Toward Graphene-Based Imaging and Drug-Delivery Agents for Breast Cancer: Cytotoxicity of Graphene Oxide Nanoribbons in Human Breast Cancer Cell Lines

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INTRODUCTION

Graphene Oxide Nanoribbon (GONR), synthesized from multi-walled carbon nanotube (MWCNT) has many potential biomedical applications due to its unique electronic and physiochemical properties.

Molecular imaging probes can consist of GONR functionalized with a targeting agent that recognizes a biomarker specific to a tumor, such as a breast cancer tumor. GONR could enhance the contrast between malignant and benign tissue in a MRI scan, making it easier to detect the tumor.

Cytotoxicity of GONR must be assessed before exploring its potential applications.

Purpose- To determine the effect of Graphene Oxide Nanoribbons (GONR) on SkBr-3 and MCF-7 breast cancer cells.

Breast cancer cells were exposed to various concentrations of GONR, ranging from 0 to 500 µg/mL of 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-polyethylene glycol (DSPE-PEG) solution and tested for cell proliferation, mitochondrial dehydrogenase activity, cellular metabolism activity, lysosomal activity and lactate dehydrogenase activity. Results showed the LD50 value to be approximately 100 µg/mL for both SkBr-3 and MCF-7 cells after 48h of treatment.

EXPERIMENTAL DATA

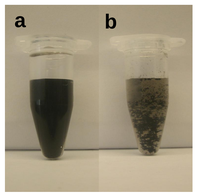

Figure 1. MWCNT dissolved in a) DSPE-PEG b) water

- GONR were dispersed in DSPE-PEG solution to form an uniform suspension and reduce aggregates (Fig. 1)
- The Raman signatures for GONR in DSPE-PEG shows characteristic D band at ~1338cm^{-1} and G band at ~1592cm^{-1} (Fig. 2).
- The Raman spectra for GONR in water shows D band at ~1348cm^{-1} and G band at ~1608cm^{-1} (Fig. 2).
- The Raman spectra for MWCNT in DSPE-PEG shows D band at ~1358cm^{-1} and G band at ~1618cm^{-1} (Fig. 3)
- The G/D ratio for GONR in DSPE-PEG was ~0.7893444, while the ratio for MWCNT in DSPE-PEG was ~0.9745542
- Comparing the intensity curve of GONR in water and GONR in DSPE-PEG, which showed similar D peaks at ~1338 cm^{-1} and G peaks ~1592 cm^{-1} (Fig. 2), it was concluded that the DSPE-PEG did not alter the structure of GONR.

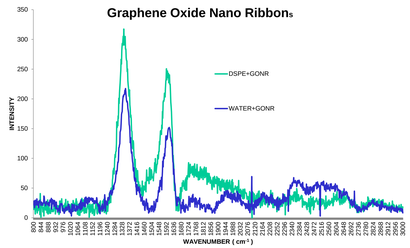

Figure 2. The Raman spectroscopy of 50µg/ml GONR dispersed in water and that in DSPE-PEG

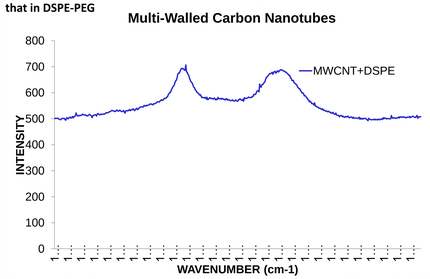

Figure 3. The Raman spectroscopy of MWCNT dispersed in DSPE-PEG

FLUORIMETRIC/COLORIMETRIC CYTOTOXICITY ASSAYS

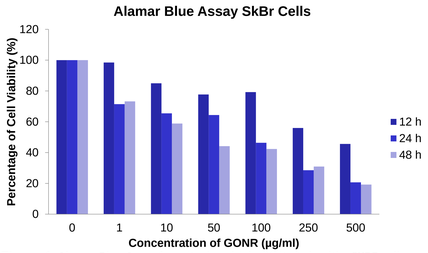

Figure 4. Alamar Blue Assay for mitochondrial activity in healthy SKBR cells

- The Alamar Blue reagents were added to cells that had been treated with the GONRs at various concentrations for 12 to 48 h. After each time point, fluorescence was measured and the data is represented as percentage viable cells.
- GONR treatment reduced the viable cells in a dose and time dependent manner. GONR treatment at 100µg/ml for 24 and 48h respectively reduced the viability to 54 and 58% respectively (Fig. 4). At 500 µg/ml of GONR treatment for 12h reduced the cell viability to 54% respectively.

