Effects of Cholesterol Enhancement on the Cell Properties in the Presence of Graphene

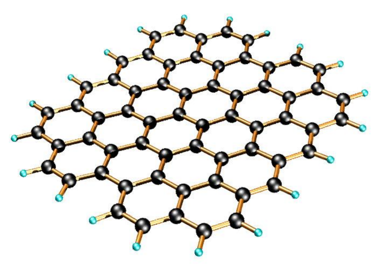
<u>Jason A. Ray-Alfaro</u>[1], **Kristina E. Kitko**[1,2], **Roman Lazarenko**[1], **Tu Hong**[3], **Da Ying**[3], **Ya-Qiong Xu**[3], **Qi Zhang**[1]

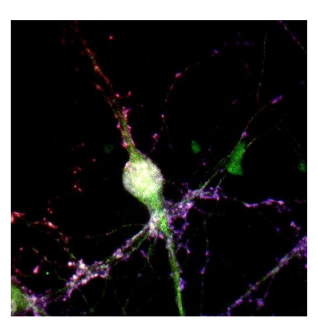
[1]Department of Pharmacology, Vanderbilt University, Nashville TN 37240

[2] Program in Interdisciplinary Materials Science, Vanderbilt University, Nashville TN 37235

[3]Department of Electrical and Computer Engineering, Vanderbilt University, Nashville, TN 37235

Introduction





Motivation: Graphene is a two-dimensional carbon crystal with remarkable mechanical strength and a singular electrical conductivity, a combination that has led to interest in biomedical application. However, this demands a clear understanding of graphene's interaction with cell surface molecules and its impact on cell function.

Objectives:

- To analyze the cholesterol concentration of culture media in the presence of graphene using spectral analysis
- To modify cell properties using graphene and demonstrate a relationship between graphene concentration and membrane resistance
- To highlight the importance of proper procedure when producing cell cultures to prevent cortex contamination

Graphene may Increase Membrane Resistance

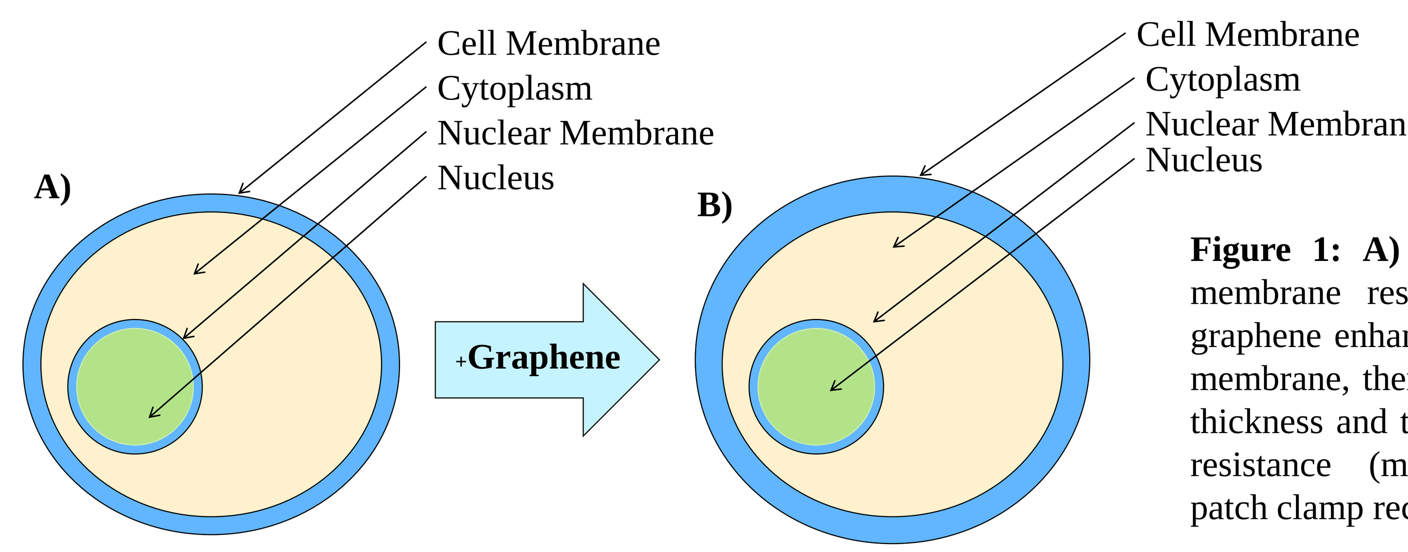


Figure 1: A) Untreated cell , normal membrane resistance. **B)** Addition of graphene enhances the cholesterol in the membrane, thereby increasing its overall thickness and thus whole cell membrane resistance (measured via whole-cell patch clamp recording) .

