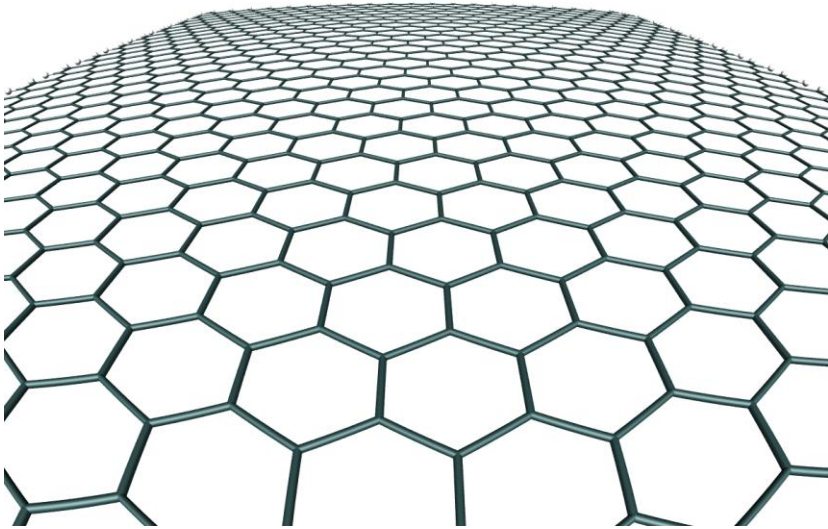
Highly Biocompatible Reduced Graphene Oxide and its Applications in Drug Delivery and Early Cancer Detection



By Assoc. Prof. Dr Kasturi Muthoosamy
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Introduction

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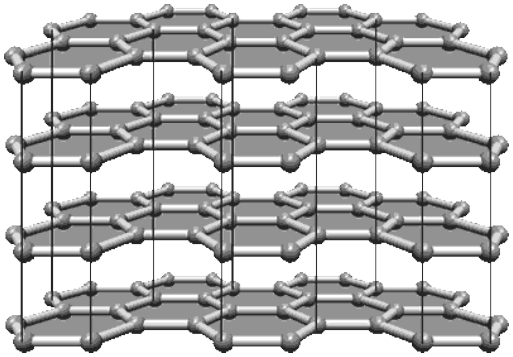
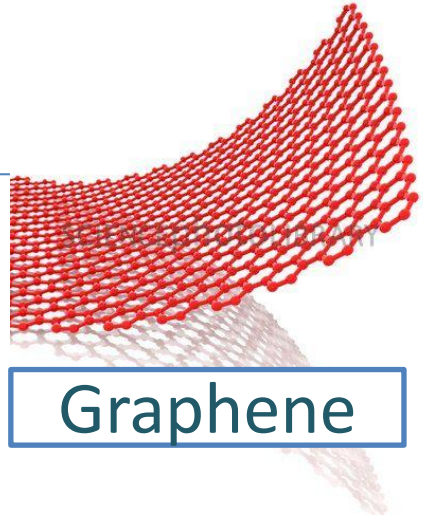
Graphene derivatives

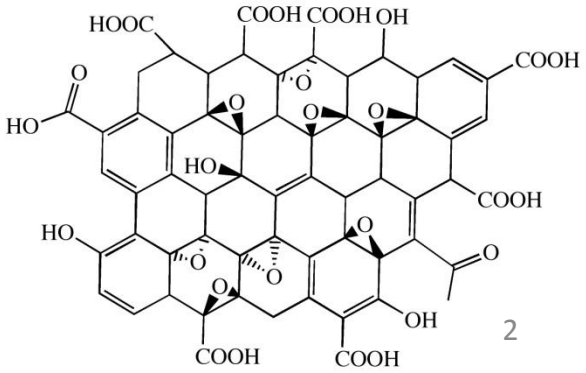
Graphite

Graphene oxide (GO)





Graphene



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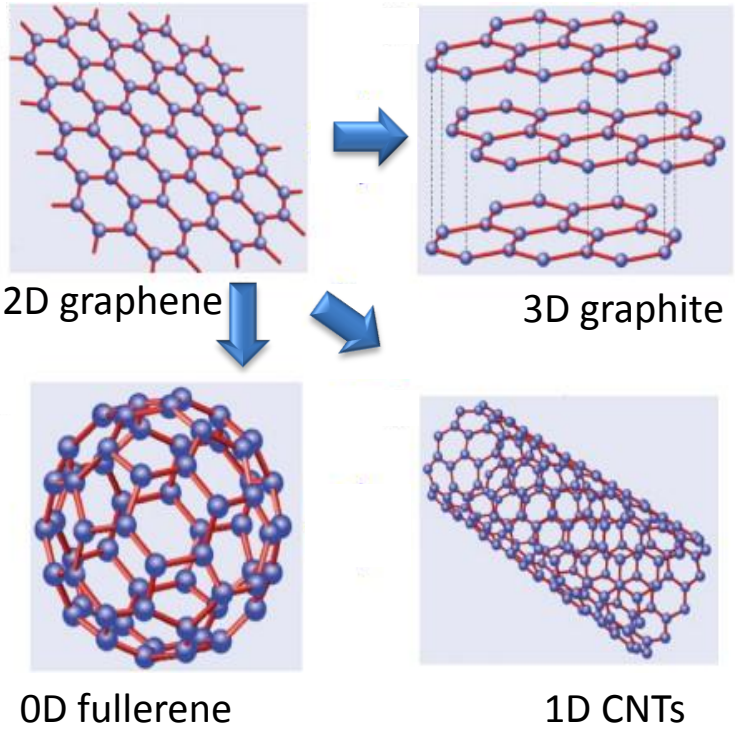
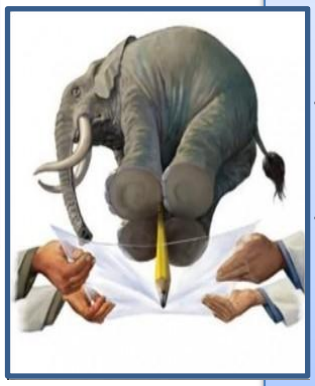
Graphene derivatives





Properties

- Superlative mechanical strength (Young's modulus of 1100 GPa)
- Unparalleled thermal conductivity (5000 W/m/K)
- Exceptional electrical conductivity (mobility charge carrier 200,000 $cm^2/V/s$)
- **High planar surface area (2630 m^2/g)**

Applications

- Energy storage devices
- Electrodes
- Photodetectors
- Biosensing
- Photothermal therapy
- Medical imaging
- **Drug delivery applications**

Introduction

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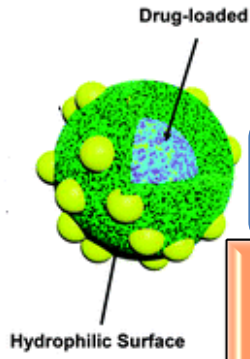
Graphene and other drug delivery cargos

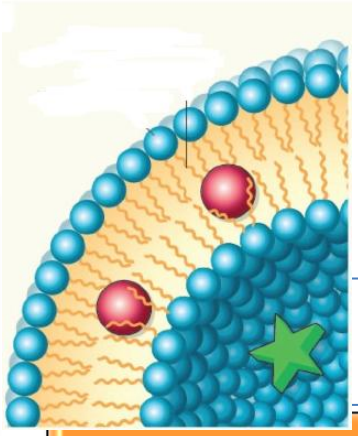


Polymeric micelles

- Drug loading efficiency (LE): 15-95%

Drawbacks:

- Tedious synthesis route
- Loading capapcity ≈20 wt.%
- Right selection of polymer
- Functional groups present
- Charge

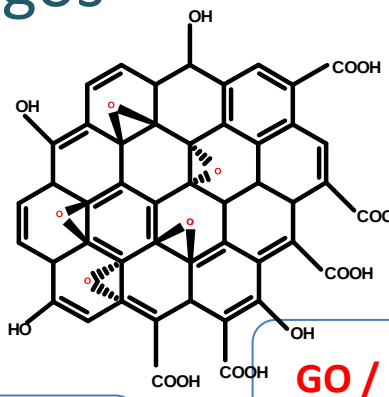


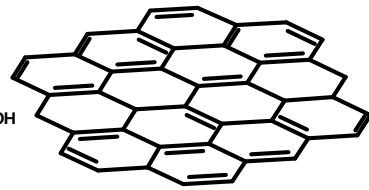
Lipids

- Drug LE: 0.2-5%

Drawbacks:

- Unpredictable gelation tendencies
- Low incorporation rate, crystalline structure





GO / reduced GO (RGO)

- Drug LE: 95%
- Ease of functionalization
- Drug delivery of hydrophobic drugs without modifications
- **High drug loading ≈200 wt.% via pi-pi stacking**

Research Background

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Conventional Graphene production

Modified Hummer's method.

Graphite powder

H_2SO_4,
H_3PO_4
$KMnO_4$

72 h

Graphene oxide (GO)

Chemical reduction

anhydrous hydrazine, hydrazine monohydrate, sodium borohydrate, hydroquinone, metal/ hydrochloric acid

Concerns
-Toxicity

Graphene/ Reduced graphene oxide (RGO)

Muthoosamy K, Bai RG, Manickam S. Graphene and graphene oxide as a docking station for modern drug delivery system. *Current Drug Delivery* 2014;11;1-18.

