Supporting Information

Cytotoxicity of Graphene Oxide and Graphene in Human Erythrocytes and Skin Fibroblasts

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1. Experimental Section

Preparation of chitosan-coated graphene oxide (GO/chitosan): The graphene oxide (GO) after 30-minute probe sonication, pGO-30, was coated with a biocompatible polymer, chitosan using a modified procedure published by Fang et al.[1] A pGO-30 suspension in D.I. water (3 mL, 0.5 mg mL^{-1}) was gradually added to a low molecular weight chitosan (Mw<6000, Sigma-Aldrich, Milwaukee, WI) solution (3 mL, 0.5 mg mL^{-1} in 0.01 M HCl) under stirring at room temperature. After 24-hour stirring, the stock GO/chitosan solution was transferred to phosphate buffered solution (PBS) and centrifuged at 10,016 g for 10 minutes. The obtained pGO/chitosan particles were further washed with PBS two times to remove any excess chitosan. The washed pGO/chitosan particles were then dispersed in 6 mL of PBS for use in the hemolysis assay.

Control experiments for the lactate dehydrogenase (LDH) assay: Cell-free control experiments were performed with the commonly employed LDH assay to see if the GO and GS react directly with the LDH enzyme. First, 100 μL of serum-free MEM, pGO-5 and GS at 25 μg/mL (in serum-free MEM) were added to each well in a 96-well plate. One μL of LDH was added to each well. The mixture was incubated at 37 ^{o}C under 5% CO_2 for 4 hours. After incubation, the plate was centrifuged at 250 g for 10 minutes and 10 μL of supernatant was transferred to another well and then 100 μL of water soluble tetrazolium salt substrate mix (WST-8 from LDH kit, BioVision, Milpitas, CA) was added. After 30 minutes, absorbance of the mixture solutions was measured at 450 nm with 655 nm as a reference, using an iMark microplate reader.

Samples	MEM+1μL LDH (Positive control)	pGO-5+1μL LDH	GS+1μL LDH
Optical density @450 nm (655 nm as reference)	1.011	0.077	0.084

Trypan blue exclusion viability assay: The cell viability was evaluated using the trypan blue exclusion assay. The human skin fibroblast cells were seeded in a 96-well plate ($6x10^4$ per well) and cultured in MEM supplemented with 10% FBS and 1% PS at 37 ^{o}C under 5% CO_2. Twenty-four hours later, the cells were washed with serum-free MEM (1% PS) two times and incubated with 100 μL of different concentrations of pGO-5 and GS particles (50-200 μg mL^{-1}) in serum-free MEM (1% PS). After 24-hour exposure, the cells were washed with serum-free MEM (without phenol red) three times and detached by adding 200 μL of trypsin-EDTA solution. Then, 10 μL of cells suspension was added to 10 μL of trypan blue (in 0.85% saline). The cell number was counted using hemocytometer. The cell viability is determined as percentage of the cell number of sample well (cells exposed to pGO-5 or GS) to control well (cells without pGO-5 or GS exposure).

Reactive oxygen species (ROS) measurements: The human skin fibroblast cells were seeded in a 96-well plate ($6x10^4$ per well) and cultured in MEM supplemented with 10% FBS and 1% PS at 37 ^oC under 5% CO_2. After 24 hours, the cells were washed with serum-free MEM (1% PS) two times and incubated with 100 μL of 20 μM of H_2DCFDA (Invitrogen, Eugene, OR) in serum-free MEM (1% PS) for 1 hour. The cells were washed with serum-free MEM (1% PS) one time and then incubated with 100 μL of different concentrations of pGO-5 and GS particles (3.125-25 μg mL^{-1}) in serum-free MEM (1% PS) for 24 hours. Then, the treated cells were washed with serum-free MEM (without phenol red) three times. After adding 100 μL of serum-free MEM (without phenol red) to each well, the fluorescence intensity of the treated cells were measured by a fluorescence microplate reader (BioTek, Winooski, VT) with excitation/emission at 485/528 nm. The ROS level is expressed as ratio of fluorescence intensity of the sample well (F_{sample}, cells exposed to pGO-5 or GS) to control well ($F_{control}$, cells without pGO-5 or GS exposure).