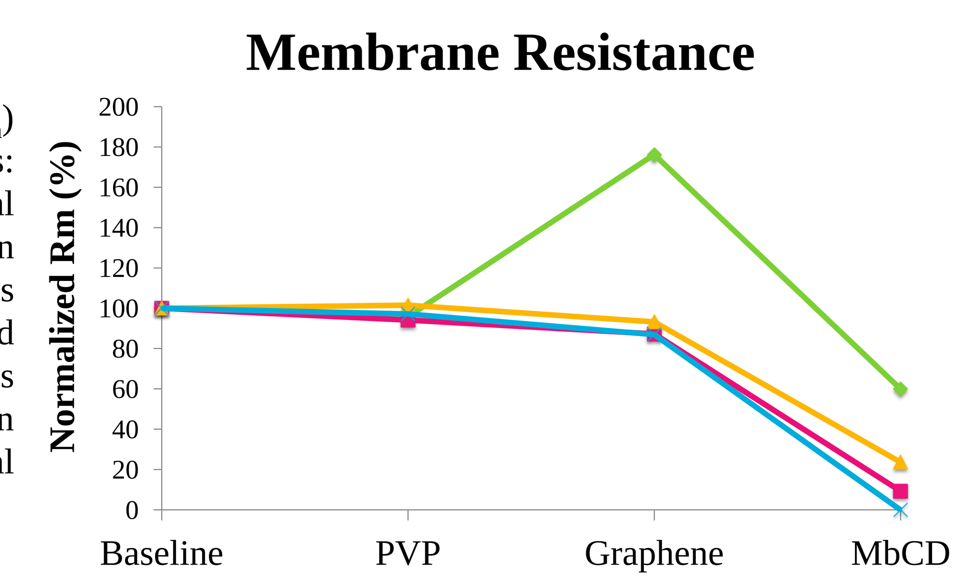
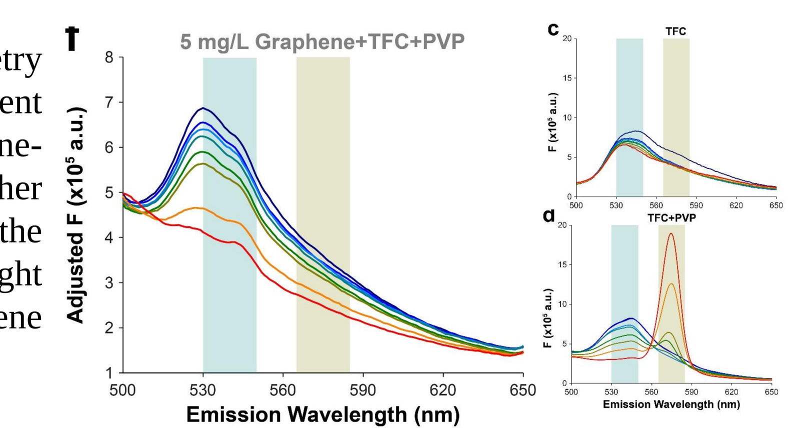


Figure 2: Data from four records of membrane resistance (R_m) measurement. Cells were treated in 4 different conditions: Baseline, Polyvinylpyrrolidone (PVP) solution, 40μm/ml Graphene in PVP solution, and Methyl-beta-cyclodextrin (MβCD). R_m is normalized with the baseline value of each cell as 100%. PVP solution is a sham control as graphene was dissolved in PVP solution. MβCD deprives membrane cholesterol and thus reduces R_m. One out of four cells showed increased R_m upon graphene application, highlighting the importance of additional recordings.

Testing the Graphene and Cholesterol Interaction

Figure 1: Spectrofluorometry measurements show the time-dependent fluorescence quenching of a graphene-fluorophore solution, unique from either the fluorophore alone (right top) or the fluorophore and the solubilizer (right bottom), suggestive of a graphene interaction.



