SUPPORTING INFORMATION

Keratinocytes are Capable of Selectively Sensing Low Amounts of Graphene-based Materials: Implications for Cutaneous Applications

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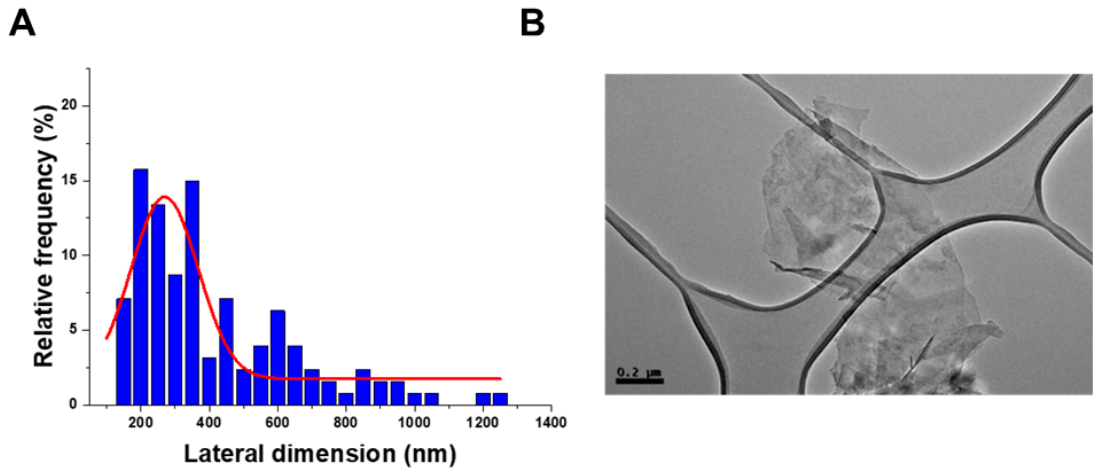
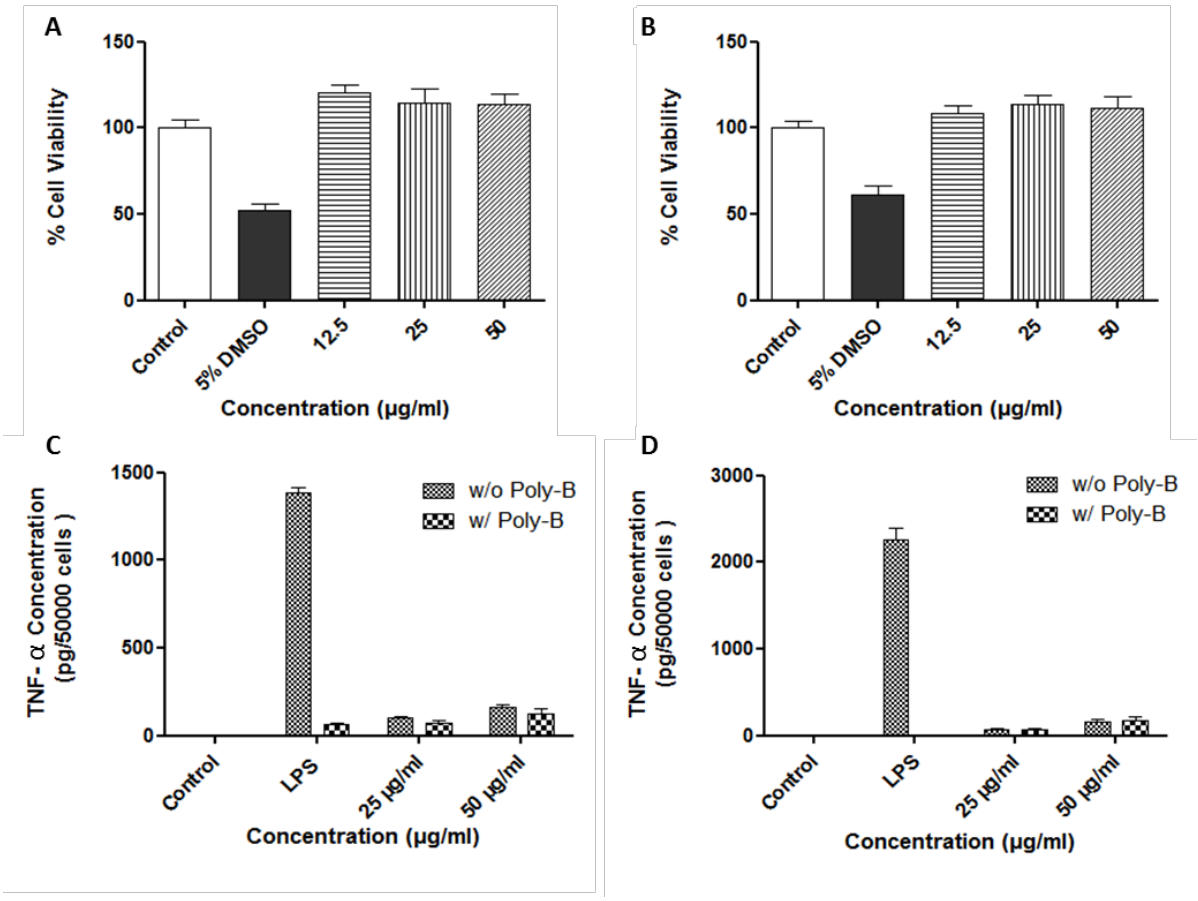
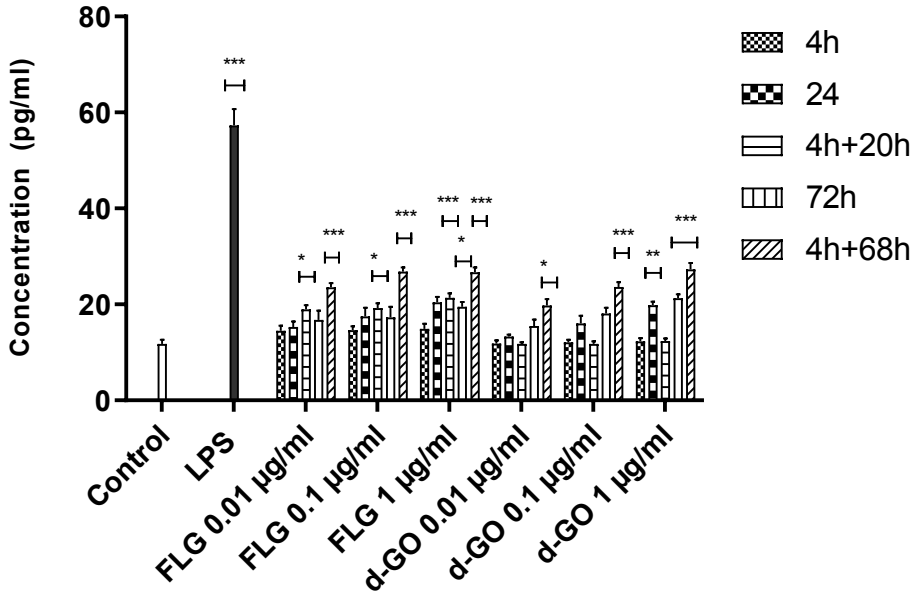