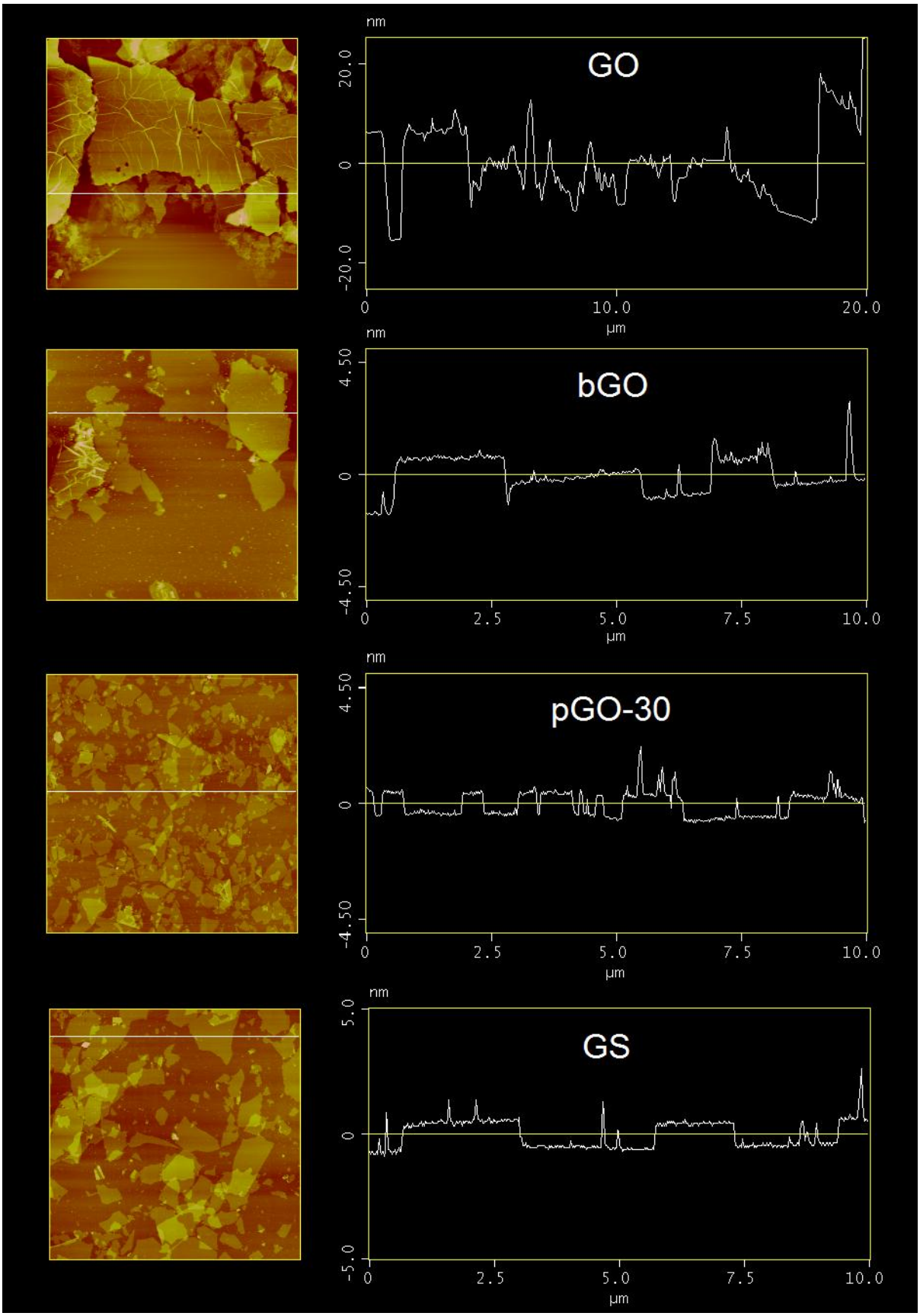

Figure S1. AFM images of GO, bGO, pGO-30, and GS with cross-sectional topography.

