Supporting Information

GO-Se nanocomposite as an antioxidant nanozyme for cytoprotection
Yanyan Huang, a,b Chaoqun Liu, a,b Pu Fang, a Zhen Liu, a Jinsong Ren,* a and Xiaogang Qu* a

Experimental Section

Reagents and materials: Graphite, selenium (IV) oxide and polyvinylpyrrolidone (PVP) were purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). Glutathione, L-Ascorbic acid (VC) and glutathione reductase were purchased from Sigma-Aldrich. Nicotinamide adenine dinucleotide phosphate (NADPH) was purchased from Sangon Biotechnology Inc. (Shanghai, China). All other reagents were of analytical reagent grade, and used as received. Ultrapure water (18.2 MΩ; Millpore Co., USA) was used throughout the experiment.

Measurements and characterizations: The scanning electron microscopy (SEM) samples were prepared by depositing a dilute aqueous dispersion of the as-prepared samples onto a silicon wafer. Transmission electron microscopy (TEM) images, high-angle annular dark-field scanning TEM (HAADF-STEM) and the energy dispersive X-ray spectroscopy (EDX) were recorded using a FEI TECNAI G2 20 high resolution transmission electron microscope operating at 200 kV. FT-IR characterization was carried out on a BRUKER Vertex 70 FT-IR spectrometer. X-ray photoelectron spectroscopy (XPS) was recorded using a Perkin Elmer PHI 5600. The crystalline structures of the as-prepared samples were evaluated by X-ray diffraction (XRD) analysis on a on a D8 Focus diffractometer (Bruker) using Cu Kα radiation (λ =
Atomic-force microscopy (AFM) measurements were performed using Nanoscope V multimode atomic force microscope (Veeco Instruments, USA). Tapping mode was used to acquire the images under ambient conditions. The UV-Vis absorption spectra were recorded using a JASCO V550 UV-Visible spectrophotometer (JASCO International Co., LTD., Tokyo, Japan). Fluorescence measurements were carried out by using a JASCO FP-6500 spectrofluorometer (Jasco International Co., Japan).

**Synthesis of Graphene Oxide (GO):** GO was prepared according to the Hummer's method. Pretreated graphite powder was put into 120 mL concentrated H₂SO₄ (0 °C). After that, 15 g KMnO₄ was gradually added into the mixture under stirring. An ice bath was used to keep the temperature of the solution below 20 °C. The mixture was further stirred under 35 °C for 4 h and then diluted with 250 mL deionized water by keeping the temperature at 50 °C. Then, inject 700 mL water into the above solution followed by adding 20 mL H₂O₂ (30 wt %) drop by drop. To remove metal ions, the mixture was filtered and washed with an aqueous HCl solution (v/v 1:10, 1L). To remove HCl, the mixture was filtered and washed with deionized water. The obtained product was further dialyzed for 1 week. Exfoliation was carried out by sonicating the GO dispersion under ambient conditions for 1 h.

**Synthesis of selenium nanoparticles:** SeO₂ (1 mM) and PVP (0.5 mg mL⁻¹) were incubated for 10 min and then L-Ascorbic acid (5 mM) was added to the mixture solution. The mixture was stirring for 2 h and then dialyzed.
Synthesis of GO-Se nanocomposites: GO (0.2 mg mL\(^{-1}\)) and SeO\(_2\) (3 mM) were incubated for 30 min and then L-Ascorbic acid (10 mM) was added to the mixture solution. The mixture was stirring for 1 h and then dialyzed.

Synthesis of FITC-midified GO-Se nanocomposites: 1 mg GO-Se nanocomposites and 1 mg fluorescein isothiocyanate (FITC) were dispersed in 2 mL anhydrous tetrahydrofuran (THF). Then the mixture was stirred under 30 °C. After 2 h, the product was centrifuged, washed with ethanol for 3 times and dried.

GPx-like activity of GO-Se nanozyme: GR-coupled assay was used to study the GPx-like capacity of GO-Se nanozyme. In a typical assay, 0.01 mg mL\(^{-1}\) GO-Se nanocomposites, 2 mM GSH, 0.4 mM NADPH, 1.7 units GR and 240 μM H\(_2\)O\(_2\) were added in 25 mM pH 7.4 phosphate buffer under 25 °C. Control experiments were carried out in the absence of at least one of the reactants.

Cell culture: The RAW264.7 cells were incubated in Dulbecco's modified eagle medium (DMEM) containing 1% Penicillin-Streptomycin and 10% fetal bovine serum under 37 °C in a humidified atmosphere of 5% CO\(_2\).

In vitro cytotoxicity assays: RAW264.7 cells were seeded at a density of 10 000 cells/well in 96-well assay plates for 24 h. After that, GO-Se nanocomposites with different concentrations were added to the cell culture medium. Cells and nanocomposites were further incubated for 24 h. To determine toxicity, 10 μL of MTT solution was added to each well of the microtiter plate and the plate was incubated in the CO\(_2\) incubator for another 4 h. After that, The cell media was removed and 100 μL DMSO was added into each well. To dissolve all formed
precipitate, the plate was then gently swirled for 2 min at room temperature at dark. Bio-Rad model-680 microplate reader was used to measure the absorbance values at 490 nm.

