Supporting Information

Cisplatin-induced self-assembly of graphene oxide sheets into spherical nanoparticles for damaging sub-cellular DNA

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Materials and Methods

Material:

Graphene oxide (4mg/ml), distilled water, proflavine (3,6-diaminoacridine hydrochloride), sodium dodecyl sulfate (SDS), cisplatin, 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and silicon wafer for FE-SEM were acquired from Sigma-Aldrich. Doxorubicin was bought from Selleck Chemicals. DMEM media and DAPI were purchased from HiMedia. Annexin-V-FITC Staining Kit was purchased from Roche. LysoTracker™ Red DND-99, LysoTracker™ Green DND-26, SlowFade® Gold Antifade Reagent were obtained from Life Technologies. Anti-PARP antibody-clone 7A10, anti-phospho-histone H2AX (Ser139) antibody-clone JBW301, GAPDH antibody were obtained from BioLegend. HeLa cells were obtained from National Centre for Cell Science (NCCS), Pune.

Synthesis of aquated cisplatin (3):

Aquated cisplatin was prepared by using the method described in reference 1.

Synthesis of GO-Prof conjugate (2):

Graphene oxide (1) (4 mg/mL, 250 µL) was dispersed in distilled water (2 mL). Aqueous solution of proflavine (5 mg, 0.023 mmol) in distilled water (1 mL) was prepared and added to the dispersed graphene oxide solution. The reaction was stirred in dark at room temperature for 24h. To remove un-reacted proflavine, the reaction mixture was dialysed against distilled water through dialysis membrane (MWCO = 1 kDa) for 24 h. Water was lyophilized to obtain GO-Prof conjugate (2).

Synthesis of GO-Prof-CDDP conjugate (4) and GPC-NPs:

1 mg of GO-Prof conjugate (2) was suspended in 1 mL distilled water and aquated cisplatin (3) (5 mg/mL, 0.019 mmol) into it. The reaction mixture was stirred at room temperature for 24h. The reaction mixture was further dialyzed (MWCO = 1 kDa) against water for 6 h to remove excess of aquated cisplatin to obtain GO-Prof-CDDP conjugate (4). 20 µL of dialyzed solution was further diluted to 1 mL by distilled water for size, shape and morphology characterization.

Synthesis of GO-Dox conjugate (5):

Graphene oxide (1) (4 mg/mL, 250 µL) was dispersed in distilled water (2 mL). Aqueous solution of doxorubicin (0.5 mg, 0.023 mmol) in distilled water (1 mL) was prepared and added to the dispersed graphene oxide solution. The reaction was stirred at room temperature for 24 h. To remove un-reacted doxorubicin, the reaction mixture was dialyzed (MWCO = 1kDa) against distilled water through for 24 h. Water was lyophilized to obtain GO-Dox conjugate (5).

Synthesis of GO-Dox-CDDP conjugate (6) and GDC-NPs:

1 mg of GO-Dox conjugate (5) was suspended in 1 mL distilled water and aquated cisplatin (5 mg/mL, 0.019 mmol) was added into it. The reaction mixture was stirred at room temperature for 24h. The reaction mixture was further dialyzed (MWCO = 1 kDa) against water for 6 h to remove excess of aquated cisplatin to obtain GO-Dox-CDDP conjugate (6). 20 µL of dialyzed solution was further diluted to 1 mL by distilled water for size, shape and morphology characterization.
Field-Emission Scanning Electron Microscopy (FESEM) of GPC-NPs and GDC-NPs

15 μL of samples (GO-Prof, GO-Dox, GPC-NPs, GDC-NPs) were diluted in 1 mL water and sonicated for 1 min. 2 μL of these solutions were placed on a silicon wafer without any dopant and it was allowed to dry at room temperature under vacuum desiccators for 2 h. The FESEM images were taken by using the method described in reference 2.

Atomic Force Microscopy (AFM) of GPC-NPs and GDC-NPs:

15 μL of samples (GO-Prof, GO-Dox, GPC-NPs, GDC-NPs) were diluted in 1 mL water and sonicated for 1 min. Then 10 μL of these solutions were placed on mica sheet and dried under the vacuum desiccators for 2 h. AFM images were taken using the method described in reference 2.