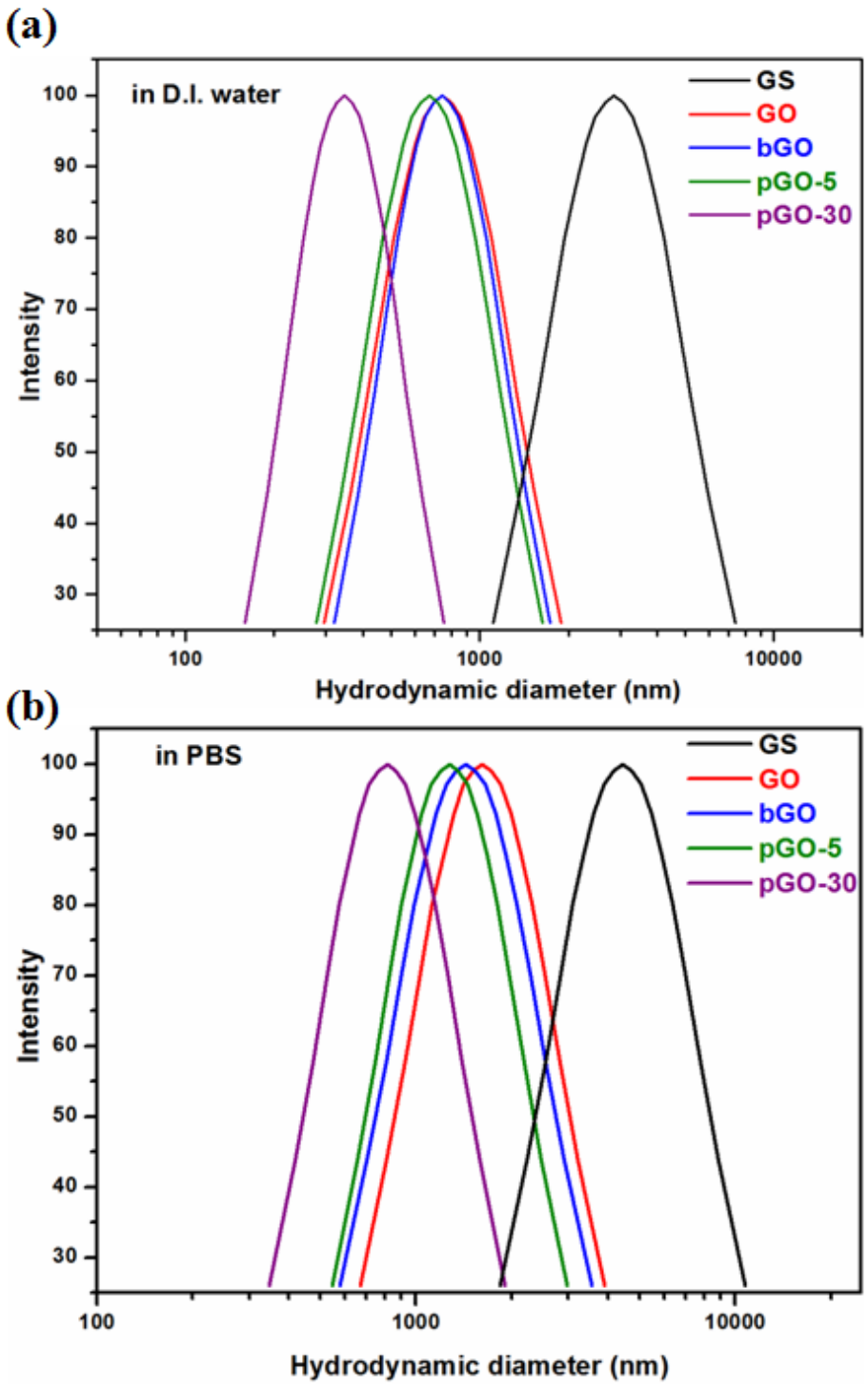

Figure S2. Hydrodynamic size distribution of 50 μg mL^{-1} GO, bGO, pGO-5, pGO-30, and GS measured by DLS at room temperature in (a) D.I. water and (b) PBS.

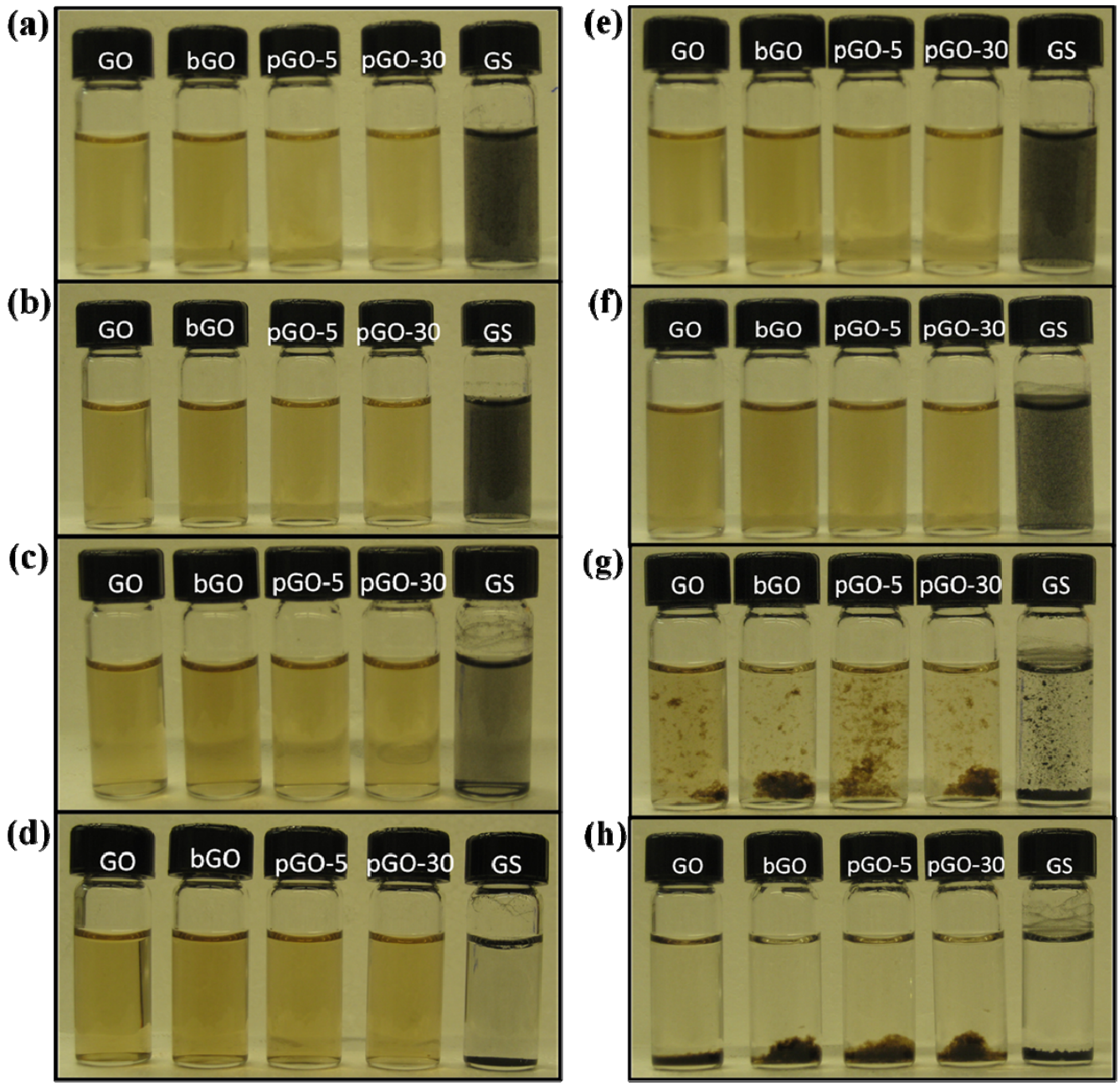

Figure S3. Photographs of GO, bGO, pGO-5, pGO-30, and GS sheets dispersed at 50 μg mL^{-1} in D.I. water (a, b, c, d) and in PBS (e, f, g, h) after various time points: samples right after preparation (0 hour) (a, e), after 3-hour mixing agitation, (b,f), after 3-hour static aging (c, g), and after 24-hour static aging (d, h). All samples were held at 37 ^{o}C.