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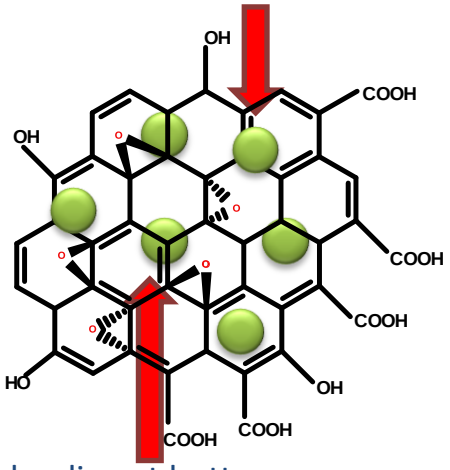
Graphene as drug delivery cargo

Drug loading on GO & RGO

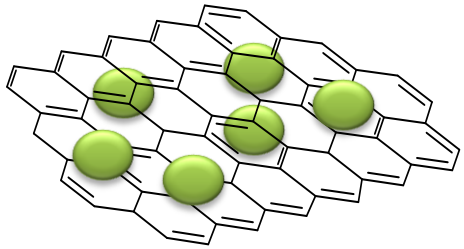
2-layered drug loading

loading on top



loading at bottom

Graphene oxide (GO)



Reduced graphene oxide (RGO)

Limitations:
-Steric hindrance

Limitations:
-Solubility

Methodology – Part 1

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Synthesis of RGO

Use of mushroom extract as an effective reducing agent.

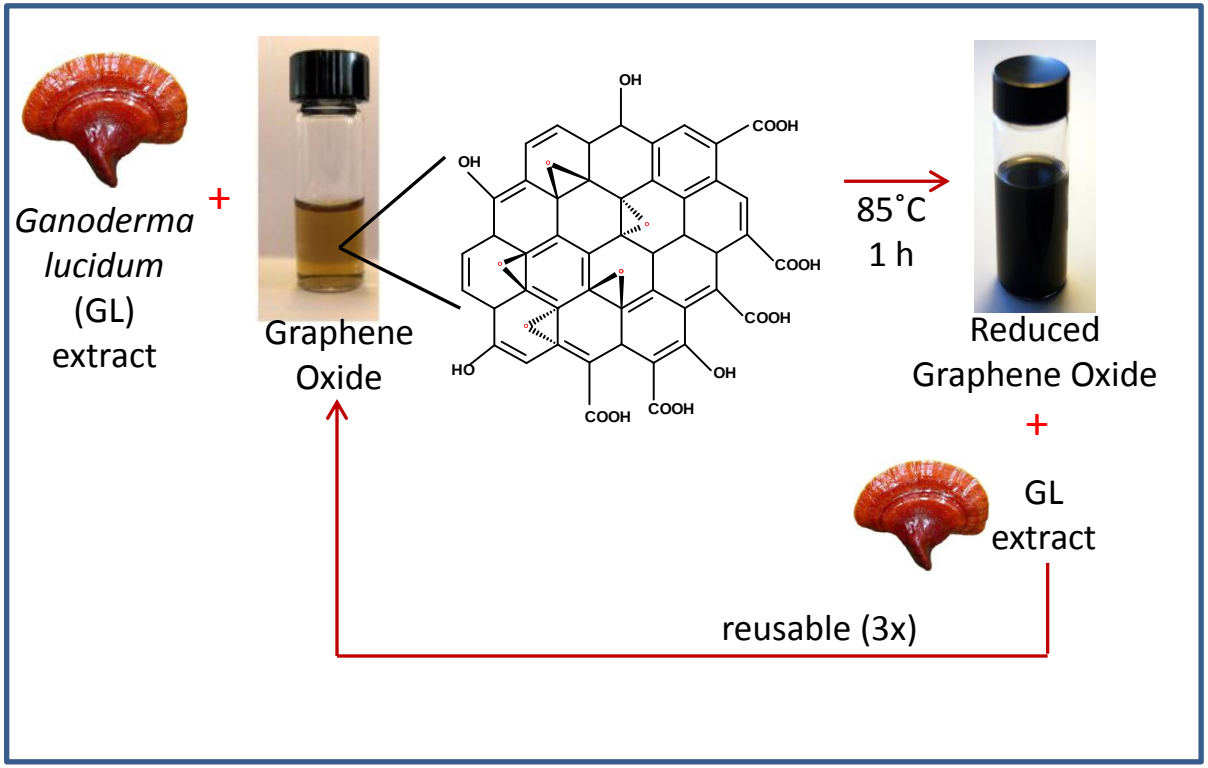


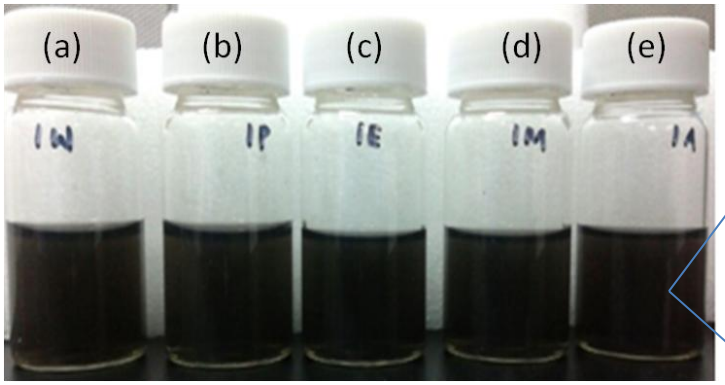
Fig. 1: Schematic representation of RGO production from the reduction of GO.

Research highlights

- *In-situ* reduction of GO with reusability of reducing agent (3x) with 75% conversion efficiency
- Green-synthesis with easy separation, purification and bulk production

Results – Part 1

Characterization of RGO



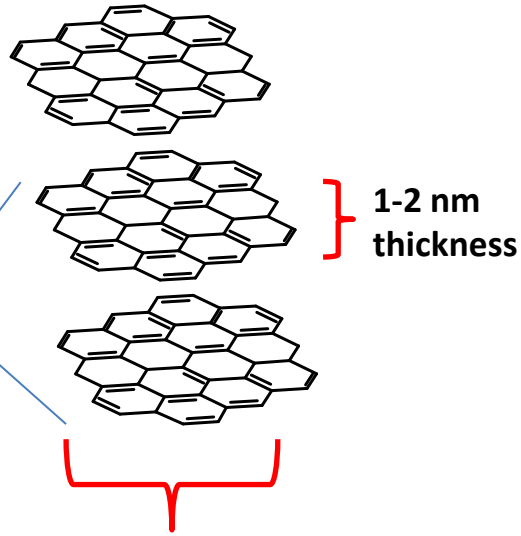


Fig. 2: Solubility of RGO in (a) water (b) PBS buffer (c) ethanol (d) methanol and (e) acetone.

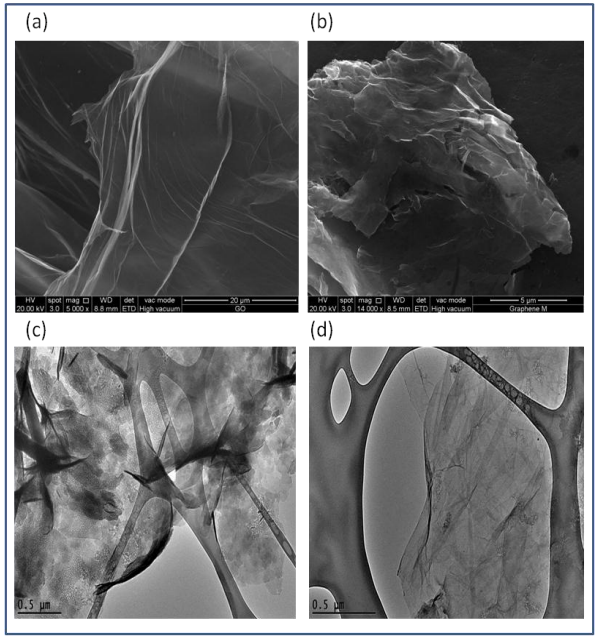


Fig. 3: FESEM images of (a) GO and (b) RGO and HRTEM images of (c) GO and (d) RGO.

Research highlights

- Dispersible in water and other solvents and stable for a year
- RGO nanosheets (3 layers thick based on Raman spectroscopy)

Results – Part 1

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Cell viability of GO and RGO

Table 1: IC_{50} values of cells upon treatment with GO and RGO.

Sample	Cell line (IC_{50} µg/ml)		
	HT-29	U87MG	MRC-5
GO	261.1	26.27	138.2
RGO	392.7	132.40	364.4

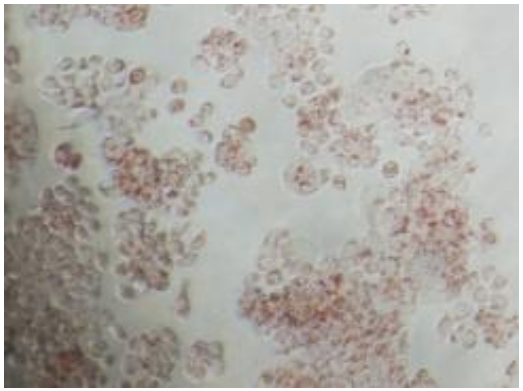

Fig .4: Representative image of cell viability assay of GO on HT-29, U87MG, MRC-5 cells

Research highlights

- RGO exceedingly biocompatible to HT-29, U87MG cancer cells and MRC-5 normal cells

Muthoosamy K, Bai RG, Abubakar IB, et al. Exceedingly biocompatible and thin-layered reduced graphene oxide nanosheets using an eco-friendly mushroom extract strategy. *Int. Journal of Nanomedicine*. 2015;10:1505-1519.

