Hyperpolarization and axonal topology alter NGF axonal transport leading to enhanced neurite outgrowth of peripheral neurons on graphene

D. Convertino[1,2]
F. Fabbri[2], N. Mishra[2], G. Signore[2], M. Mainardi[3], V. Cappello[2], L. Albertazzi[4], S. Luin[1], A. Cattaneo[3], L. Marchetti[2], C. Coletti[2]

1 NEST, Scuola Normale Superiore, Pisa, Italy
2 Center for Nanotechnology Innovation @NEST, Istituto Italiano di Tecnologia, Pisa, Italy
3 BIO@SNS Laboratory, Scuola Normale Superiore, Pisa, Italy
4 ICMS, Eindhoven University of Technology, Eindhoven, The Netherlands

camilla.coletti@iit.it; laura.marchetti@unipi.it; domenica.convertino@iit.it

In recent years, different strategies have been proposed to improve axon regeneration and neural activity. Among them, graphene displays a great potential as a conductive peripheral neural interface thanks to its electrical and tribological properties (1,2,3).
In this work, we investigated graphene influence on neonatal dorsal root ganglion (DRG) neurons. We adopted graphene synthesized via chemical vapor deposition (CVD) and transferred on culture plates, envisioning its integration in neural guides.
We observed an increased axonal length on graphene with respect to control, which was significantly increased up to 17% after two days in culture. This confirms a trend we previously reported for embryonic DRG neurons on epitaxial graphene (4). Axonal transport of nerve growth factor (NGF), a protein that supports survival and axon elongation of sympathetic neurons (5), was investigated via fluorescence microscopy. Surprisingly, the results show that graphene did not alter neurotrophin internalization, but simply modified vesicles mobility, drastically reducing the number of retrogradely transported vesicles in favour of an immobile population that might determine a local effect on axon elongation.
Spectroscopy and microscopy were used to investigate whether neurotrophin immobilization correlated with altered electrical and structural properties of axons on graphene. A charge redistribution on graphene in the cell proximity was observed via Raman spectroscopy. Accordingly, electrophysiological recordings showed a hyperpolarized resting membrane potential and reduced excitability of graphene-cultured DRG neurons with respect to glass-cultured controls. Ultrastructural analysis of the axonal cytoskeleton revealed a different topology and a reduced microtubule distance in neurons on graphene, which can be involved in altered vesicle trafficking and increased elongation.
These results help to shed light on the molecular mechanisms underpinning the effect of a growth substrate on living cells, a critical issue for its application in regenerative medicine. Ultimately, we envision transferring graphene on transparent and flexible biocompatible substrates, thus allowing the realization of advanced neural conduits for improved nerve regeneration.

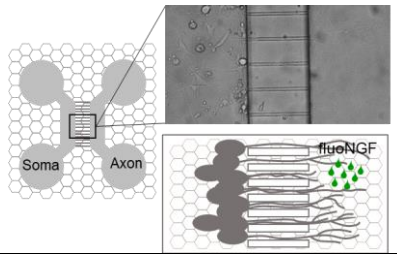

Figure 1: Schematic of compartmentalized DRG neuron culture on graphene for axon elongation and NGF transport measurements.

References

[1] Shin, Advanced Drug Delivery Reviews, 105 (2016) 255-274.
[2] Liu, ACS Appl. Mater. Interfaces, 9, (2017) 14677−14690.
[3] Qian, Nature Communications, 9 (2018) 323.
[4] Convertino, Front. Neurosci., 12 (2018)
[5] Levi-Montalcini, Science, 4819 (1987) 1154-1162.