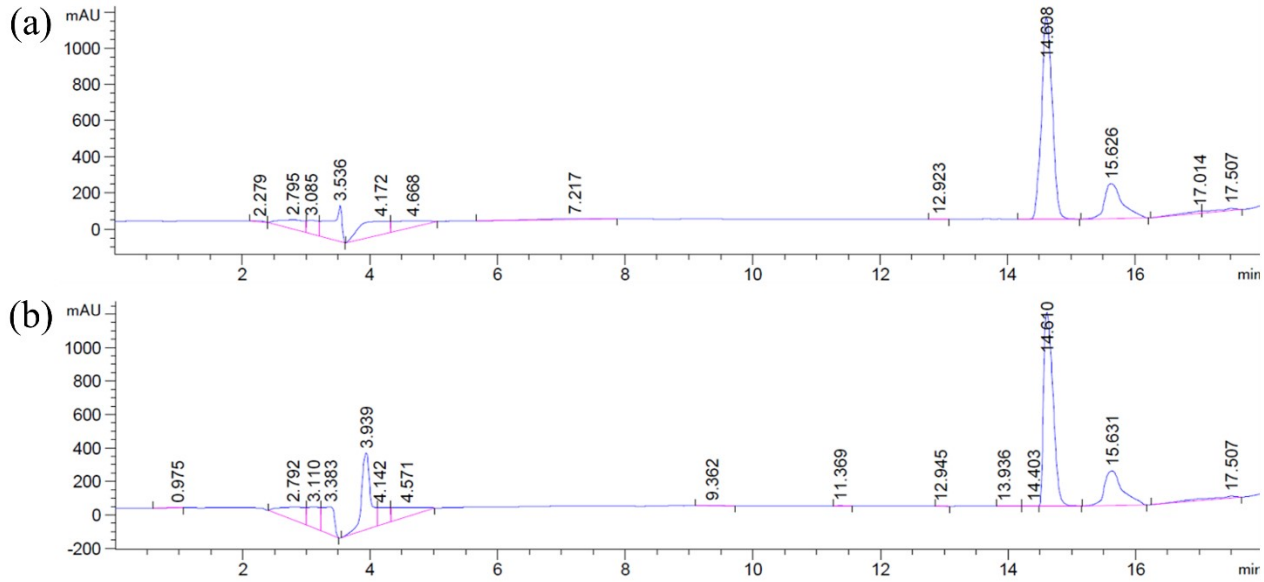

Figure S2. Representative chromatograms obtained by HPLC analysis showing the retention times and intensity of the peaks of reference control of cysteine-containing peptide solubilized in acetonitrile (a) or in water (b), according to OECD TG 442C. The peptide has a retention time of 14.6 min.