Methodology – Part 2

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Application of RGO – drug delivery of hydrophobic drugs Curcumin (Cur) and Paclitaxel (Ptx)

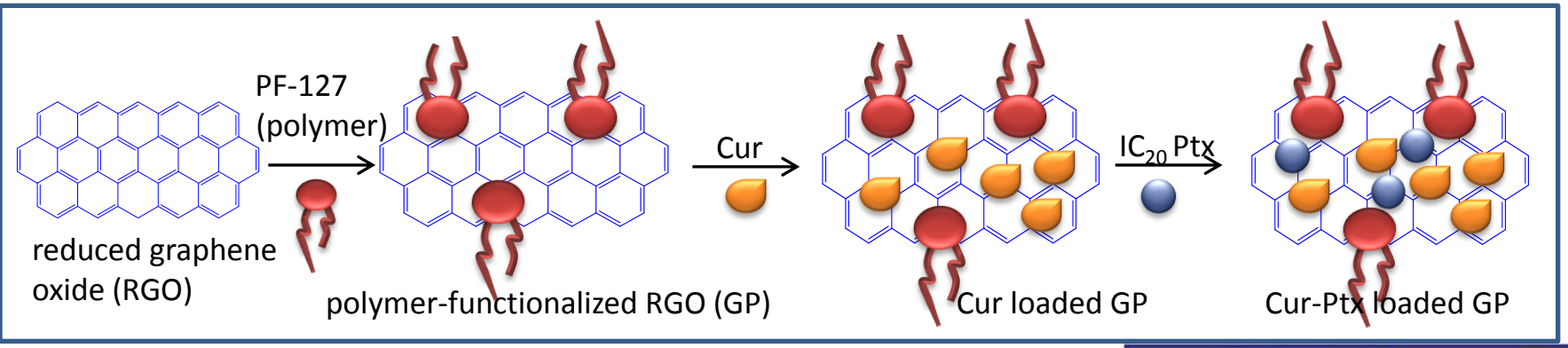

Fig. 5: Schematic representation of drug loading onto RGO.

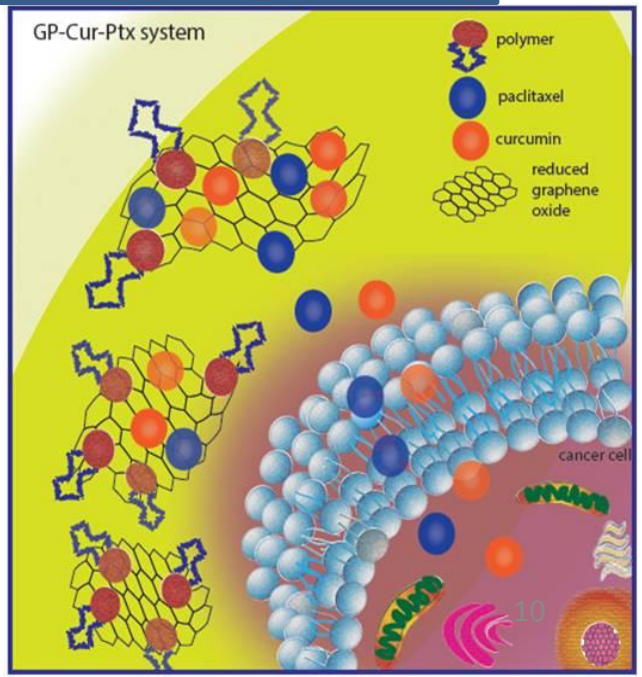

Fig. 6: Cur and Ptx delivery into cancer cells using a GP cargo.

Muthoosamy K, Abubakar IB, Bai RG, et al. Exceedingly higher co-loading of Curcumin and Paclitaxel onto polymer-functionalized reduced graphene oxide for highly potent synergistic anticancer treatment. *Sci. Rep., 6, 32808 (2016).*

Results - Drug loading and unloading

Drug loading and principles

- Cur loading efficiency (%) = 97 ± 0.07
- Cur loading capacity = 678 wt. %
- Ptx loading efficiency (%) = 98 ± 0.15

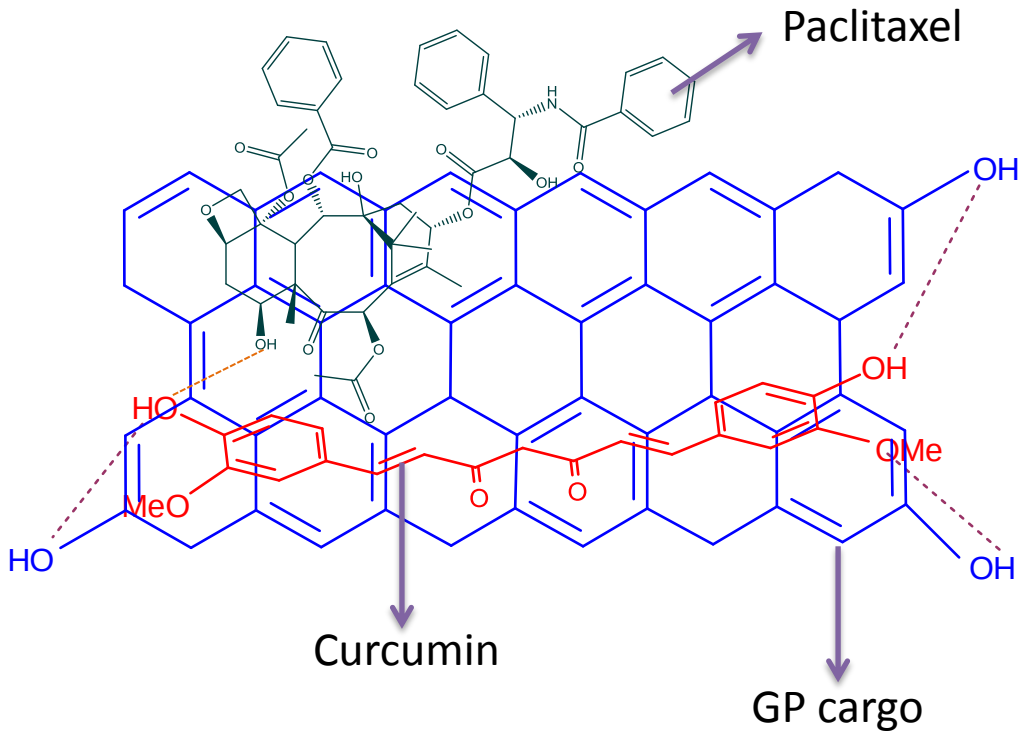

Fig.7: Besides pi-pi interactions, drugs are also held by weak hydrogen bonding.

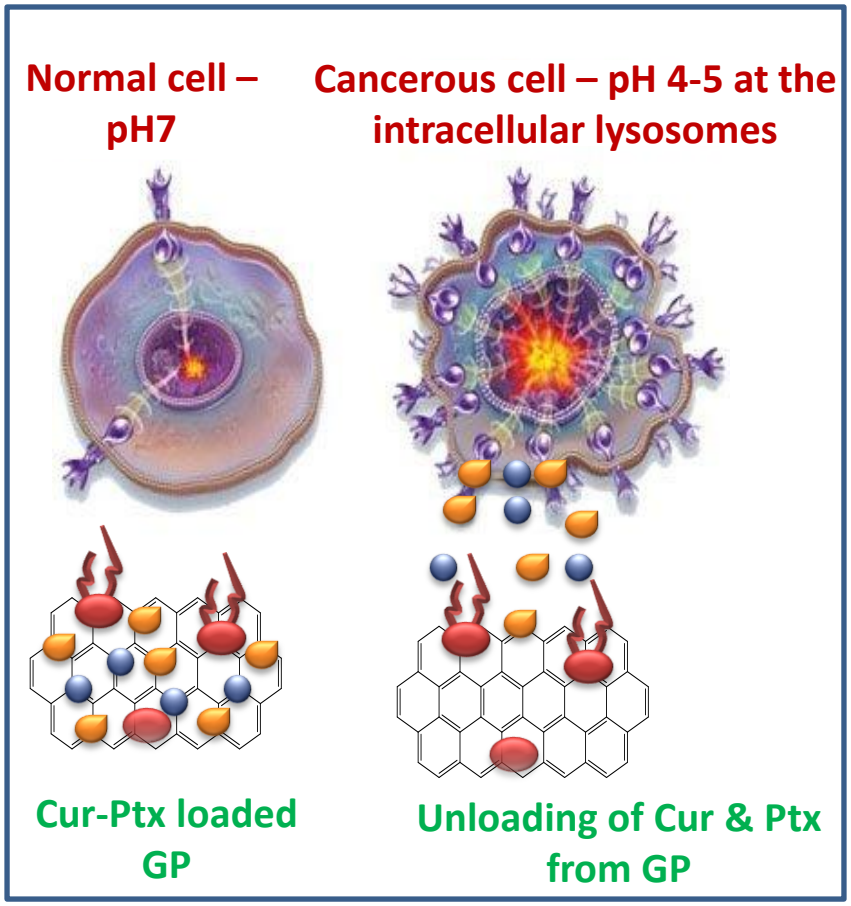

Fig. 8: Schematic representation of pH sensitive drug unloading from RGO.

