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 multiwalled carbon nanotube (MWCNT) has many potential medical applications due to its wide-ranged absorption spectra and near-infrared fluorescence.

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- to reduce unwanted side effects and possible permanent damage could occur in throughout these applications.

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

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

SYNTHESIS OF GONR

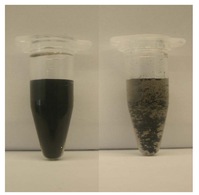

Figure 1. MWCNT dissolved in a) DSPE-PEG b) water

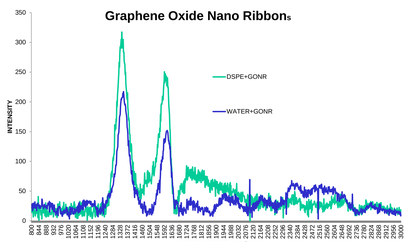

Figure 2. The Raman spectroscopy of 50µg/ml GONR dispersed in water and that in DSPE-PEG

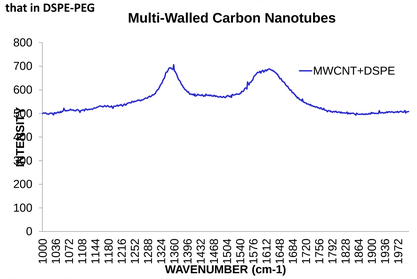

Figure 3. The Raman spectroscopy of MWCNT dispersed in DSPE-PEG

- Nanoparticles were covalently grafted with DSPE-PEG to disperse the nanoparticles uniformly and not to form the aggregates, which tend to clump together (Fig. 1)
- The Raman spectra of GONR in DSPE-PEG includes D peak located at ~1338cm^{-1} with intensity value of 317.4362 arbitrary units (a.u.) and G peak at ~1592cm^{-1} with intensity value of 250.5665 a.u. (Fig. 2)
- The Raman spectra of GONR in water includes D peak located at ~1348cm^{-1} with intensity value of 217.3682 a.u. and G peak at ~1608cm^{-1} with intensity value of 151.9878 a.u. (Fig. 2)
- The Raman spectra of MWCNT in DSPE-PEG include D peak located at ~1358cm^{-1} with intensity value of 706.7489 a.u. and G peak at ~1618cm^{-1} with intensity value of 688.7651 a.u. (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
- By 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.

Fluorometric/Colorimetric Cytotoxicity Assays

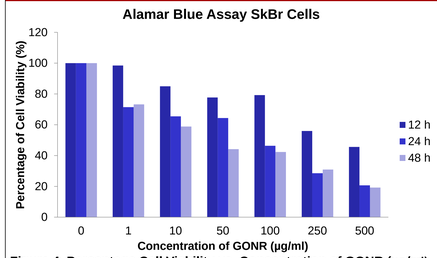

Figure 4. Percentage Cell Viability vs. Concentration of GONR (µg/ml) in SkBr Cells for Alamar Blue Assay.

- 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 12 h time point, fluorescence was measured
- 46% viability of SkBr-3 cells was determined at 500 µg/ml of GONR for 12h time point, and approximately 46% and 42% viability was determined at 100µg/ml of GONR for 24 and 48h respectively (Fig. 4)

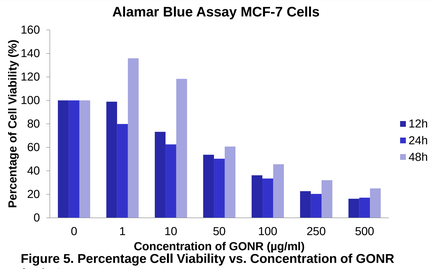

Figure 5. Percentage Cell Viability vs. Concentration of GONR (µg/ml) in MCF-7 Cells for Alamar Blue Assay

- The percentage of SkBr-3 and MCF-7 cell viability is a 100% at each time point with 0µg/ml of GONR.
- The percentage of MCF-7 cell viability is 100% at 0µg/ml. Approximately 54% and 50% viability was determined at 50 µg/ml GONR for 12 and 24 h time point, and approximately 46% viability was determined at 100 µg/ml for 48h time point (Fig. 5)

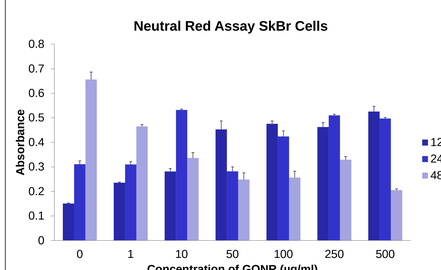

Figure 6. Absorbance (a.u.) vs. Concentration of GONR (µg/ml) in SkBr Cells for the Neutral Red Assay. Bars represent positive standard deviation.