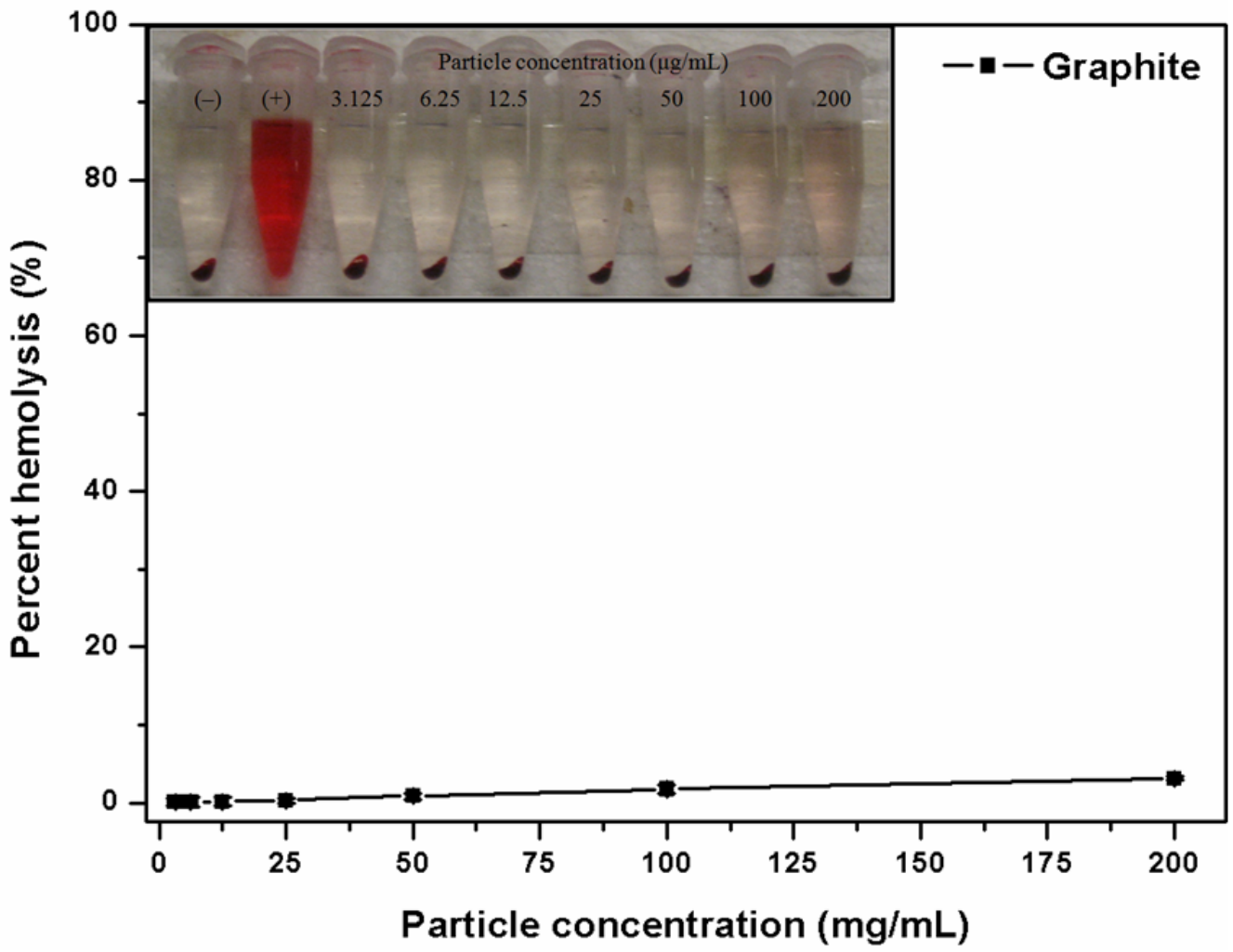

Figure S4. Percent hemolysis of RBCs incubated with different concentrations (3.125 to 200 μg mL^{-1}) of graphite for 3 hours at 37 ^{o}C with agitation. Data represent mean ± SD, n=3. Inset: photographs of RBCs after 3-hour exposure to graphite with negative and positive controls. (+) and (-) symbols represent positive control and negative control, respectively.