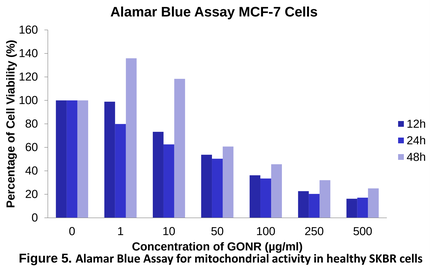

Figure 5. Alamar Blue Assay for mitochondrial activity in healthy SKBR cells

- GONR treatment show a dose and time dependent response in MCF-7 cells. Approximately 54% and 50% viability was observed at 50 µg/ml GONR for 12 and 24 h time point, and approximately 46% viability was observed at 100 µg/ml for 48h time point (Fig. 5). The LD50 for GONR is 100ug/ml in SKBR cells and about 50ug/ml in MCF-7 cells.

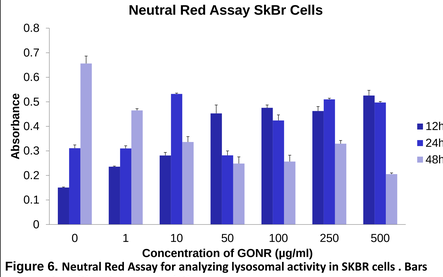

Figure 6. Neutral Red Assay for analyzing lysosomal activity in SKBR cells . Bars represent positive standard deviation.

- Neutral red is uptaken and localized into the lysosomes of by viable cells. Greater the absorbance greater is the viability. For SkBr-3 cells, the treatment of GONRs showed a positive trend between the absorbance value and the concentration of GONR for 12 h time point and 24 h time point.
- Opposite trend resulted in 48 h time point of SkBr-3 cells

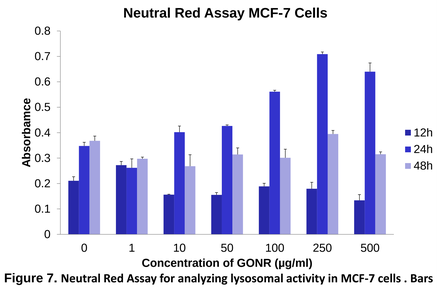

Figure 7. Neutral Red Assay for analyzing lysosomal activity in MCF-7 cells . Bars represent positive standard deviation.

- For MCF-7 Cells, the treatment resulted with increasing absorbance trend until the 250µg/ml of GONRs for 24 and 48 h time points
- Steady absorbance values shown at 12 h time point of MCF-7 cells (Fig. 7)

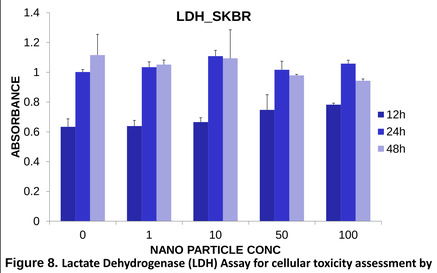

Figure 8. Lactate Dehydrogenase (LDH) Assay for cellular toxicity assessment by membrane destabilization in SKBR cells

- LDH is a cellular cytosolic enzyme that is released when toxic materials destabilize the cell membrane. The extent of LDH release provides direct indication of the toxic potential of the nanoparticles. GONRs showed dose and time dependent release of LDH from SKBR cells up to 24 h. Varying concentrations of GONR treatment had no effect on the LDH release at 48 h .