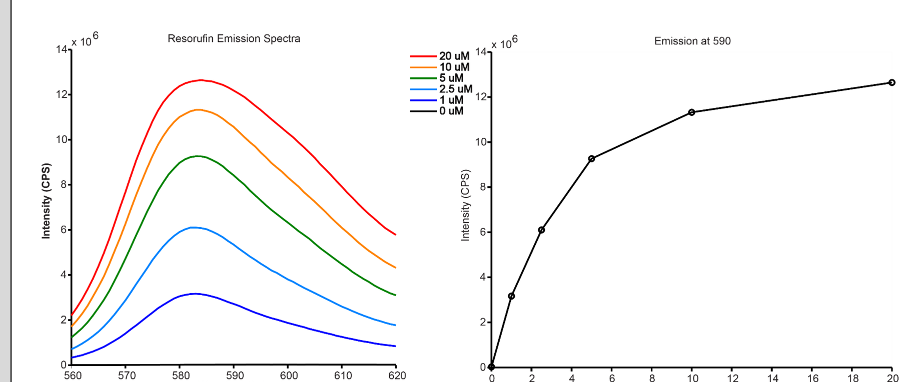
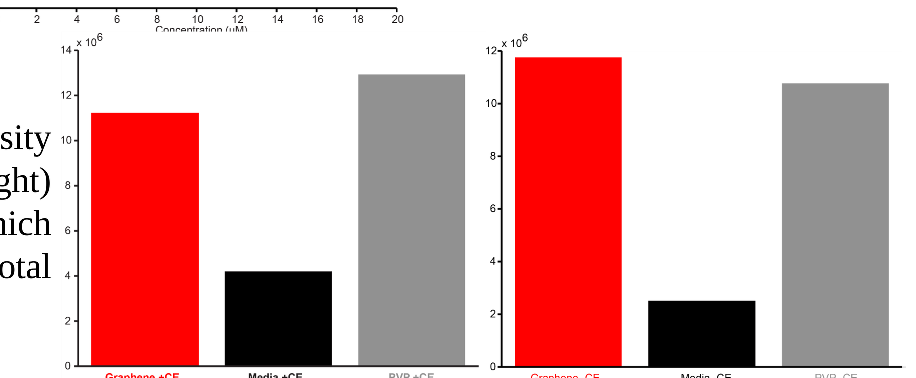


Figure 2: Resorufin emission spectra calibration. Resorufin is an end product of the cholesterol reaction, thus used as a baseline to verify assay functionality.

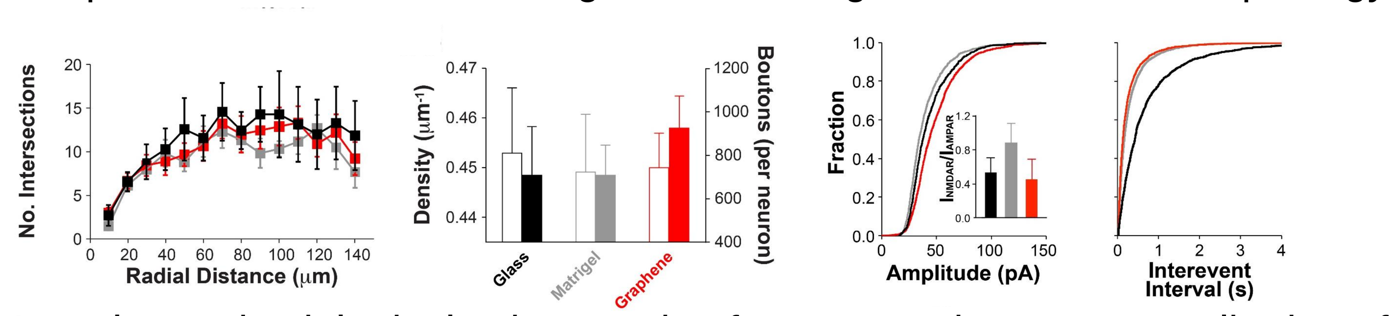
Figure 3: Fluorescence intensity with (left) and without (right) cholesterol esterase, which quantifies both free and total cholesterol, respectively.