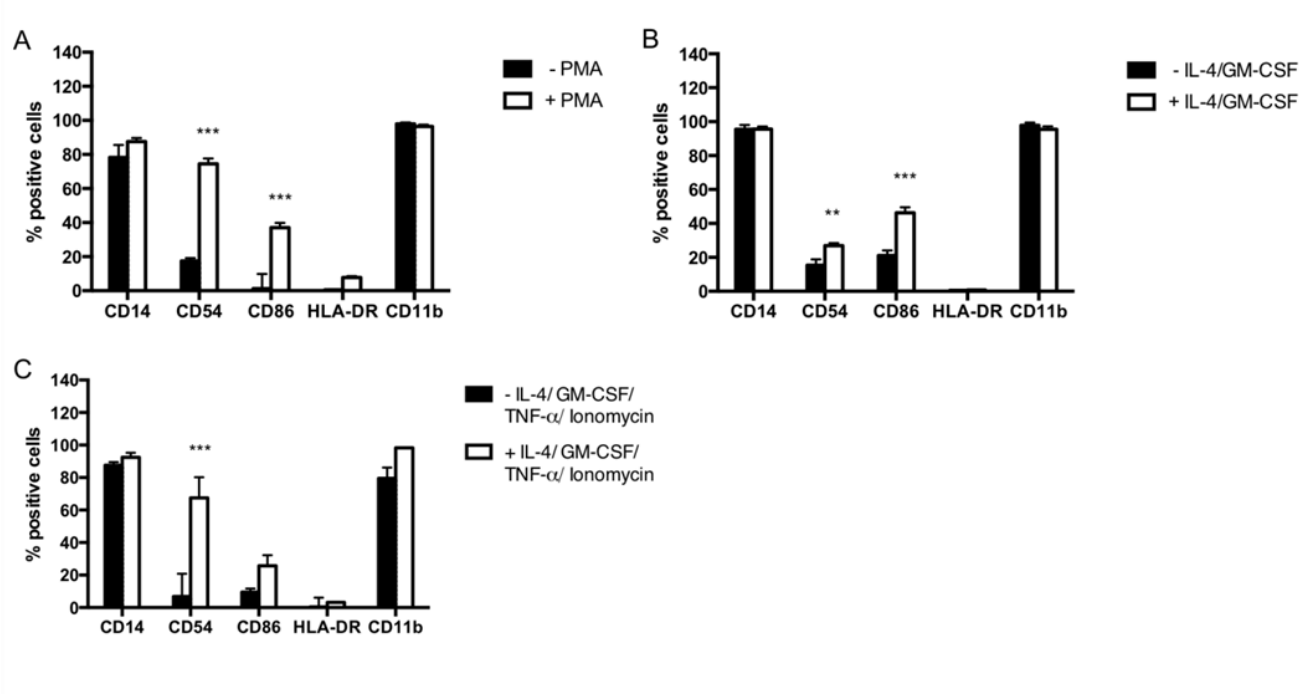
Supplementary Figure S1. Characterization of FLG samples before the thermal treatment was conducted by means of total reflection X-ray fluorescence (TXRF), transmission electron microscopy (TEM) and elemental analysis (EA). The latter gave average values of: C 95.77 ± 0.51 wt%, H 0.42 ± 0.04 wt% and N 0.43 ± 0.01 wt%, being the amount of oxygen lower than 4 wt%. From the %N obtained in EA we estimate that the content of melamine within FLG powder is 0.64 wt%. In addition, since a stainless-steel flask was used during the ball-milling process, TXRF was performed to ensure the absence of metals, especially Fe, in the graphene sample, revealing a Fe concentration of only 0.026 mg/L. Regarding the morphology of the sheets, TEM was used to determine the shape and the lateral size of FLG. The results confirmed a wide size distribution (~100-1300 nm) with an average lateral dimension of 413.8 ± 261.2 nm (A). Representative FLG sheet shown in (B). The characterization of GO powder before the thermal treatment has been previously published.[8a] EA resulted in C 41.88 ± 1.06 wt%, H 3.04 ± 0.14 wt% and N 0.04 ± 0 wt%, being the amount of oxygen ~ 53 wt%. Regarding the size of GO flakes, the lateral dimension gave an average value of 979 ± 498 nm.[8a]