Results – Part 2

Drug release profile

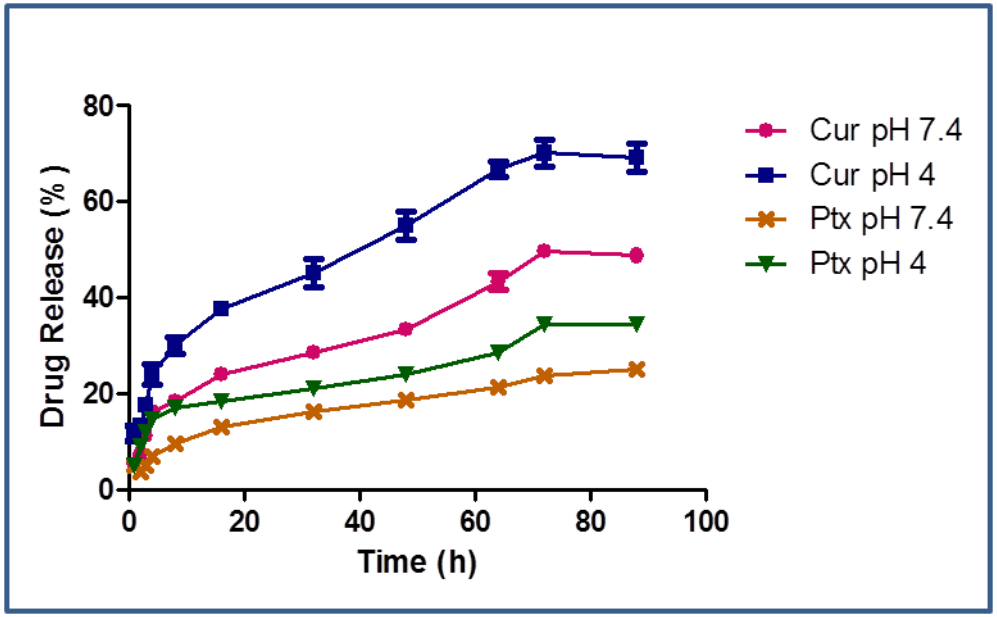

Fig. 9: Release profile of Cur and Ptx from the GP-Cur-Ptx system in PBS buffer at pH 4 and at 7.4, monitored for 90 h.
Data presented are mean ± SD of triplicates (n=3).

- By 48 h, 50% of Cur was released, only 25% of Ptx, at pH 4.
- Drug release at pH 4 > pH 7.4.
- Slow release suggest good stability of GP-Cur-Ptx hybrid system.
- Release of Cur in advance would allow chemosensitization of cancer cells, which in turn increase therapeutic efficacy of Ptx.

Results – Part 2

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Cell viability assay on A549 and MDA cells

Table 2. Combination index (CI) analysis of GP-Cur-Ptx against A549 and MDA cells and the effect of these respective doses on MRC-5 cells, in terms of IC_{50} values.

No	Cells	IC_{20} dose of Ptx (ng/ml)	Dose of GP-Cur which induced 50% growth inhibition in combination with IC_{20} of Ptx (µg/ml)	CI
1	A549	69.7	**13.24 ± 1.8**	0.54
2	MDA	46.7	**1.450 ± 1.9**	0.43
3	MRC-5	69.7	25.71 ± 1.2	-
4	MRC-5	46.7	37.50 ± 1.2	-

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Percentage of ROS generation

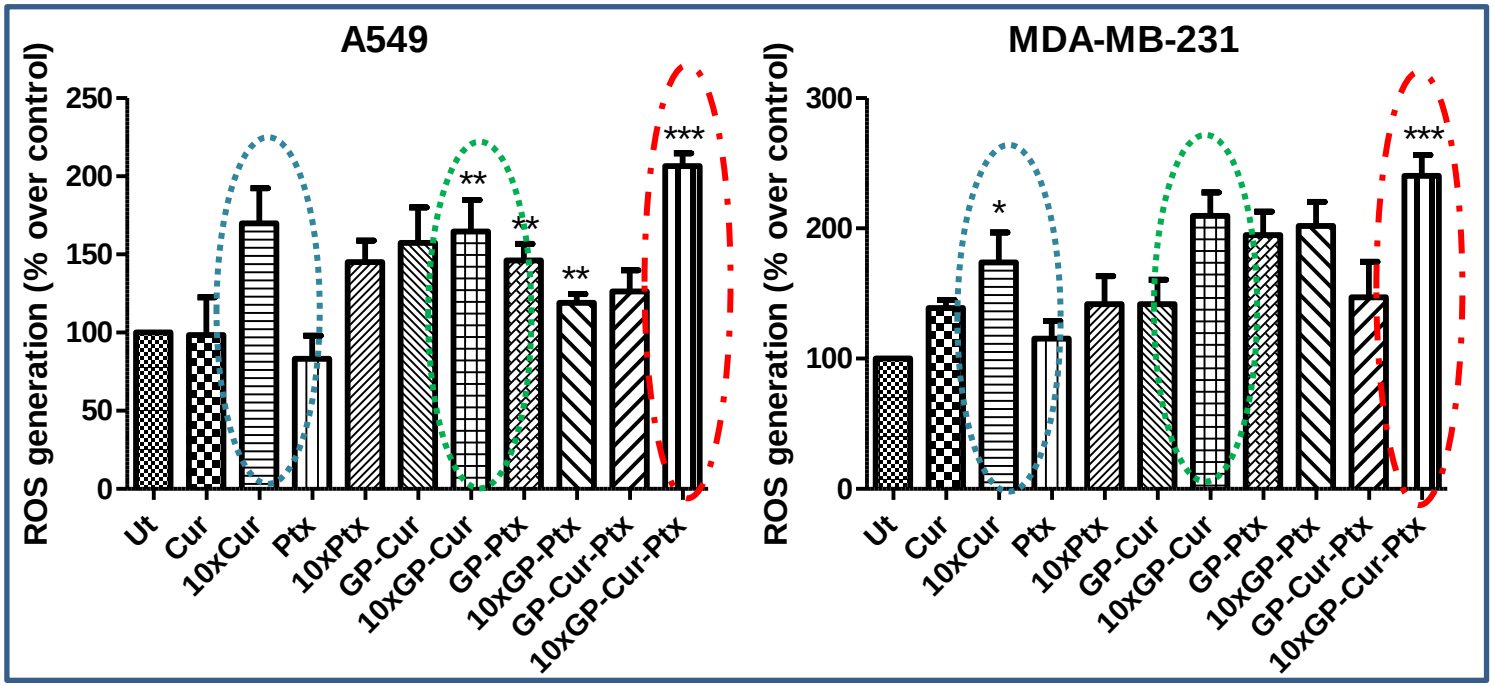
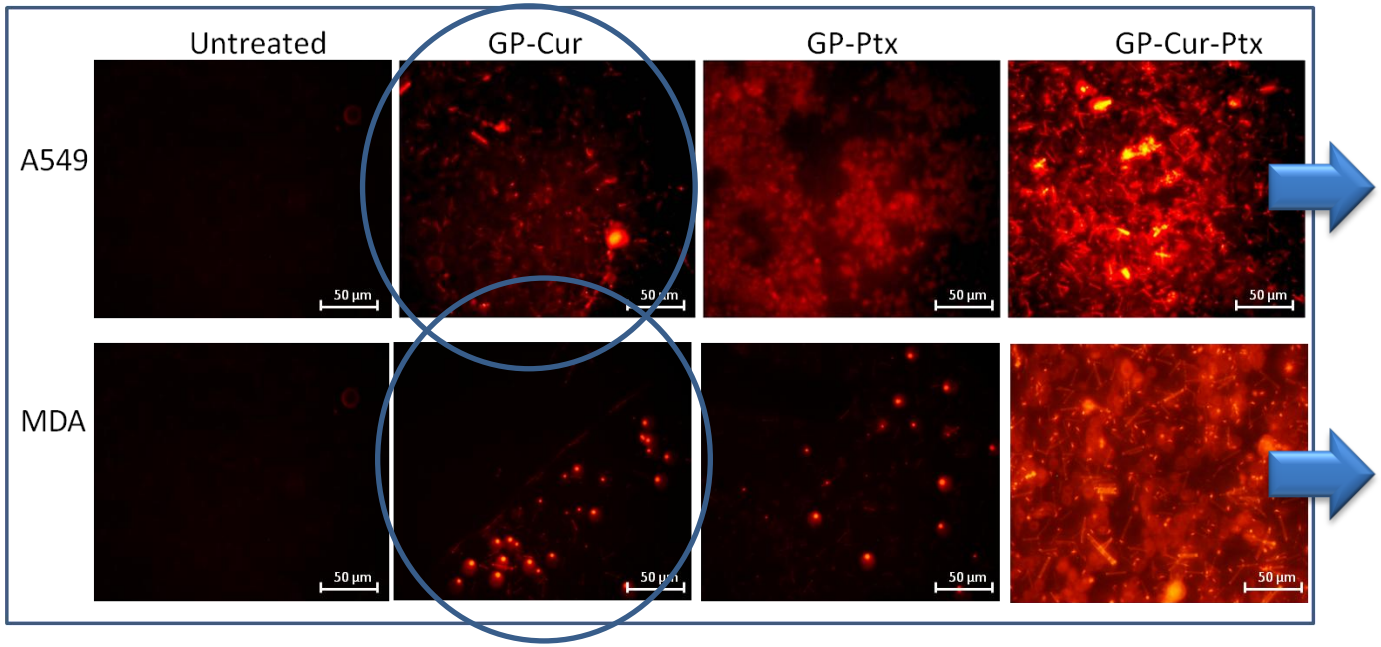


Fig. 10: ROS generation of A549 and MDA cells.

