Supplementary Material (ESI) for Nanoscale



**Electronic supplementary information**

## Experimental Section

**Synthesis:** To prepare pure graphene (*p*-G), thermal exfoliation of graphite oxide was performed. Graphite oxide was prepared by reacting graphite with a mixture of conc. nitric acid and sulfuric acid with potassium chlorate at room temperature for 5 days. Thermal exfoliation of graphitic oxide was carried out in a long quartz tube at 1050 $^{\circ}$C under Argon atmosphere.

**Functionalization of Graphene:** Graphene (25 mg) was refluxed with dilute nitric acid (2 M) for 12 h. The product was washed with distilled water and centrifuged repeatedly to remove traces of acid. Graphene thus obtained functionalized with hydrophilic groups, could be dispersed in water or physiological medium.

**Characterization:** Raman spectra were recorded with LabRAM HR High Resolution Raman spectrometer (Horiba Jobin Yvon, USA), using a He–Ne Laser ($\lambda$=632.8 nm). TEM images were obtained with JEOL JEM 3010 (JEOL, Japan). AFM measurements were performed using a Dimension 3100 Nanoman AFM (Veeco, NY).

**Contact angle measurement**: Hydrophobic/hydrophilicity of pristine and COOH-functionalized graphene samples were obtained by measuring the contact angle of spreading sessile drops, with distilled water as the contacting solvent. A drop shape analyzing system (DSA 100 EasyDrop Contact Angle Measuring System, KRÜSS, Germany) was used to determine the surface contact angles.

**Cellular Uptake and Cell viability:** Cells were grown on 35 mm μ-Dish (ibidi, Germany) and analyzed in confocal microscopy (Leica TCS SP5 II, Leica Microsystems, USA). Alamar blue (Invitrogen, CA, USA) assay was used to evaluate the cell viability. When cells reached 80% confluency, they were harvested and $3 \times 10^4$ cells/ml were seeded in 24 well plates and incubated for 24 h at 37 °C. The cells were then treated with different concentrations of *p*-G and *f*-G for 24 h at 37 °C and Alamar Blue assay was performed. Fluorescence was recorded using a fluorescence microplate reader (Beckman Coulter DTX 880 Multimode Detector, USA) using 560/590 nm ex/em filter settings.

**LDH release:** After incubation with different concentrations of graphene for 24 h at 37 °C the cell culture medium was collected and centrifuged at 10000 rpm for 10 min. LDH level in the extracellular medium was assessed using a commercial test kit (Sigma, St. Louis, USA) using manufacturer's protocol. An aliquot of 50 $\mu$l culture medium was used to measure LDH leakage and optical absorbance was measured in a microplate spectrophotometer (Biotek PowerWave XS, USA) at 490 nm with 690 nm set as the reference wavelength.

**Cytoskletal imaging:** After treatment with *p*-G and *f*-G the Vero cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. Cells were then stained with Alexa Fluor 488 conjugated Phalloidin (Invitrogen, CA, USA) specific for F-actin filaments. Nuclei were further stained with propidium iodide (PI). Cytoskeletal alignment was visualized using confocal laser scanning microscopy (He-Ne and Ar laser).

**Apoptosis:** Annexin V–FITC and PI assay (BD Biosciences, CA, USA) was employed to detect apoptotic and necrotic cells. After incubation with a dose range of graphene for 24 hr at 37 $^{\circ}$C, the cells were washed and stained with Annexin V and PI. Typically $2 \times 10^5$ cells were resuspended in 100 $\mu$l of binding buffer and 5 $\mu$l of FITC-conjugated Annexin V (Annexin V–FITC) and 5 $\mu$l of propidium iodide (PI) were added sequentially at room temperature in the dark. After incubation for 15 min, stained cells were diluted with 400 $\mu$l of binding buffer and directly analyzed in flow cytometry (BD FACSAria; BD Biosciences, CA, USA), measuring the fluorescence emission at 530 nm and 575 nm.