- After each 12 h time point, absorbance was measured at 540nm using BioTek El800. 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

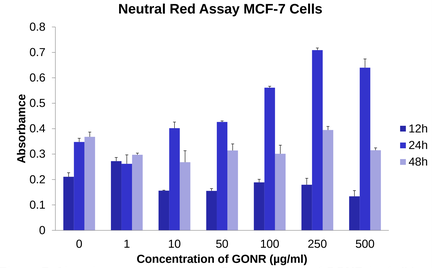

Figure 7. Absorbance level (a.u.) vs. Concentration of GONR (µg/ml) in MCF-7 cells for Neutral Red Assay. Bars represent positive standard deviation

- 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)

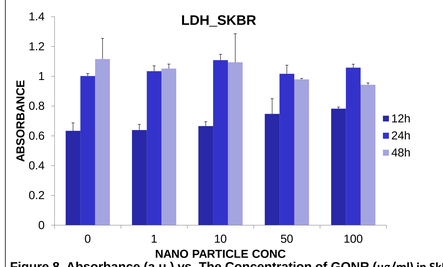

Figure 8. Absorbance (a.u.) vs. The Concentration of GONR (µg/ml) in SkBr Cells for the Lactate Dehydrogenase (LDH) Assay

- After each 12 h time point, absorbance was measured at 490nm using BioTek El800. For SkBr-3 cells, the treatment of GONRs showed direct relationship between the absorbance value and the concentration of GONR for 12 h time point
- If the absorbance increases, then the number of viable cells decreases.

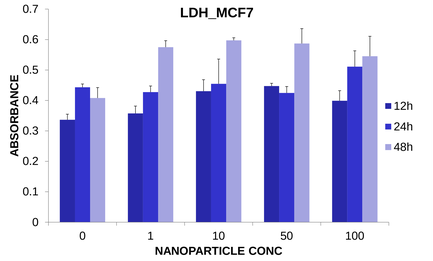

Figure 9. Absorbance (a.u.) vs. The Concentration of GONR (µg/ml) in MCF-7 Cells for the LDH Assay

- For MCF-7 Cells, the treatment resulted with increasing absorbance trend until the 100µg/ml of GONRs for 12, 24 and 48 h time points
- As time and/or concentration of GONR increases, the cell viability decreases.

Fluorometric/Colorimetric Cytoxicity Assays

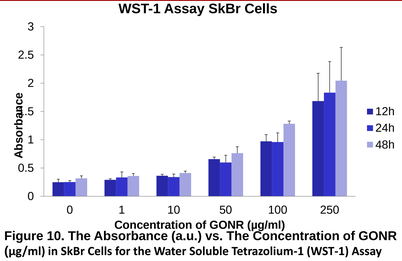

Figure 10. The Absorbance (a.u.) vs. The Concentration of GONR (µg/ml) in SkBr Cells for the Water Soluble Tetrazolium-1 (WST-1) Assay

- After each 12 h time point, absorbance was measured at 540nm using BioTek El800.
- At the concentrations of 0, 1, and 10 ug/ml, the absorbance values are constant. However, at 50, 100, and 250 ug/ml an increasing trend is observed

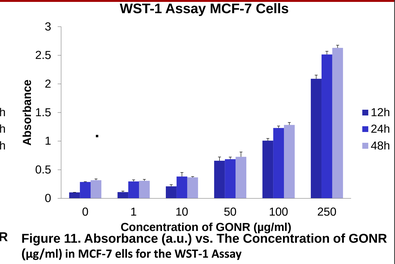

Figure 11. Absorbance (a.u.) vs. The Concentration of GONR (µg/ml) in MCF-7 cells for the WST-1 Assay

- Increasing trend displays that the nanoparticles interfere with absorbance values, and that this assay may not be an accurate way to measure viability without accounting for the interference

Noncolorimetric Assay

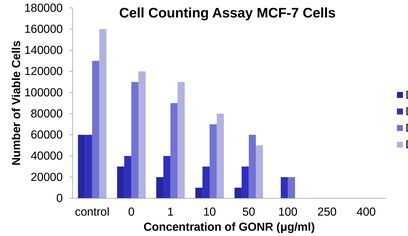

Figure 12. The Number of Viable Cells vs. The Concentration of GONR (µg/ml) in MCF-7 Cells for the Cell Counting Assay

- Cells were plated at about 5,000 cells/ml and were given 24 hs to seed. Cells were trypsinized 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

The LD50 value was both concentration and time dependent, and was determined to be 500µg/ml GONR after 12h of exposure and 100 µg/ml GONR after 24 and 48h of exposure for SkBr-3 cells; for MCF-7 cells, the LD50 value was determined to be 50µg/ml GONR after 12 and 24h of exposure and 100µg/ml GONR after 48h of exposure. This means that a sample cell population will be reduced 50% at that concentration of GONRs at that time point.

Several conclusions can be drawn from this. One, when the GONR concentration exceeds 100 µg/ml of GONR before 48h in biomedical applications, it is likely that increase in cytotoxicity would be observed. Two, when GONR concentration stays the same but the exposure time is longer than 48h, there would be flux in the LD50 value.

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 assays such as clonogenic assay could be conducted
- assess the cytotoxicity of GONRs *in vivo*
- molecular studies on the cellular uptake mechanism of the nanoparticles and the actual cause of cell termination after
- treatment with nanoparticles

Acknowledgements

This work was kindly supported by the Great Neck Breast Cancer Coalition, and the Huntington Breast Cancer Action Coalition, and the Wallace H. Coulter Foundation, .