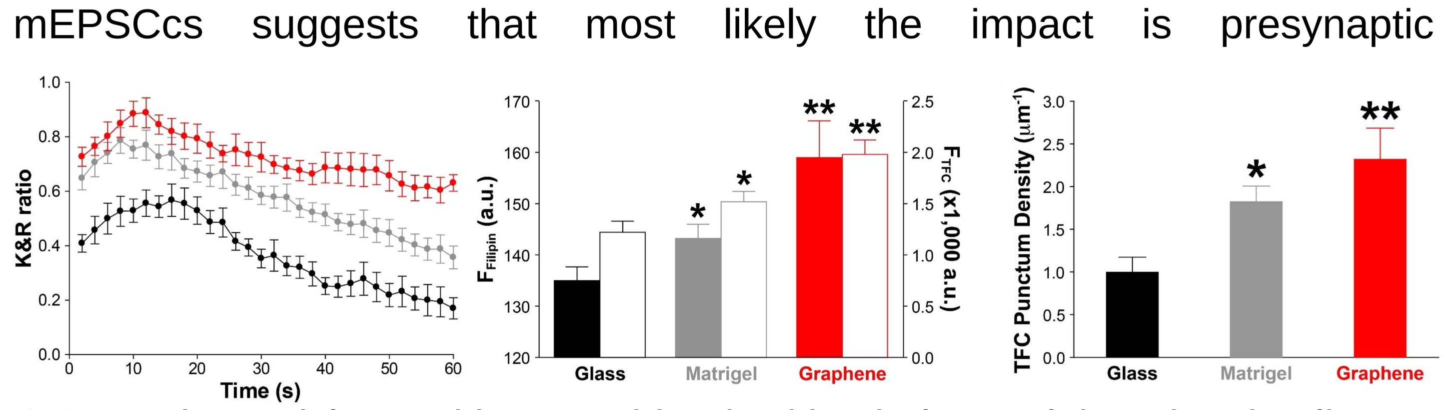
Previous Research

Previous research concluded:

❖ Graphene does not cause significant changes in neuronal morphology



❖An electrophysiological change in frequency, but not amplitude of mEPSCcs suggests that most likely the impact is presynaptic



❖Synaptic vesicle tracking combined with cholesterol imaging implicates that graphene increases both membrane cholesterol content and membrane curvature

❖As such changes are limited and defined, bare graphene may be used as both a growth substrate and a field effect transistor to detect membrane potential changes

Impact of Cortex on Hippocampal Networks

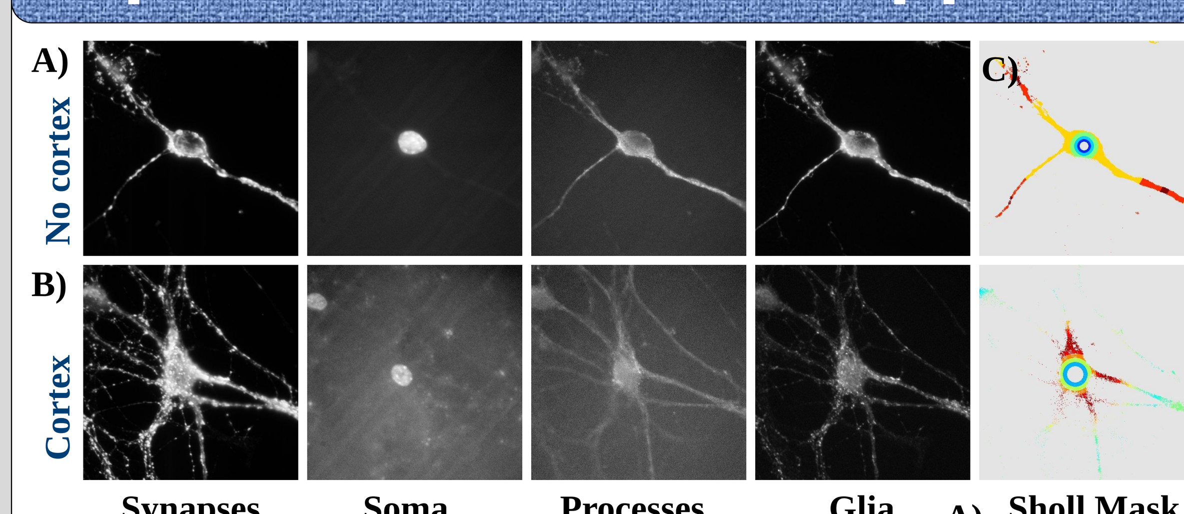


Figure 1: A) Hippocampal neuron sample with minimal cortex contamination. Images were produced following immunocytochemistry and applying a specific wavelength of light to excite fluorophores. **B)** Hippocampal neuron sample with cortex contamination. Note debris in the soma image. **C)** Sholl analysis intersection masks. This process works by creating concentric circles and counting the number of intersections as a function of radius.