*Intracellular Determination of ROS:* 2',7'-dichlorofluorescein diacetate (DCFH-DA) is one nonfluorescent compound which reacts with intracellular free radicals and generates the fluorescent product dichloro-fluorescein (DCF). In our system, it was used to monitor the production of reactive oxygen radicals and the fluorescence intensity depends on the amount of reactive oxygen radicals in cellular environment.

To perform the experiment, RAW264.7 cells were first seeded in a 6-well plate and cultured for 24 h. After that, cell medium was removed and the adherent cells were incubated with 20 μg mL⁻¹ GO-Se nanocomposites for 4 h under 37 °C. To remove the excess nanoparticles, cells were washed three times with pH 7.4 phosphate buffer. The cells were then incubated at 37 °C for another 1 h with 50 μg mL⁻¹ Rosup. Then, the RAW264.7 cells was further incubated with DCFH-DA at 37 °C for 1 h. BD FACS Aria was used to measure the flow cytometric data. The excitation wavelength was 488 nm and signals were collected at FITC channel 500-560 nm.

*Lipid peroxidation:* RAW264.7 cells were first seeded in a 6-well plate and cultured for 24 h. After that, cell medium was removed and the adherent cells were incubated with 20 μg mL⁻¹ GO-Se nanocomposites or selenium nanoparticles for 4 h under 37 °C. To remove the excess nanoparticles, cells were washed three times with pH 7.4 phosphate buffer. The cells were then incubated at 37 °C for another 1 h with 50 μg mL⁻¹ Rosup. Treated or untreated cells were harvested and sonicated at 30%
amplitude for 3 pulses of 1 min. After that, trichloroacetic acid (1 mL, 70 mg mL⁻¹) and TBA (1 mL, 5 mg mL⁻¹) were added into the above cell lysate (1 mL). The mixture was then incubated under 80 °C for 1 h. Finally, the solutions were centrifuged at 12,000 rpm for 10 min and the supernatant was collected for UV-Vis absorption spectra measurement.
Figure S1. The TEM image of GO nanosheets.
Figure S2. The AFM pattern of GO nanosheets.
Figure S3. TEM image of GO-Se nanocomposites.
Figure S4. EDX pattern of the GO-Se nanocomposites.
Figure S5. XPS spectra of GO-Se nanocomposites. Inset was Se3d spectrum of the nanocomposites.
Figure S6. A) Fourier Transform Infrared Spectroscopy of GO, Se and GO-Se nanomaterials; B) Fourier Transform Infrared Spectroscopy of GO and GO-Se nanomaterials.
Figure S7. XRD pattern of GO, Se and GO-Se nanomaterials.
Figure S8. UV-Vis spectra of GO, Se and GO-Se nanomaterials. Inset was their corresponding photograph.
Figure S9. The SEM image of Se nanoparticles.
Figure S10. The TEM image of Se nanoparticles.
Figure S11. Time-dependent absorbance changes of NADPH in the presence of different concentrations of GO-Se nanocomposites.
Figure S12. The Michaelis-Menten kinetics of GO-Se nanocomposites. Lineweaver-Burk plots of GPx activity of nanocomposites corresponding to the variation of GSH concentration (A and B) and H₂O₂ concentration (C and D), respectively; E) the Maximum initial velocity (V_{max}) and Michaelis-Menten constant (K_m) of GO-Se nanocomposites, V_2O_5 nanowires and natural GPx.

<table>
<thead>
<tr>
<th>Catalyst</th>
<th>Substrate</th>
<th>K_m [mM]</th>
<th>V_{max} [M s⁻¹]</th>
</tr>
</thead>
<tbody>
<tr>
<td>GO-Se</td>
<td>GSH</td>
<td>0.02</td>
<td>0.2⋅10⁻³</td>
</tr>
<tr>
<td>V_2O_5</td>
<td>GSH</td>
<td>2.22</td>
<td>1.4⋅10⁻³</td>
</tr>
<tr>
<td>GPx</td>
<td>GSH</td>
<td>10</td>
<td>-</td>
</tr>
<tr>
<td>GO-Se</td>
<td>H₂O₂</td>
<td>0.04</td>
<td>5.0⋅10⁻⁷</td>
</tr>
<tr>
<td>V_2O_5</td>
<td>H₂O₂</td>
<td>0.11</td>
<td>3.2⋅10⁻⁸</td>
</tr>
<tr>
<td>GPx</td>
<td>H₂O₂</td>
<td>0.025</td>
<td>-</td>
</tr>
</tbody>
</table>
Figure S13. The effect of ionic strength and pH on our system: the enzyme-mimic capacities of our nanocomposites were measured under different phosphate buffer. 1) 25 mM pH 7.4; 2) 100 mM pH 7.4; 3) 25 mM pH 5.5; 4) 100 mM pH 5.5.
Figure S14. The FL emission spectra of FITC modified GO-Se nanocomposites. Inset was the corresponding photograph under the UV plate exposure.
Figure S15. Fluorescence microscopy images of untreated RAW 264.7 cells (A-C) and FITC-modified GO-Se nanocomposites treated cells (D-F).