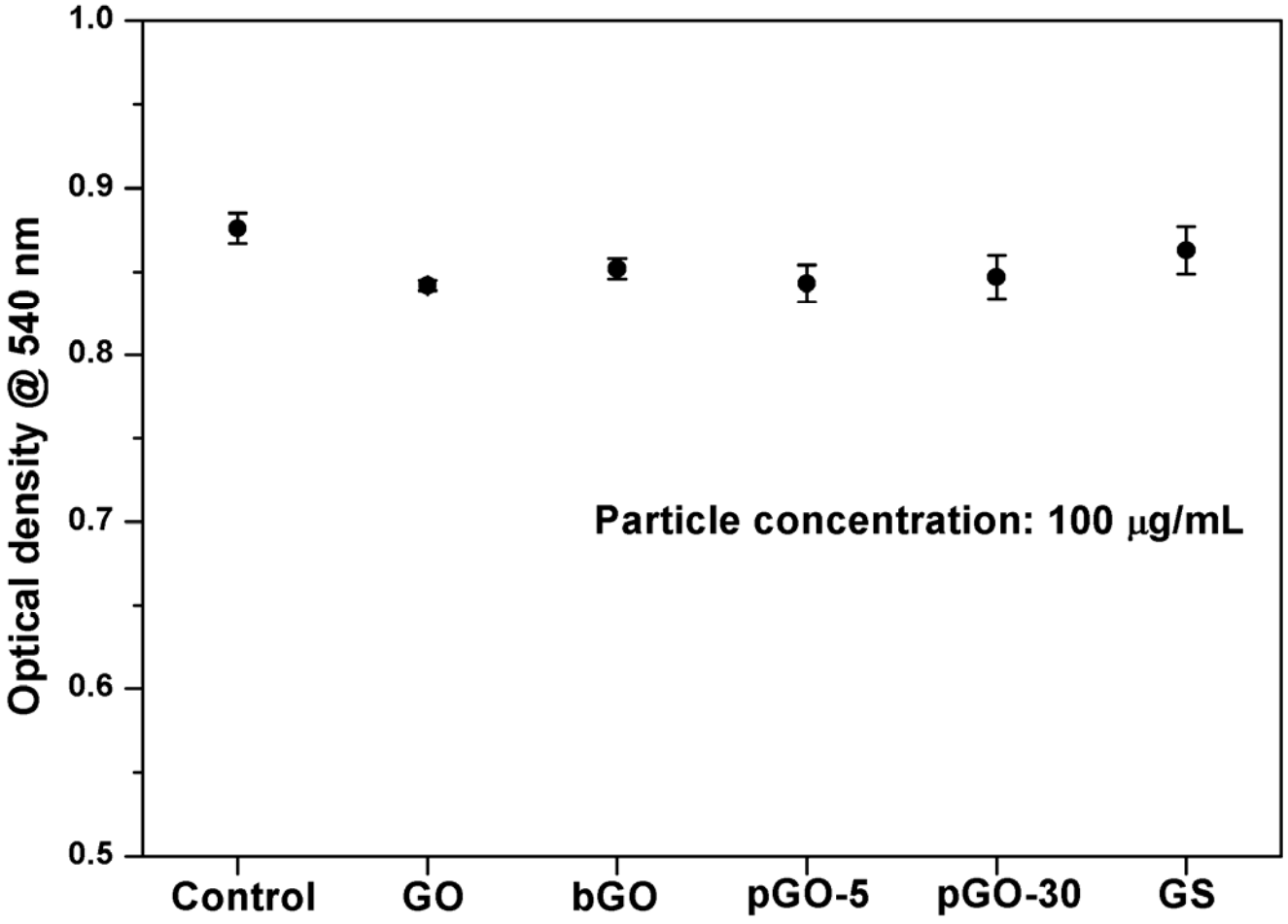

Figure S5. None of the five GO and GS samples adsorb significant amounts of hemoglobin. Hemoglobin was mixed with GO, bGO, pGO-5, pGO-30, and GS (100 μg mL^{-1} in PBS) and incubated for 3 hours at 37 ^{o}C. Each data point is the average of three measurements of optical density of the supernatant.