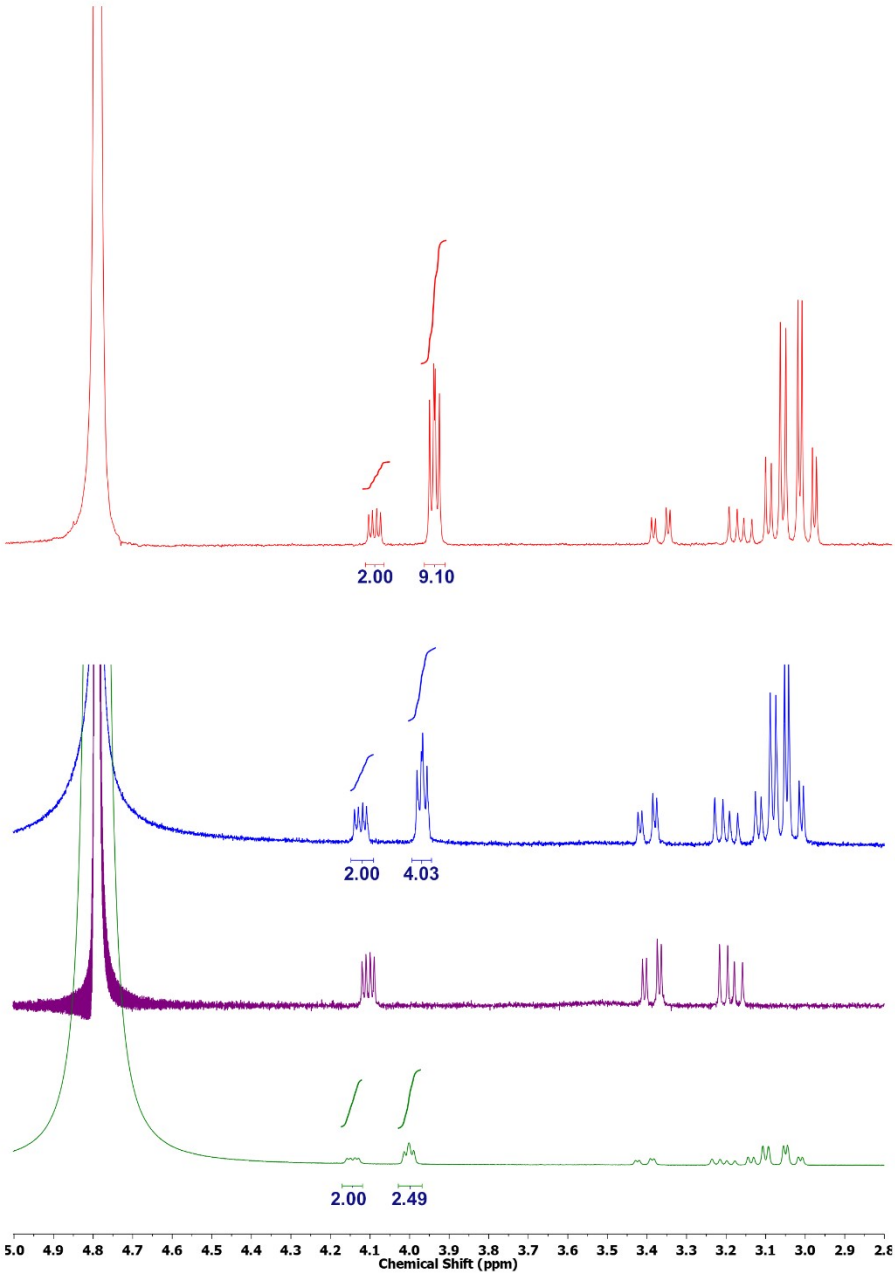

Figure S3. Representative ^{1}H-NMR spectra of a 5 mM L-cysteine solution in D_2O, after 24 h incubation in the absence of GBMs (red) and after 24 h incubation with GO, rGO, and GNP (in green, purple, and blue, respectively). The integrals show the ratio L-cysteine/cystine. The signal at 4.12 ppm corresponds to cystine, meanwhile the signal at 3.9 ppm corresponds to L-cysteine. To calculate the ratios, 2H of cystine corresponds to 1 cystine molecule and 1H of L-cysteine corresponds to 1 L-cysteine molecule.