Results – Part 2

ROS generation images



➢In **GP-Cur-Ptx**, Cur also acts as antioxidant, which induces adverse effect on Ptx, thus increased ROS level.

Fig. 11: ROS generation images of A549 and MDA cells.

➢In **GP-Cur**, Cur as pro-oxidant: induce ROS accumulation.

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ROS generation images

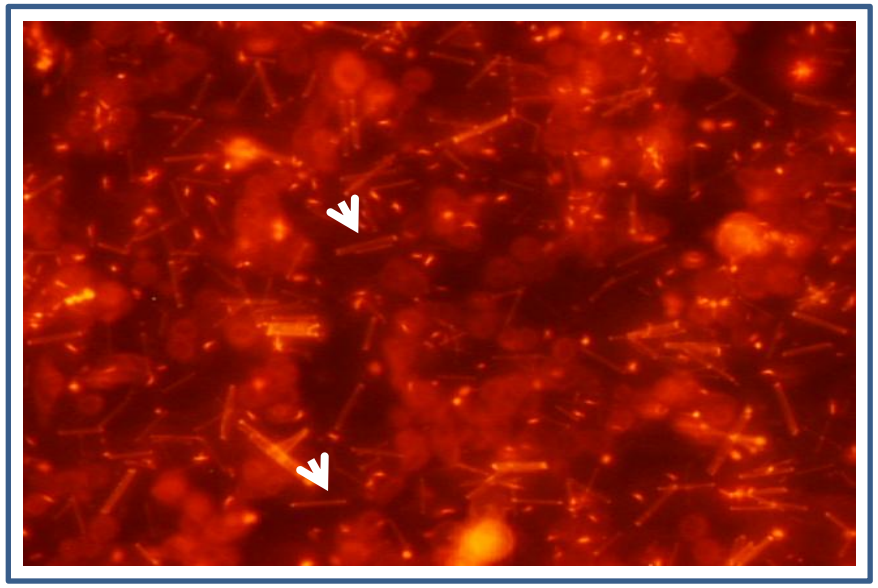

Fig. 12: Representative images of ROS generation in MDA cells.

Rod-like structure in A549/MDA cells:
-Only drugs were internalized.
-GP serves as drug delivery vehicle.

?

Is the elevated ROS due to the GP cargo?

Results – Part 2

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Morphology of cells treated with concentrated GP cargo

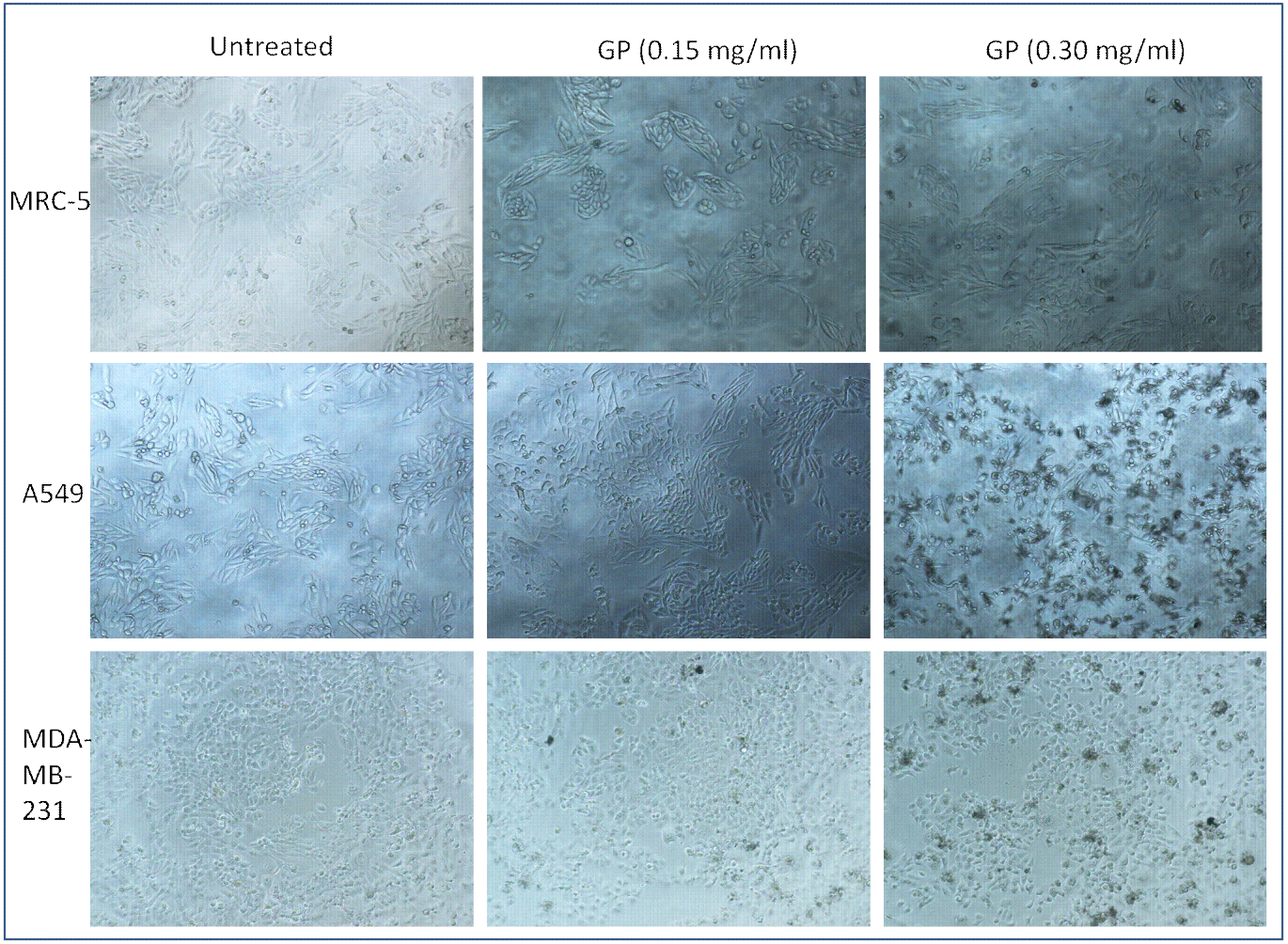

Fig. 13: Morphology images of untreated cells and cells treated with the drug carrier, GP at 0.15 and 0.30 mg/ml for MRC-5, A549 and MDA-MB-231 cells.

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Percentage of apoptosis in A549 cells

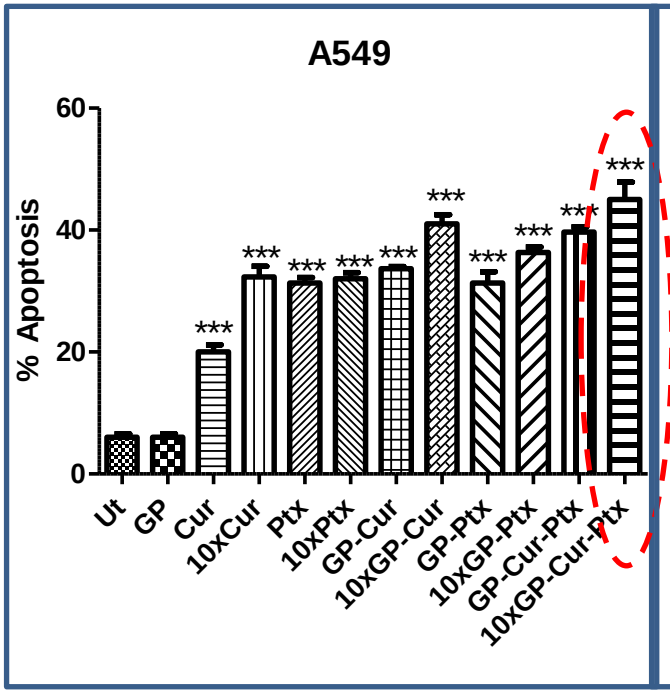

Fig. 14: Percentage of apoptosis induced by the treatment groups on A549 cells.