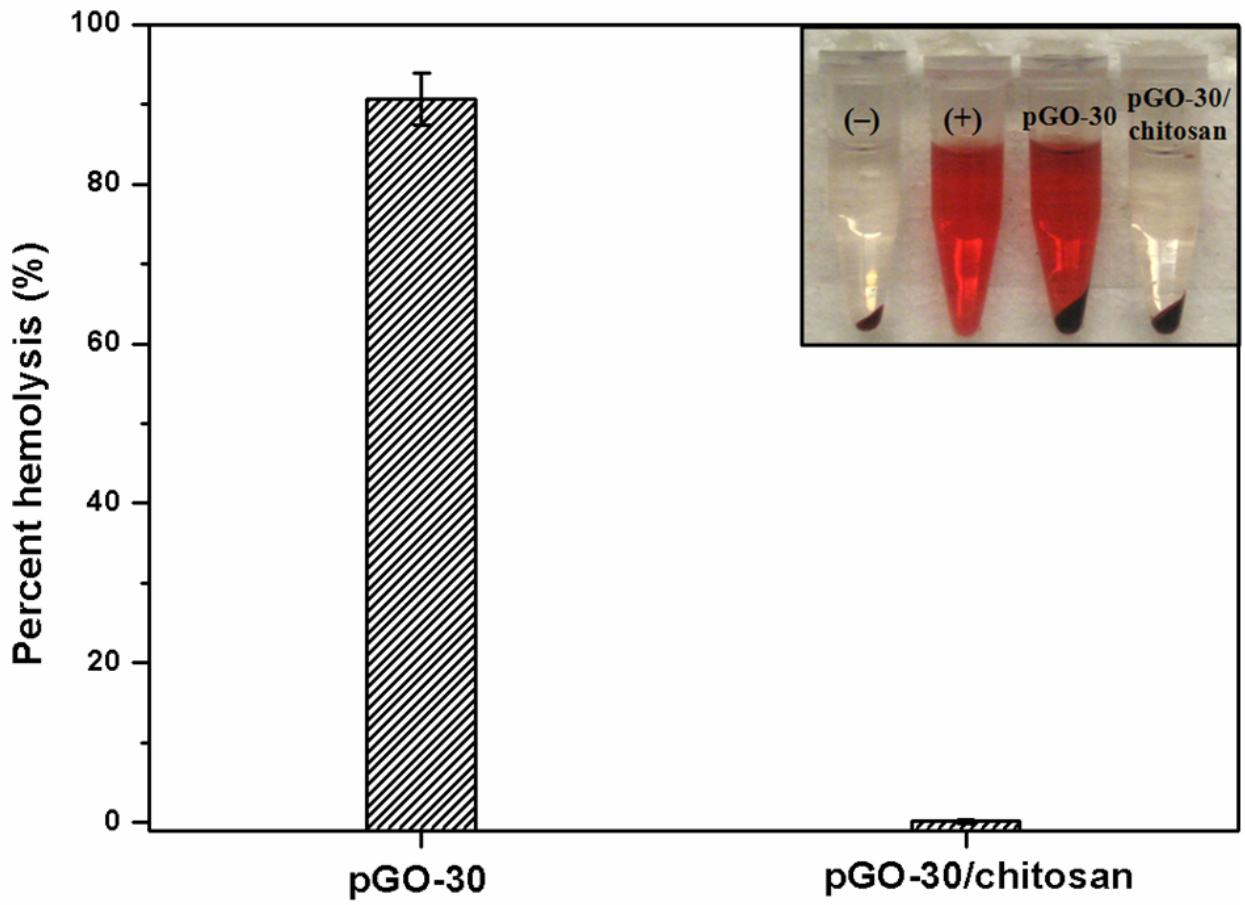

Figure S6. Percent hemolysis of RBCs incubated with 100 μg mL^{-1} of pGO-30 and pGO-30/chitosan for 3 hours at 37 ^{o}C with agitation. Data represent mean ± SD from three independent experiments. Inset: photographs of RBCs after 3-hour exposure to pGO-30 and pGO-30/chitosan with negative and positive controls. (+) and (-) symbols represent positive control and negative control, respectively.