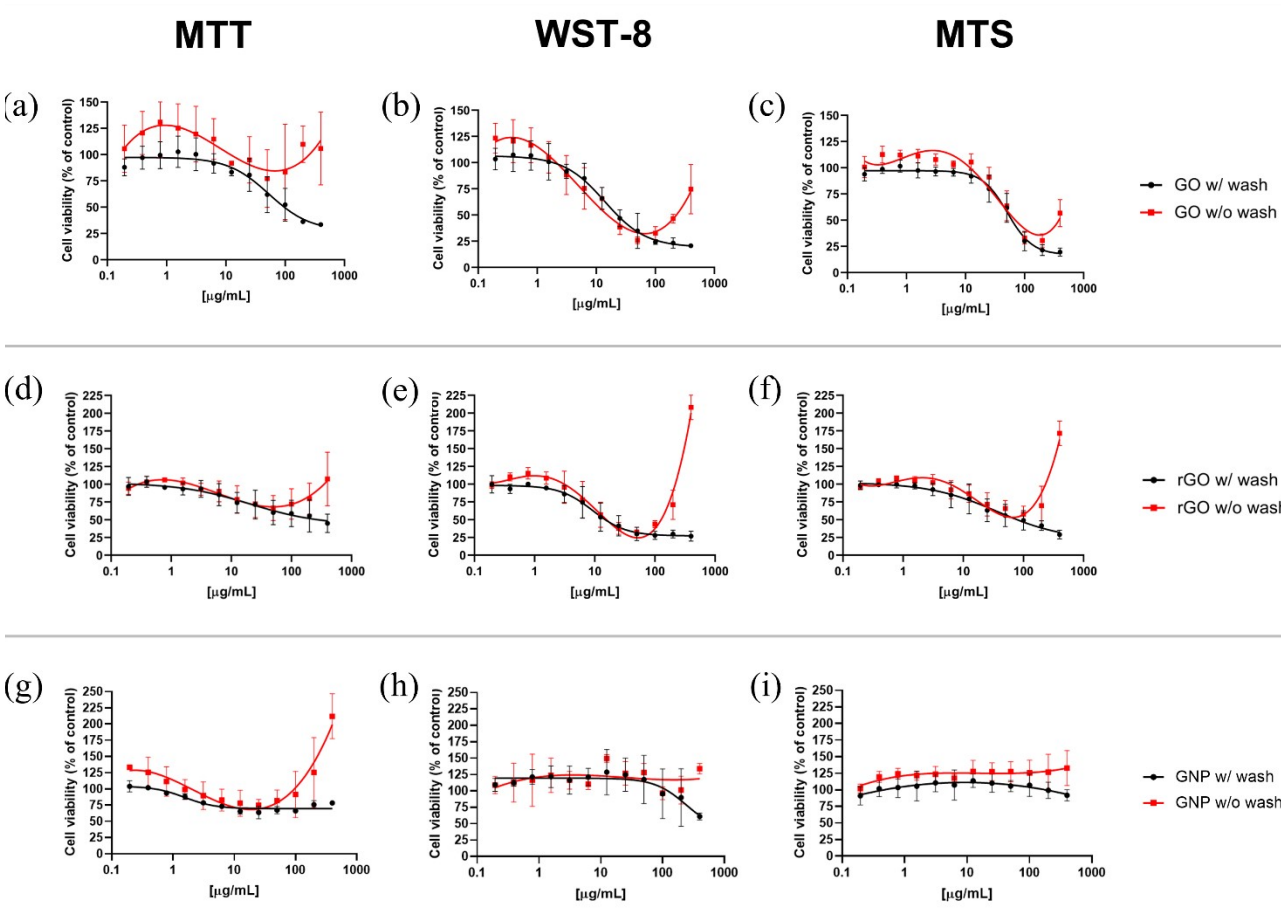

Figure S4. Comparison between the effects of 48 h exposure to GO (a, b, c), rGO (d, e, f) or GNP (g, h, i) (0.2 – 400.0 µg/mL) on KeratinoSens™ cells viability using the MTT (a, d, g), WST-8 (b, e, h) and MTS (c, f, i) assays, with or without two washing steps with 200 µL/well PBS. Data are the mean ± SE of three independent experiments performed in triplicate.