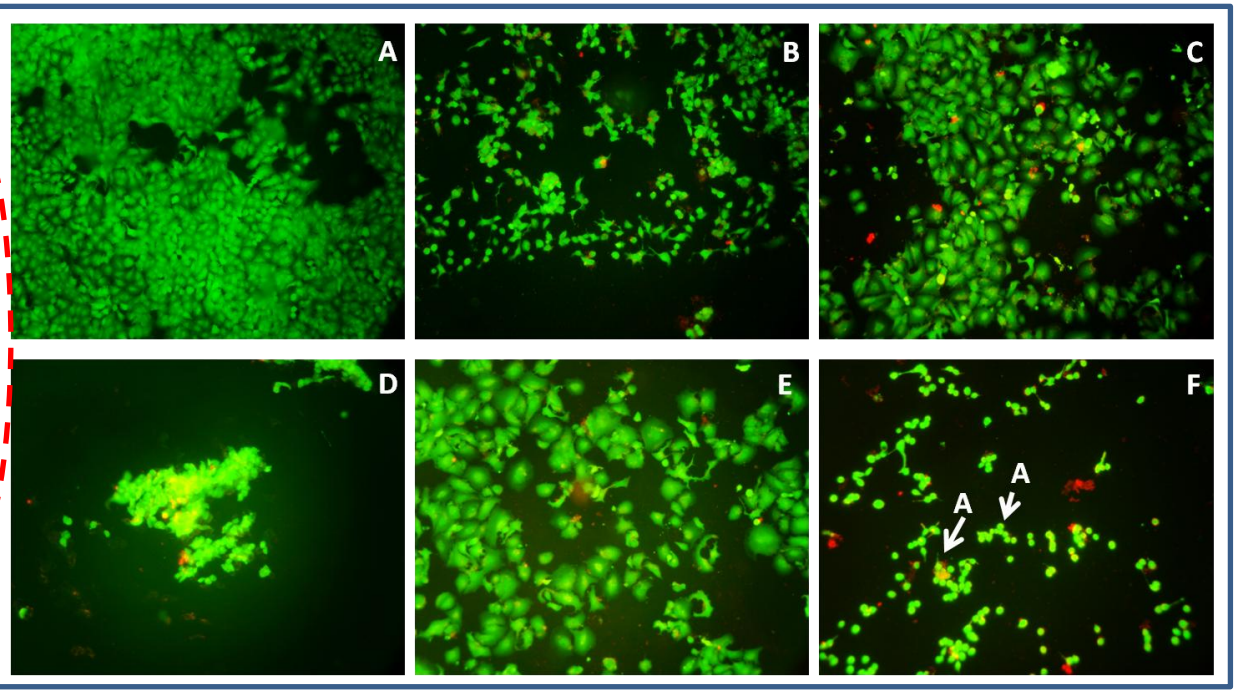

Fig. 15: Merged images of A549 cells after staining with Annexin-Cy3 and 6-CFDA. (A) untreated cells, and cells treated with: (B) Cur; (C) Ptx; (D) GP-Cur; (E) GP-Ptx and (F) GP-Cur-Ptx.
Arrows labelled 'A' are representation of typical features of cells undergoing apoptosis.

Results – Part 2

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Percentage of apoptosis in MDA-MB-231 cells

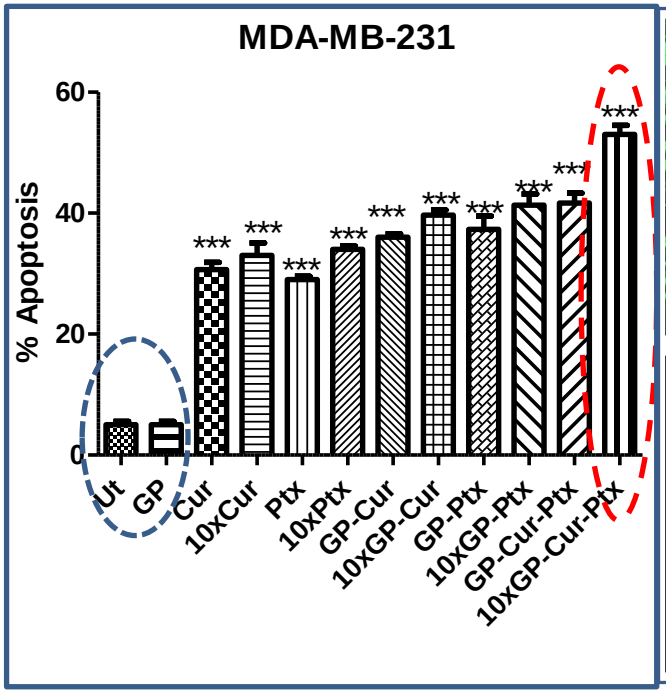

Fig. 16: Percentage of apoptosis induced by the treatment groups on MDA-MB-231 cells.

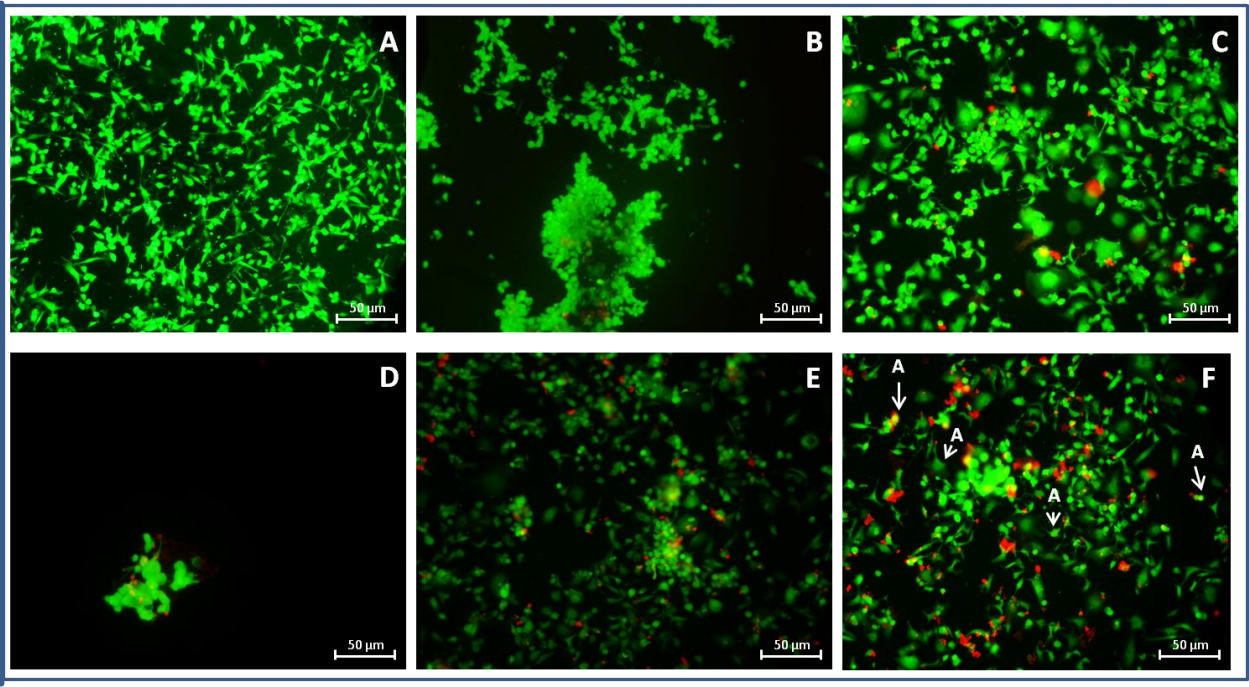

Fig. 17: Merged images of MDA-MB-231 cells after staining with Annexin-Cy3 and 6-CFDA. (A) untreated cells, and cells treated with: (B) Cur; (C) Ptx; (D) GP-Cur; (E) GP-Ptx and (F) GP-Cur-Ptx. Arrows labelled 'A' are representation of typical features of cells undergoing apoptosis.

Application 2- Biosensing

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Folic acid (FA) modified RGO (RGO-FA)

Methodology

- **RGO preparation by mushroom mediated reduction of GO.**
- **FA attachment to RGO by direct loading.**