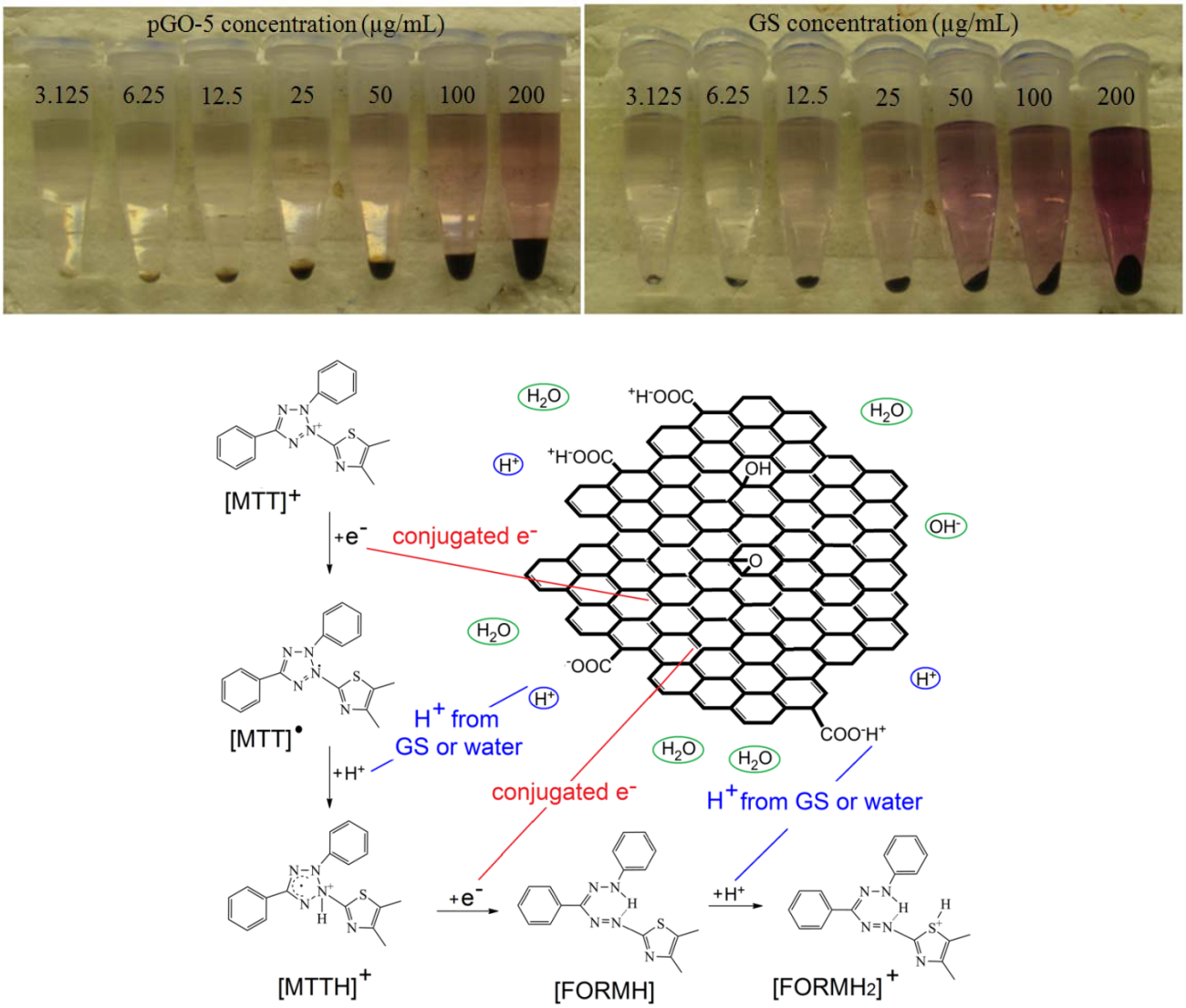

Figure S7. Photographs of MTT formazan (in the DMSO supernatant) produced by pGO-5 and GS particles. [MTT]+ will react with the conjugated electrons from GS to form the radical intermediate [MTT]•. Then [MTT]• will react with the protons to form a protonated cation [MTTH]+ under at least pH<13 environment. [MTTH]+ will then react with another electrons from GS to form [FORMH], followed by a further protonated reaction with another proton mainly from GS to form [$FORMH_2$]+.

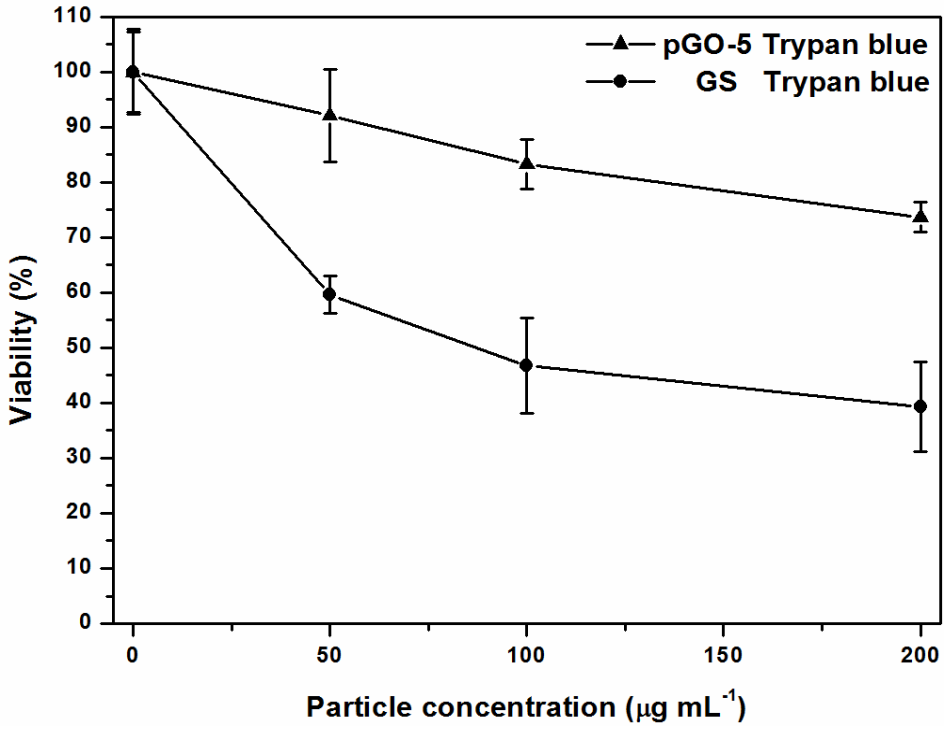

Figure S8. Cell viability of human skin fibroblast cells determined from trypan blue exclusion assay after exposure to different concentrations of pGO-5 and GS. Data represent mean ± SD, n=3.