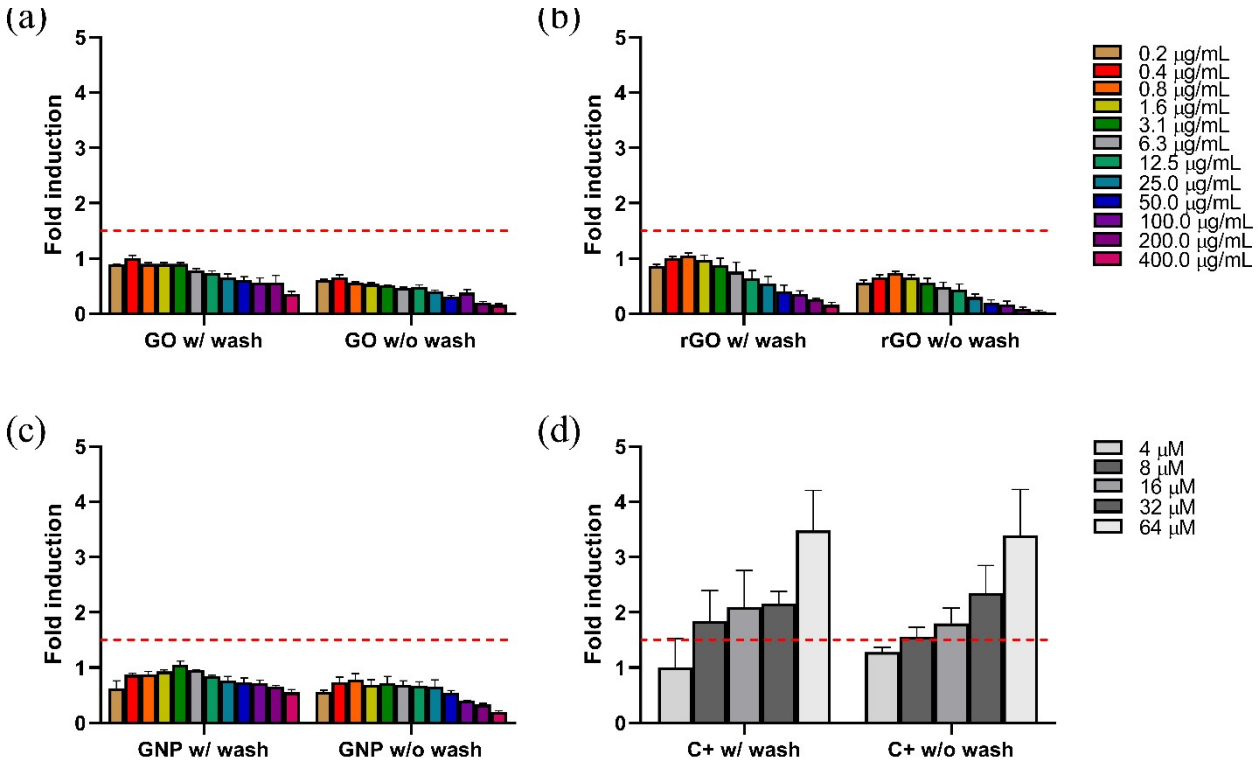

Figure S5. Induction of luciferase activity in the KeratinoSens™ cell line. The cells were exposed for 48 h with GO (a), rGO (b), GNP (c) (0.2 - 400.0 µg/mL) or positive control (d; cinnamic aldehyde; 4-64 µM) with or without two washings with 200 µL/well PBS. Data are expressed as mean ± SE of three independent experiments performed in triplicate.