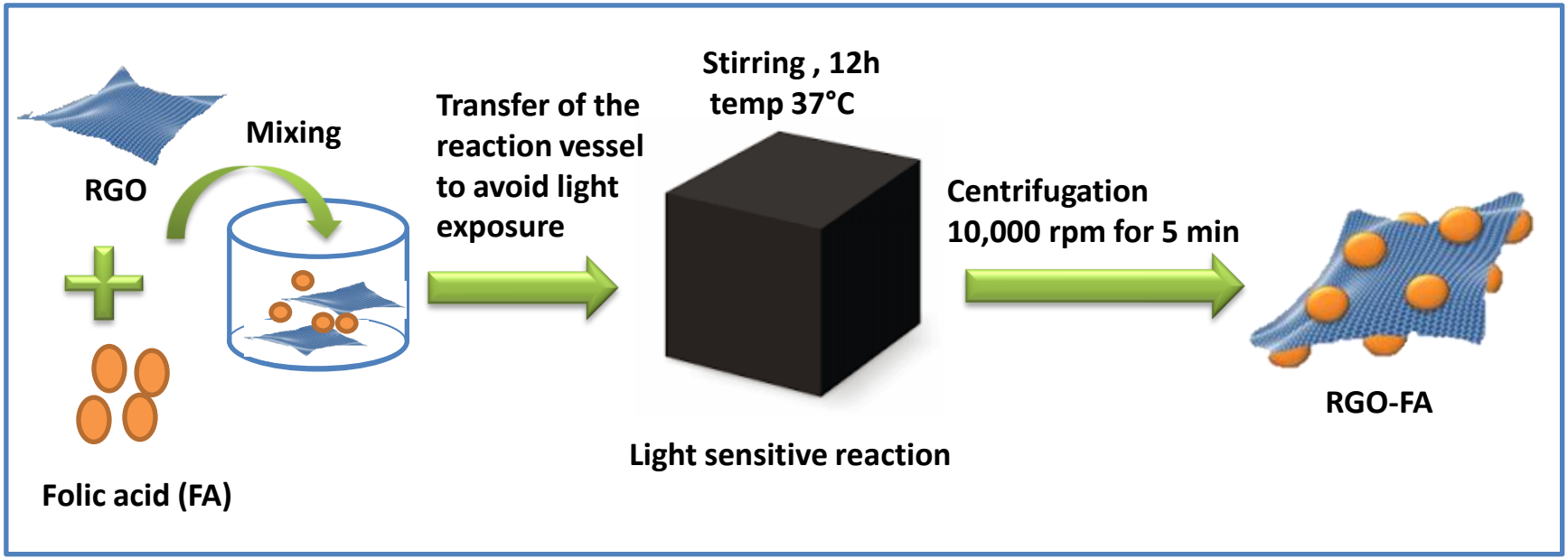

Fig. 18: Schematic diagram of RGO-FA preparation.

Bai RG, Muthoosamy K, Huang NM, et al.Highly sensitive enzyme-less biosensor for cancer detection using folic acid modified reduced graphene oxide. In Press, 2017.

Methodology

Detection of folate receptor (FR) using RGO-FA

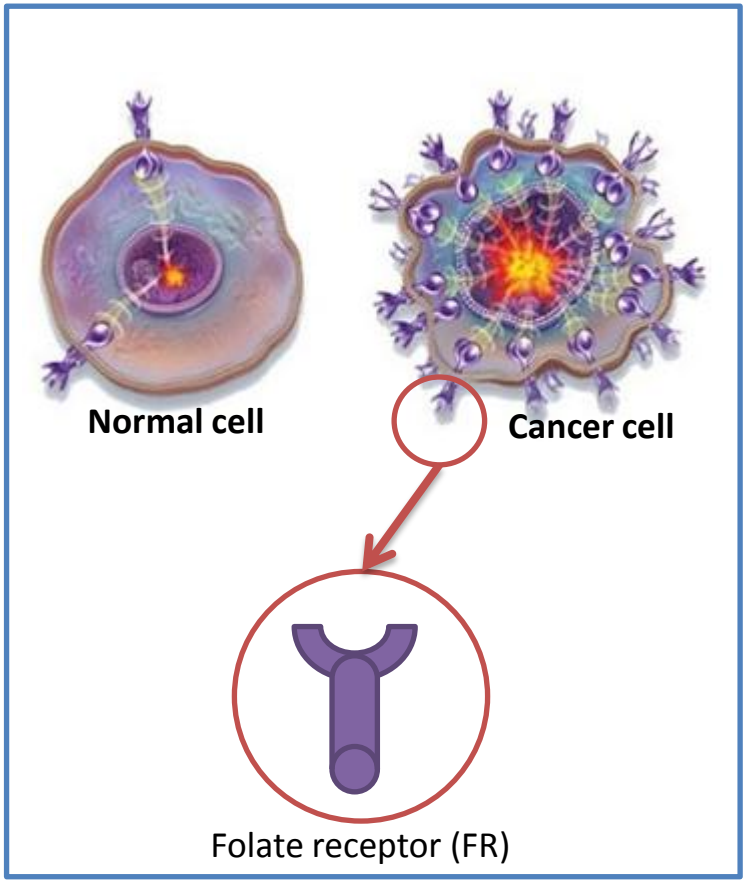

Fig. 19: Illustration of over-expression of FRs in cancerous cells.

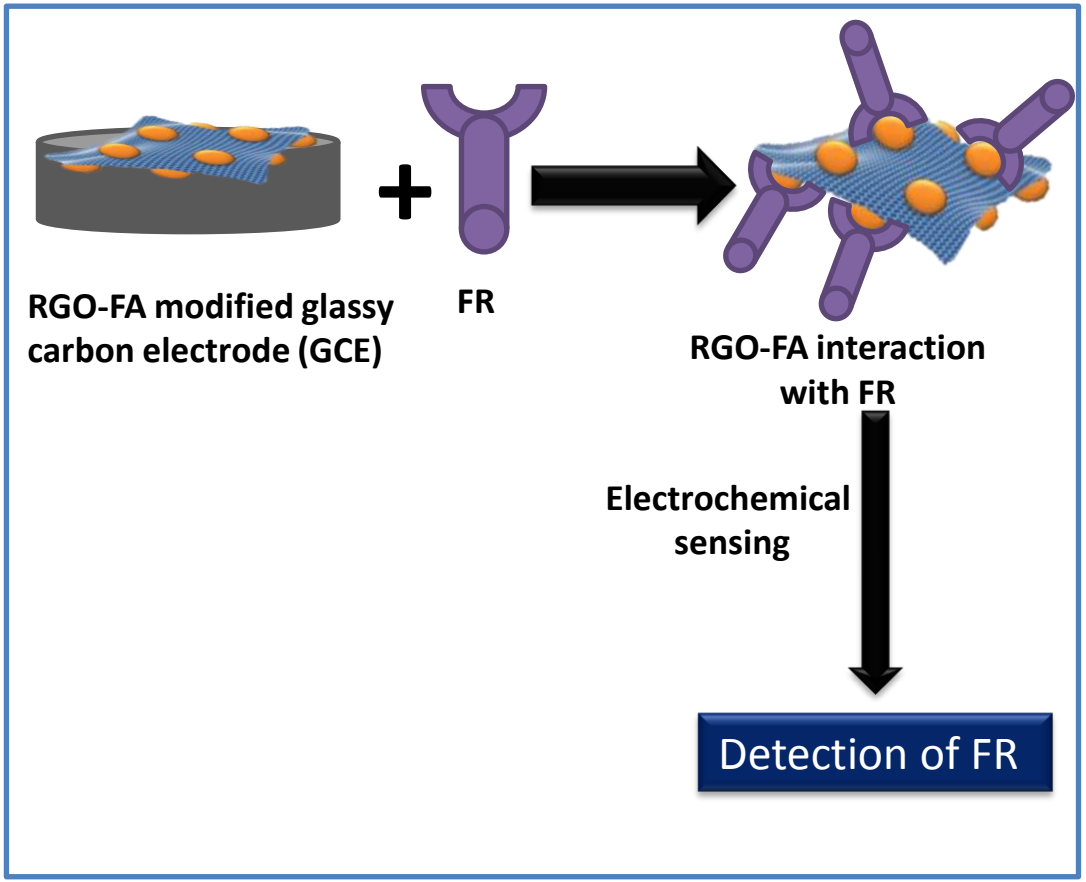

Fig. 20: Schematic representation of RGO-FA modified GCE and its use in FR detection.

Results – Part 3

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RGO-FA sensing of FR using CV and EIS

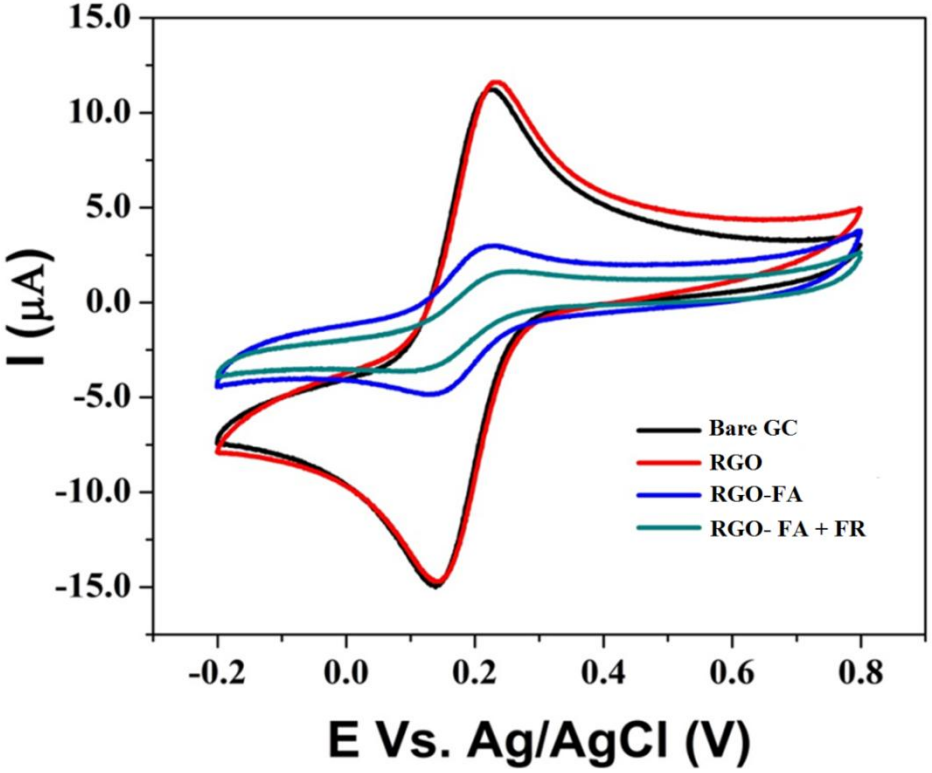

Fig. 21: CV analysis of bare GCE, RGO/GCE and RGO-FA/GCE and RGO-FA/GCE + 100 pM FR in 0.1 M $[Fe(CN)_6]^{3-/4-}$.