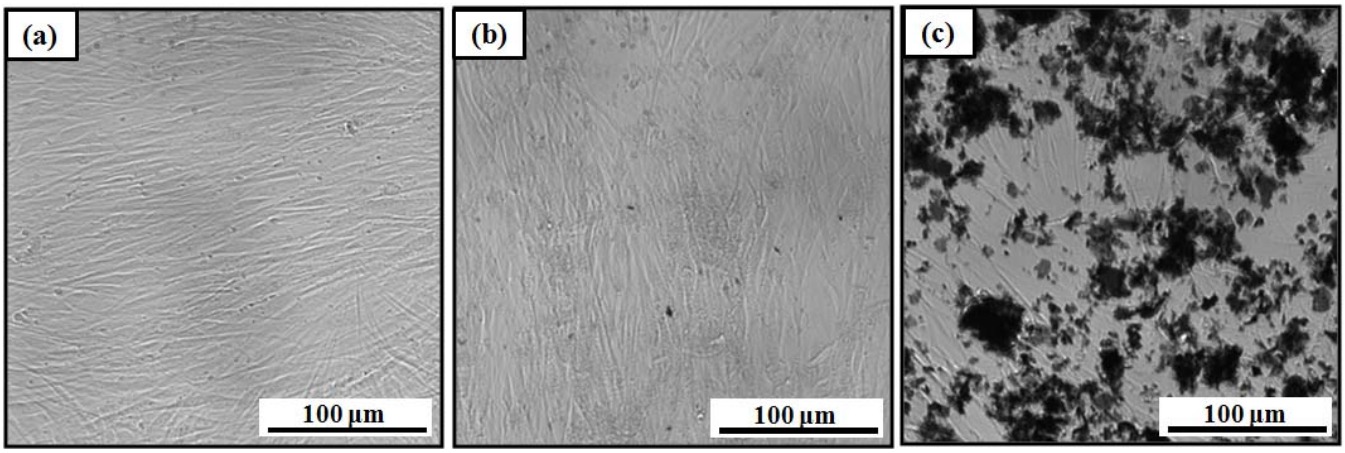

Figure S9. Optical microscopy images of human skin fibroblasts after 24-hour incubation at 37 ^{o}C (5% CO_2) in the presence of (a) medium (control), (b) 50 μg mL^{-1} of pGO-5, and (c) 50 μg mL^{-1} of GS

particles.

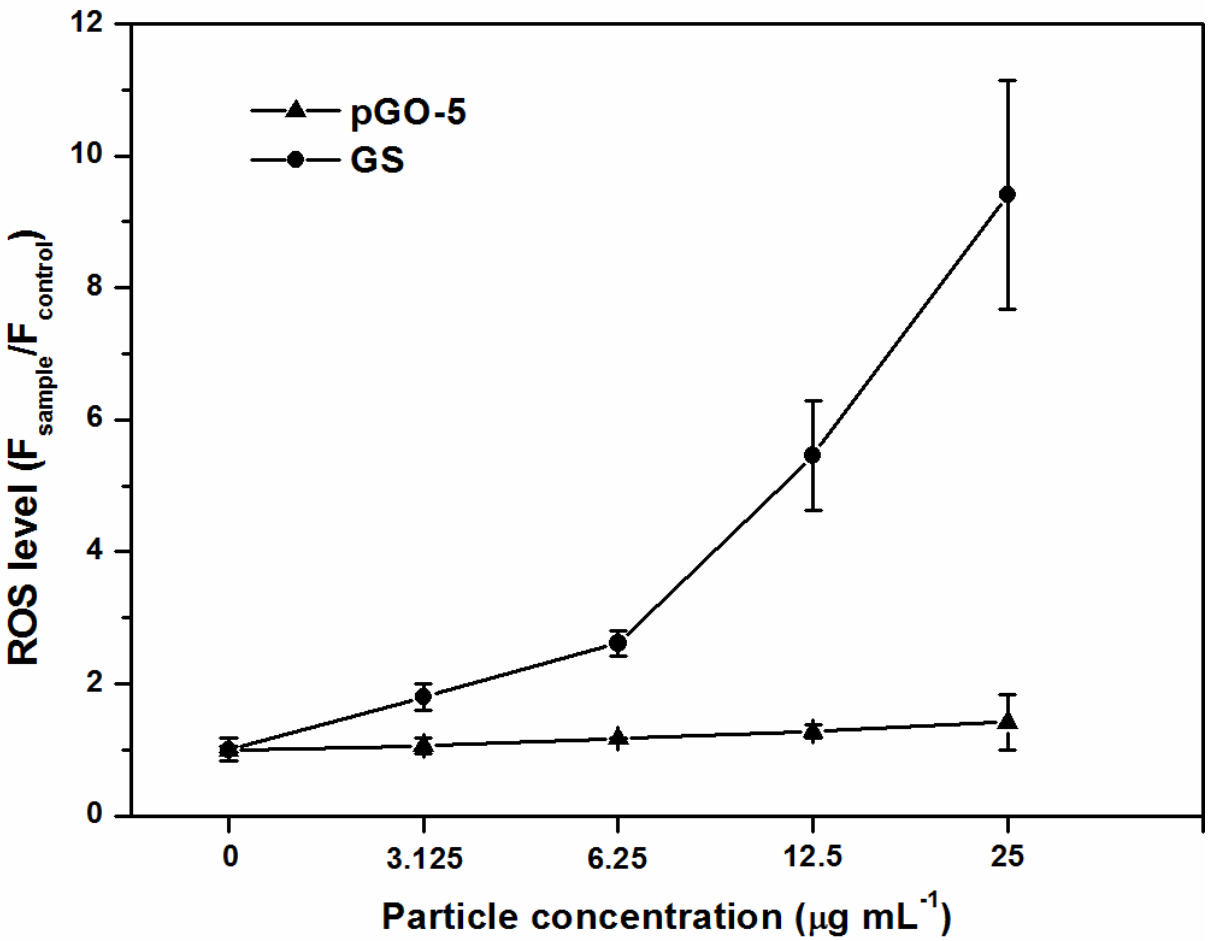

Figure S10. Effect of pGO-5 and GS on the generation of ROS in human skin fibroblast cells.

Reference:

1. Fang, M.; Long, J.; Zhao, W.; Wang, L. Chen, G., *Langmuir*, **2010**, *26*, 16771-16774.