Transmission Electron Microscopy (TEM) of GPC-NPs and GDC-NPs:

15 μL of GPC-NPs and GDC-NPs were diluted in 1 mL water and sonicated for 1 min. Then 15 μL of the GO-NPs was placed on a TEM copper grid. After 30 min, this sample drop was wicked off by using filter paper and then 15 μL of freshly prepared 0.25% uranyl acetate solution was placed on the TEM copper grid. After 1 min, uranyl acetate solution was wicked off and the sample was washed three times with 15 μL dd water each time. The sample was dried overnight on a clean dust free surface under a funnel. The TEM images were captured by using the method described in reference 2.

Resonance Raman Spectroscopy:

Resonance Raman spectra were collected using a Lab RAM HR 800 (Horiba scientific) using laser excitation wavelength of 532 nm excitation with a 50X objective at room temperature. 532 nm was chosen as the excitation to guarantee a good signal/noise ratio. Prior to analysis the baseline of the spectrum was extracted using the software NGSLabSpec.

Powder X-ray diffraction (PXRD):

Powder X-ray diffraction (PXRD) spectra of GO, GO-Prof, GPC-NPs and GO-CDDP in different concentrations were collected using a Bruker D8-Advance X-ray powder diffractometer (Cu Kα radiation; λ=1.5418 °A) in the range 3-40° (0.010° step size, 175 s holding time).

X-ray photoelectron spectroscopy (XPS):

XPS spectra were acquired in an ultra high vacuum equipment (10⁻¹ mbar) using a hemispherical electron energy analyzer and an Mg K-alpha X-ray source (1253.6 eV). The XPS spectra of the different regions were decomposed in lorentzian Gaussian curve components. All samples were measured under the same conditions and fitted with the same.

In vitro assays:

Cellular internalization, cell viability by MTT assay, apoptosis detection by fluorescence-activated cell sorting (FACS), and Western blot analyses were performed by using the procedure described in reference 2 and reference 3.

Quantification of drug release from GPC-NPs and GDC-NPs:

Quantification of dual drug loading and release from the nanoparticles were determined by the dialysis method described in reference 4.
**Fig. S1:** Synthetic scheme of GO-Prof-CDDP and GO-Dox-CDDP conjugates.

**Fig. S2:** (a-c) AFM images of GO, GO-Prof and GO-Dox conjugates showing height increase after stacking proflavine and doxorubicin on GO surface.
Fig. S3: (a-c) AFM images of GO, GO-Prof and GO-Dox showing the sheet like morphology.

Fig. S4: (a-b) Fluorescence emission spectra of GO-Prof and GO-Dox showing fluorescence quenching of proflavine and doxorubicin upon stacking on GO surface.
**Fig. S5**: (a-b) Histograms determined from FESEM images to show the mean diameter and polydispersity index (PDI) for GPC-NPs and GDC-NPs respectively.

**Fig. S6**: (a-b) TEM images of GPC-NP and GDC-NP.
**Fig. S7:** (a-b) Fluorescence emission spectra of GPC-NPs and GDC-NPs showing fluorescence quenching of proflavine and doxorubicin upon stacking on GO surface.

**Fig. S8:** EDX of GPC-NPs from FESEM images showing the presence of cisplatin.
**Fig. S9:** EDX of GDC-NPs from FESEM images showing the presence of cisplatin.

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<tr>
<th>Element</th>
<th>Weight%</th>
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<td>C</td>
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**Fig. S10:** (a-b) XPS of GPC-NPs showing the presence of nitrogen from proflavine moiety and Pt(II) from cisplatin.
Fig. S11: (a-e) FESEM images of GO-CDDP composites in different weight ratios to evaluate cisplatin mediated self-assembly.

Fig. S12: EDX of GO-CDDP-NPs at different weight ratios to show the presence of cisplatin
**Fig. S13:** PXRD of GO-CDDP composites in different weight ratios.