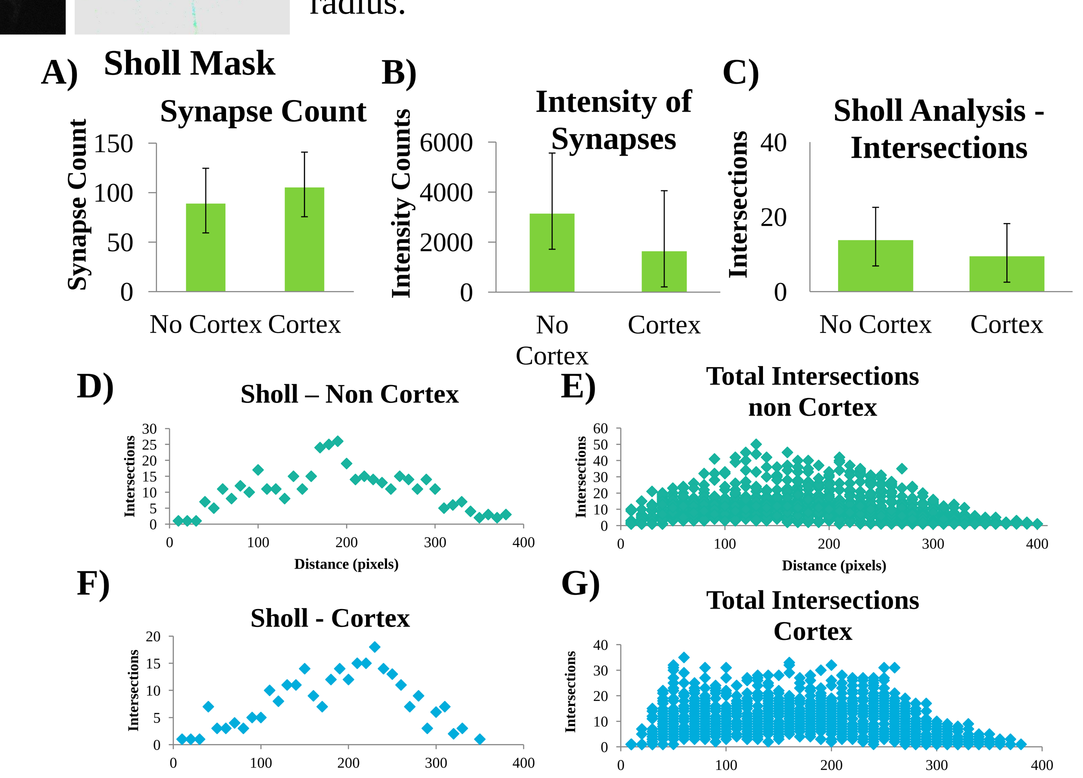


Figure 2: A) Number of synapses for cortex contamination vs. pure hippocampal culture. There was a slightly greater number of synapses in cortex, which may have been due to debris interference. **B)** Fluorescence intensity of synapses. No Cortex showed a greater intensity. **C)** Number of intersections via Sholl analysis. Overall averages were the same. **D)** Single Sholl analysis performed in a neuron with no cortex contamination. Peak of intersections occurred half-way in average. **E)** Total amount of intersections for non-Cortex. The total data fits a Gaussian curve proficiently. **F)** Sholl Analysis of a single neuron with cortex contamination. A peak is less prominent, but a bell curve can be seen. **G)** Total number of intersections for Cortex. Very similar to pure culture, but at higher intersections it loses shape, affecting the overall validity of the data.

Results and Future Work

- Cortex has a slight negative impact on hippocampal networks. The debris increases the uncertainty of analysis done via immunocytochemistry as the antibodies will also stain the cortex
- Preliminary data shows implicates that graphene may indeed increase membrane resistance, although additional verification is necessary
- Further spectral analysis of fluorophore interaction with graphene reveals a time-dependent fluorescence decay that is unique from that of the fluorophore, consistent with an interaction involving energy transfer

<u>Future Work</u> will include additional patch clamping experiments on different cell types to further study the relationship between chronic or acute graphene exposure and membrane resistance

Acknowledgements

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References:

Graphene Diagram: Hamilton, Christopher E. "Graphene." *OpenStax CNX*. RICE, 2012. Web.