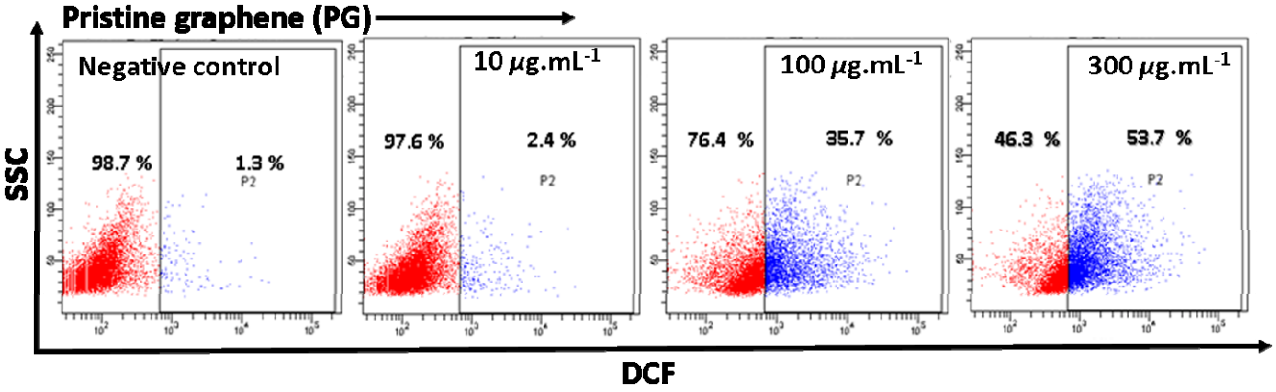
**Intracellular ROS:** Intracellular ROS generation was measured using an oxidation sensitive dye 2,7-dichlorofluorescin diacetate (DCFH-DA; Invitrogen, CA, USA). DCFH-DA is a non-fluorescent dye that undergoes intracellular de-acetylation, followed by ROS mediated oxidation to a fluorescent dichlorofluorescin (DCF) which has an excitation/emission maxima of 495 nm/ 529 nm. Typically $2 \times 10^5$ Vero cells after 24 h at 37 $^{\circ}$C of exposure to graphene were re-suspended in HBSS containing 5 $\mu$M of DCFH-DA for 30 min and intracellular ROS generation was evaluated using flow cytometry.

**SI Video 1** Video showing contact angle measurement of pristine graphene (p-G).

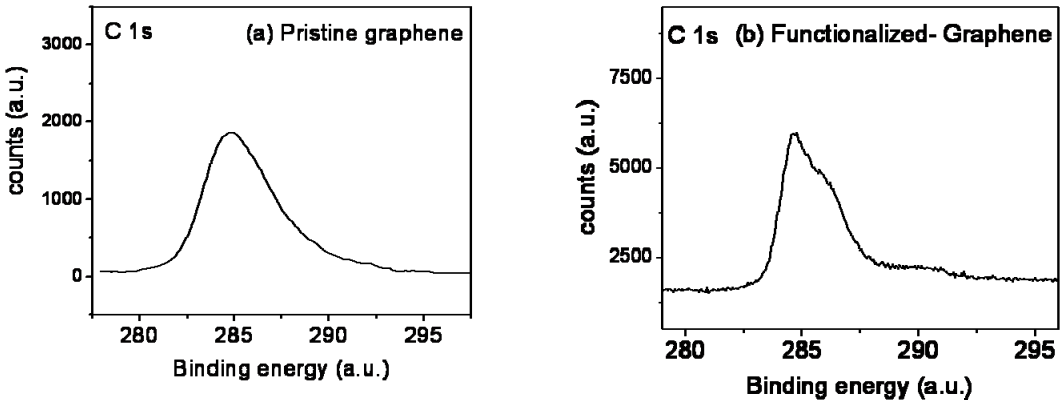
**SI Video 2** Video showing contact angle measurement of COOH-functionalized graphene (f-G).

**SI Video 3** Video portraying confocal microscopy images of pristine graphene (p-G) treated Vero cells by changing depth in the z axis. Confocal optical z sections in 1-µm intervals were taken through the whole cells.

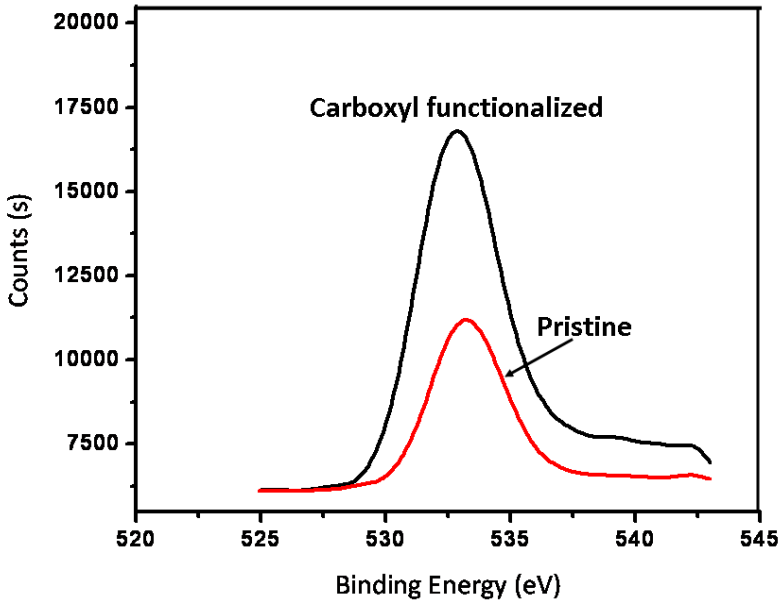
**SI Video 4** Video portraying confocal microscopy images of pristine graphene (f-G) treated Vero cells by changing depth in the z axis. Confocal optical z sections in 1-µm intervals were taken through the whole cells.




**SI Fig. 1** Dose dependant expression of ROS (P2 region) in cells treated with *p*-G.




SI Fig. 2 XPS C1s spectra of graphene samples (a) Pristine (b) Functionalized graphene



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**(c)** XPS O1s spectra spectra of graphene samples