## Graphene oxide-Glial interfaces for the selective modulation of brain signalling

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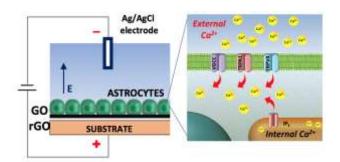
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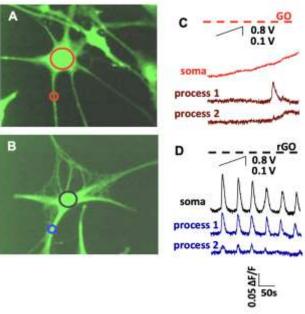
Brain implant devices for brain stimulation and recording interact not only with neurons but also with glial cells, called astrocytes. Understanding the biophysical mechanisms behind the cell/material interaction is a critical challenge, and it would be important to use electrodes to study and modulate the functionality of astrocytes. In particular, astrocyte Ca<sup>2+</sup> signalling is important for the regulation of cognitive and vascular functions, and gets compromised in many neurological disorders such as stroke, epilepsy and spreading depression. Among 2D nanomaterials, Graphene has emerged as a biomaterial interface due to its outstanding properties, including electrical conductivity, mechanical flexibility and biocompatibility. Here, we investigate the unique combination of Graphene oxide (GO) and reduced GO (rGO) coated electrodes to modulate Ca2+ signalling in astrocytes by electrical stimulation. Our results indicate that GO/rGO films are biocompatible coating interfaces, promoting the cell growth with no adverse gliotic reactivity [1]. Noteworthy, we found that the electrical stimulation can trigger distinct intracellular Ca<sup>2+</sup> responses in astrocytes, in vitro and in brain slices, depending on the electrical properties of rGO/GO interfaces. Astrocytes stimulated by conductive rGO electrodes show rapid Ca<sup>2+</sup> response with oscillating peaks, exclusively mediated by Ca2+ release from intracellular stores. Conversely, electrical stimulation delivered by insulating GO electrodes causes slower, sustained Ca2+ response, mainly due to external Ca<sup>2+</sup> influx through membrane channels [2]. We propose a bioelectrical model, hypothesizing that the different conductivity of the substrate influences the electric field at the cell/GO or cell/electrolyte interfaces, inducing, respectively, the extracellular Ca2+ influx or the intracellular Ca2+

release. Graphene-glial interfaces might be extremely promising for neural engineering and neuroscience investigation, offering a new way to dialogue and selectively interact with glial cells in the Nervous System [3].

## Figures



**Figure 1.** Scheme of the experimental setup with astrocyte cells deposited on GO/rGO for electrical stimulation. The inset on the right shows details of the cellular ions channels which shall be triggered.



**Figure 2.** Images of differentiated astrocytes plated on A) GO and B) rGO. C,D) Representative traces of the Ca<sup>2+</sup> variations recorded in the main body of the astrocyte (soma, red/black traces) and in their elongations (called processes, brown/blue traces) on GO (C) and rGO (D).

## References

- M. Durso, A. I. Borrachero-Conejo, C. Bettini, E. Treossi, A. Scidà, E. Saracino, M. Gazzano, M. Christian, V. Morandi, G. Tuci, G. Giambastiani, L. Ottaviano, F. Perrozzi, V. Benfenati, M. Melucci, V. Palermo, J. Mater. Chem. B., 6(2018), 5335.
- [2] I. Borrachero-Conejo, E. Saracino, M. Natali, F. Prescimone, S. Karges, S. Bonetti, G. P. Nicchia, F. Formaggio, M. Caprini, R. Zamboni, F. Mercuri, S. Toffanin, M. Muccini, V. Benfenati, Adv. Healthc. Mater., 8(2019), 1801139.

[3] R. Fabbri, E. Saracino, E. Treossi, R. Zamboni, V. Palermo, V. Benfenati, Nanoscale, 13(2021), 4390.