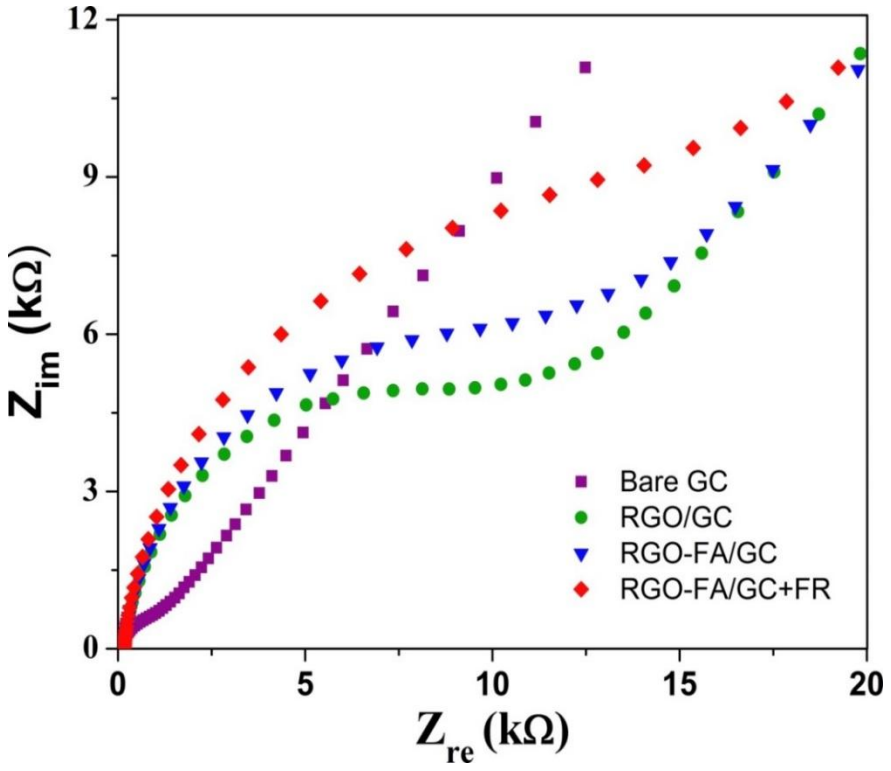

Fig. 22: EIS spectra of bare GCE, RGO/GCE and RGO-FA/GCE + 100 pM FR in 0.1 M $[Fe(CN)_6]^{3-/4-}$.

Results – Part 3

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RGO-FA sensing of FR using DPV

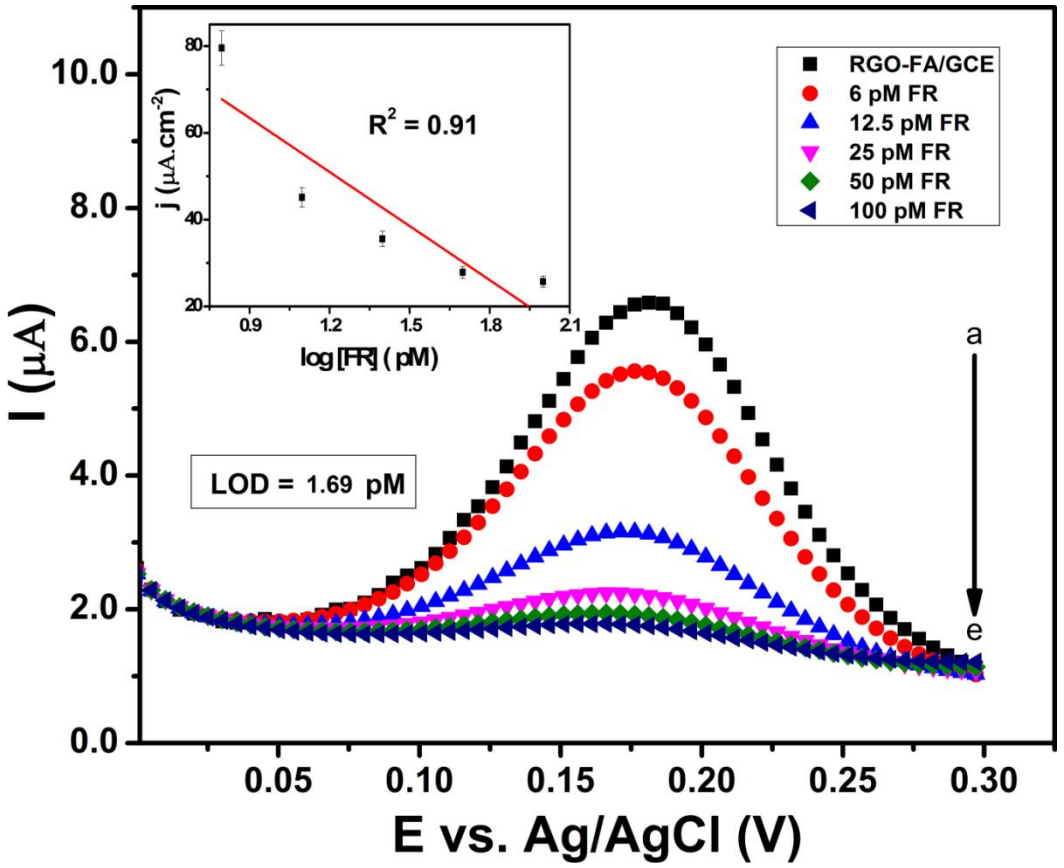

Fig. 23: DPV analysis of RGO-FA with the addition of FR at predetermined intervals.

Linear range: 6-100 pM, s/n=3, sensitivity 0.037 μA/pM.

RGO-FA interference studies

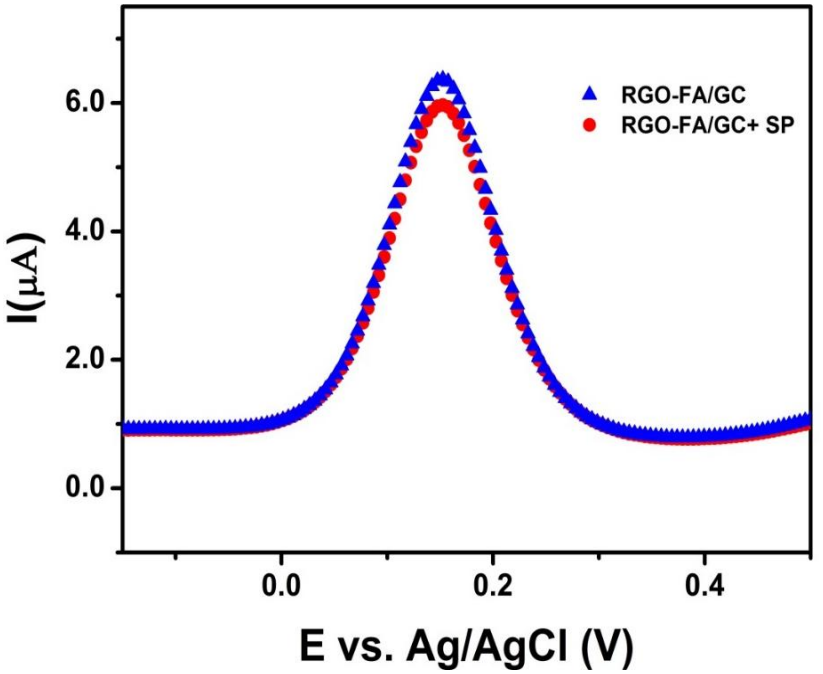

Fig. 24: Interference analysis of RGO-FA with the addition of serum protein (SP) (100 pM).

Conclusion

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- **Efficient reduction** of GO to RGO using commercial grade mushroom powder.
- A readily water soluble and stable (1 year) RGO.
- **Cur loading onto GP cargo:**
  - **687 wt%**
  - Highest observed so far
- **GP-Cur-Ptx system:**
  - Potent against **A549 (13.24 µg/ml)** and **MDA-MB-231 (1.450 µg/ml)** cancer cells (compared to treatments with single drugs).
  - Highly biocompatible and cell-specific (the same dose tested on MRC-5 (normal cells) shows no toxicity).

Conclusion

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- **GP-Cur-Ptx system:**
  - Only the drugs were engulfed by cells, leaving behind the GP cargo.

- **Biosensing studies:**
  - RGO-FA based cancer cell detection targeting FR showed **LOD of 1.69 pM** (DPV).
  - RGO-FA sensor showed good specificity towards FR even in presence of interfering proteins.

Acknowledgments

Research Team:

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Lee Xinjie,

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Revathi Raviandaran,

Wong Xin Yi.

THANK YOU