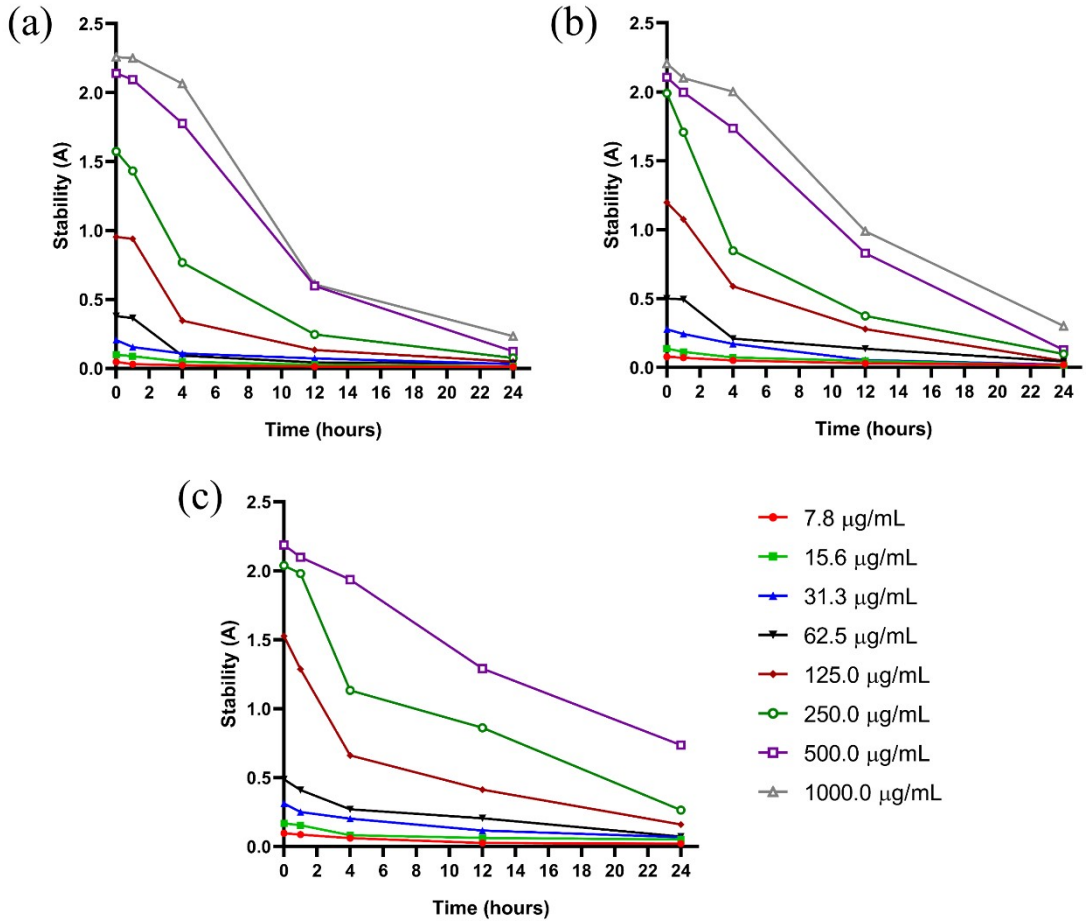

Figure S6. Dispersion stability of GO (a), rGO (b) and GNP (c) in complete culture medium up to 24 h assessed with UV-Vis analysis, measuring the absorbance (A) at 660 nm. The data are the mean of the absorbance values of the GBM suspensions recorded at different times (0, 1, 2, 4, 12 and 24 h). Results are the mean of 3 independent results.

Table S1. Assessment of skin sensitization properties of GO, rGO or GNP using the h-CLAT assay (OECD TG 442E). For each tested substance, the table reports the changes (RFI %) in the surface marker expression (FL1) and cell viability assessed by means of PI uptake (FL3) analyzed by flow cytometry. Classification was defined on the basis of the threshold given by the OECD TG 442E.

Material	Concentration (µg/mL)	RFI % CD86	RFI % CD54	Viability (PI)	Viability (Trypan Blue)	Classification
LA	1000	71.2%	87.3%	98.4%	99.3%	Negative
DNCB	4	324.7%	205.3%	63.5%	65.9%	Positive
$NiSO_4$	100	170.1%	484.1%	79.3%	76.1%	Positive
GO	7.8	126.5%	69.8%	97.6%	98.1%	Negative
	6.5	148.8%	55.7%	97.4%	98.5%	Negative
	5.4	141.8%	66.8%	98.0%	97.9%	Negative
	4.5	115.8%	75.9%	98.2%	98.6%	Negative
	3.8	98.3%	77.5%	97.9%	97.8%	Negative
	3.1	104.2%	61.2%	98.1%	97.5%	Negative
	2.6	103.7%	62.2%	98.1%	98.9%	Negative
rGO	7.8	96.3%	90.7%	98.3%	99.1%	Negative
	6.5	103.0%	74.7%	98.2%	99.6%	Negative
	5.4	116.7%	105.2%	98.4%	98.9%	Negative
	4.5	98.8%	121.4%	98.2%	99.1%	Negative
	3.8	81.3%	107.9%	98.2%	98.9%	Negative
	3.1	88.3%	136.2%	98.2%	98.7%	Negative
	2.6	105.4%	124.3%	98.1%	98.3%	Negative
GNP	7.8	103.5%	89.9%	98.1%	98.8%	Negative
	6.5	115.0%	126.07	97.7%	98.5%	Negative
	5.4	108.6%	142.4%	98.3%	98.9%	Negative
	4.5	98.5%	71.0%	97.9%	99.0%	Negative
	3.8	95.3%	129.9%	97.8%	98.6%	Negative
	3.1	95.5%	124.7%	97.7%	99.1%	Negative
	2.6	90.7%	74.8%	98.0%	98.9%	Negative