Supplementary Figure S2. Primary human monocyte-derived macrophages (HMDMs) were exposed to endotoxin-free d-GO (A) or FLG (B) or 5% DMSO for 24 h and cell viability was evaluated using the Alamar Blue assay. TNF-α secretion was monitored in HMDMs exposed to non-cytotoxic doses of FLG (C) and d-GO (D) for 24 h. Cells were exposed in the presence or absence of the LPS inhibitor, polymyxin B (10 µM), as described.[11] LPS (0.1 µg/mL) was included as a positive control. Data shown are mean values ± S.D. of 3 independent experiments each performed in triplicate.



Supplementary Figure S3. FLG and d-GO elicit GM-CSF responses in keratinocytes at low doses. GM-CSF profiling released by HaCaT cells exposed to FLG or d-GO continuously (4, 24 and 72 h) and following a brief exposure and recovery (4h+20h and 4h+68h). LPS (10 µg/mL) was included as positive control. Results are shown as mean values ± S.D. (n=3). * p value <0.05, ** p<0.01, *** p<0.001.



Supplementary Figure S4. Expression of differentiation markers in THP-1 cells differentiated into (A) macrophages (10^{-7} M PMA, for 72 h), (B) iDCs (100 ng/mL IL-4 and GM-CSF, for 120 h) and (C) mDCs (200 ng/mL IL-4, 100 ng/mL GM-CSF, 10 ng/mL TNF-α and 200 ng/mL ionomycin, for 48 h). Results are expressed as % of positive cells for the markers considered and are the averages of three experiments (black bars: undifferentiated cells; white bars: differentiated cells). Statistical differences *vs*. undifferentiated cells: **, $p < 0.01$; ***, $p < 0.001$ (one-way ANOVA and Bonferroni post-test).