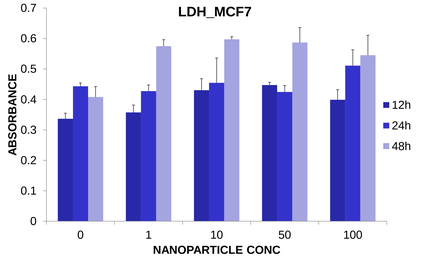

Figure 9. Lactate Dehydrogenase (LDH) Assay for cellular toxicity assessment by membrane destabilization in MCF-7 cells

- GONR treatment showed a time and dose dependent increase in the LDH release.
- GONR up to 50 µg/ml had no significant effect on the LDH release till 24 h.
- These results show that GONR up to 50 µg/ml do not affect the membrane architecture of MCF-7 cells up to 24 h as compared to SKBR cells which are not affected at all the doses and time points studied.

FLUORIMETRIC/COLORIMETRIC CYTOTOXICITY ASSAYS

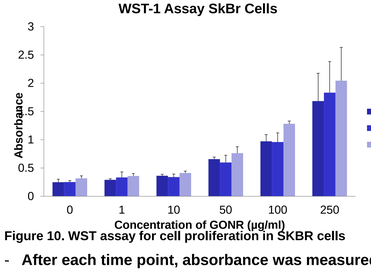

Figure 10. WST assay for cell proliferation in SKBR cells

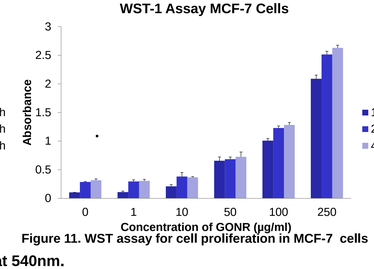

Figure 11. WST assay for cell proliferation in MCF-7 cells

- After each time point, absorbance was measured at 540nm.
- At the concentrations of 0, 1, and 10 µg/ml, GONR had no significant effect on SKBR cell proliferation. However, MCF-7 cells showed significant in crease in cell proliferation. GONR treatment at 50, 100, and 250 µg/ml significantly increased the cell proliferation in both the cell types as compared to control. This could be due to contribution of GONR interaction with the WST absorbance.

NON-COLORIMETRIC ASSAY

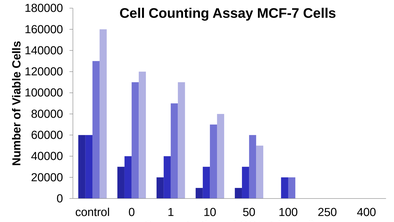

Figure 12. Trypan blue assay for cell viability

- Cells were plated at about 5,000 cells/ml and were given 24 h to seed. Cells were trypsinized, stained with trypan blue dye and manually counted using hemocytometer for every 24 h time point .
- MCF-7 cells were nonexistent from 250-400µg/ml GONR concentrations
- cells were nonexistent for days 5 and 6, so these time values not included in the graphs
- Note from control to 0µg/ml of GONR, the number of cells decreased for the cell line, and the sudden increase of number of viable cells starting from day 3 Fig. (12).

CONCLUSIONS/ FUTURE WORK

Conclusions:

- The LD50 values for GONR in SKBR cells was 500µg/ml after 12h of exposure and 100 µg/ml GONR after 24 and 48h
- LD50 values for GONR in MCF-7 cells was 50µg/ml GONR after 12 and 24h of exposure.
- From these studies, it is speculated that different cell types have variable sensitivities towards these nanoparticles, hence differential LD50 values was observed.
- One unexpected finding was that the DSPE-PEG itself had notable cytotoxicity to human cells; this was represented by the decrease in cell number from control to DSPE-PEG only, 0 µg/ml GONR concentration (Fig. 12). This can mean that the DSPE-PEG influences the overall toxicity of GONR with DSPE-PEG.
- It is essential that these results are kept account for future *in vitro* and *in vivo* toxicity studies as well as implementing GONRs for wide range of biomedical applications such as drug delivery and imaging.

Future Work:

- More non-colorimetric based assays, such as clonogenic assay, will be conducted to verify the cytotoxicity and colony formation ability across different cell types
- The cellular uptake mechanism of the nanoparticles will be studied.
- *In vivo* studies to assess the biocompatibility, biological response and MR imaging capabilities of GONR will be studied.

ACKNOWLEDGEMENTS

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