**Fig. S14:** (a-b) FESEM images of GO-CDDP-Proflavine-NPs and GO-CDDP-Dox-NP.
Fig. S15: CLSM images of HeLa cells after incubating with GPC-NPs at 1h, 3h and 6h time points. Lysosomes and nuclei were stained with LysoTracker Red and DAPI (blue). Merged images show the colocalization of GPC-NPs in lysosomes in a time dependent manner. Scale bar = 10 μm.
Fig. S16: High resolution CLSM images of HeLa cells after incubating with GPC-NPs at 1h, 3h and 6h time points. Lysosomes and nuclei were stained with LysoTracker Red and DAPI (blue). Merged images show the colocalization of GPC-NPs in lysosomes in a time dependent manner. Scale bar = 10 μm.
Fig. S17: CLSM images of HeLa cells after incubating with GDC-NPs at 1h, 3h and 6h time points. Lysosomes and nuclei were stained with LysoTracker Green and DAPI (blue). Merged images show the colocalization of GDC-NPs in lysosomes in a time dependent manner. Scale bar = 10 μm.
**Fig. S18**: High resolution CLSM images of HeLa cells after incubating with GDC-NPs at 1h, 3h and 6h time points. Lysosomes and nuclei were stained with LysoTracker Green and DAPI (blue). Merged images show the colocalization of GDC-NPs in lysosomes in a time dependent manner. Scale bar = 10 μm.
Fig. S19: CLSM images of HeLa cells after incubating with GO-Prof composite at 1h, 3h and 6h time points. Lysosomes and nuclei were stained with LysoTracker Red and DAPI (blue). Merged images show the colocalization of GO-Prof composite in lysosomes and in nucleus. Scale bar = 10 μm.
Fig. S20: High resolution CLSM images of HeLa cells after incubating with GO-Prof composite at 1h, 3h and 6h time points. Lysosomes and nuclei were stained with LysoTracker Red and DAPI (blue). Merged images show the colocalization of GO-Prof composite in lysosomes and in nucleus. Scale bar = 10 μm.
Fig. S21: CLSM images of HeLa cells after incubating with GO-Dox composite at 1h, 3h and 6h time points. Lysosomes and nuclei were stained with LysoTracker Green and DAPI (blue). Merged images show the colocalization of GO-Dox composite in lysosomes and in nucleus. Scale bar = 10 μm.
Fig. S22: High resolution CLSM images of HeLa cells after incubating with GO-Dox composite at 3h and 6h time points. Lysosomes and nuclei were stained with LysoTracker Green and DAPI (blue). Merged images show the colocalization of GO-Dox composite in lysosomes and in nucleus. Scale bar = 10 μm.
Fig. S23: FACS analysis of HeLa cells pre-treated with different endocytosis inhibitors followed by GPC-NPs.

Fig. S24: FACS analysis of HeLa cells pre-treated with different endocytosis inhibitors followed by GDC-NPs.
Fig. S25: (a-b) Time dependent release of proflavine/cisplatin and doxorubicin/cisplatin from GPC-NPs and GDC-NPs respectively at pH = 5.5 mimicking lysosome environment. (c) Plausible mechanism of dual drug release at pH = 5.5 leading to the dis-assembly of spherical nanoparticles into sheet like structures.
**Fig. S26:** FESEM images of GPC-NPs at different time points at pH = 5.5 mimicking lysosome environment.

**Fig. S27:** FESEM images of GDC-NPs at different time points at pH = 5.5 mimicking lysosome environment.
**Fig. S28:** (a-b) Quantification of γH2AX and PARP after treating HeLa cells with GPC-NPs and GDC-NPs from western blot analysis.

**Fig. S29:** (a-c) Cell viability assay of GO-CDDP-NPs, free doxorubicin and free proflavine respectively in HeLa cells at 48 h post-incubation.
Fig. S30: (a-b) Concentration dependent cell viability assay of GPC-NPs and GDC-NPs in L929 mouse fibroblast cells respectively at 24h post-incubation. (c) Concentration dependent cell viability assay of GO in L929 mouse fibroblast cells at 24h post-incubation.

References:


