Irene de Lázaro[1]
Sandra Vranic[1], Maurizio Buggio[1], Paola Posocco[2] and Kostas Kostarelos[1]
[1]Nanomedicine Lab, Faculty of Biology, Medicine and Health & National Graphene Institute, University of Manchester, AV Hill Building, Upper Brook St, M13 9PL, Manchester, UK
[2]Molecular Simulation Engineering Laboratory, University of Trieste, 34127, Trieste, Italy

irene.delazarodelrey@manchester.ac.uk

A Graphene Oxide 2D Platform for Intracellular siRNA Delivery

Development of efficient and safe nucleic acid delivery vectors remains an unmet need holding back clinical translation of such gene therapeutics. Graphene oxide (GO), among other two-dimensional (2D) nanomaterials, could help resolve this bottleneck thanks to its large surface area, versatile chemistry and biocompatibility, which could boost transfection efficiency while providing a less controversial safety profile than that of viral vectors [1]. However, complexation of double-stranded nucleic acids onto the GO surface is thought to be compromised by electrostatic repulsion and by steric hindrance of hydrophobic and π-π interactions from the nucleobases. To deliver double-stranded oligonucleotides, GO is decorated with positively-charged materials that generally induce cytotoxicity [2]. Here, we demonstrate for the first time the capacity of bare GO, without cationic functionalization, to complex a short, double-stranded nucleic acid of biological relevance (siRNA) and mediate its intracellular delivery. Atomistic molecular dynamics simulations, in combination with a variety of experimental techniques, demonstrated the binding between GO and siRNA. Confocal microscopy and stem-loop RT-qPCR allowed monitoring the efficient uptake of GO:siRNA complexes in a primary mouse cell culture **(Figure 1)**. 4 h after transfection, GO:siRNA complexes delivered sufficient oligonucleotide levels to induce significant gene silencing. However, time-lapse tracking of internalized GO and siRNA evidenced a sharp decrease of intracellular siRNA from 4 to 12 h and the entrapment of GO in large intracellular vesicles. Such intracellular behavior may explain the deficient biological performance of GO:siRNA complexes compared to a lipid-based benchmark transfection reagent. Overall, these results underline the potential of bare GO flakes to act as 2D siRNA delivery platforms, avoiding cationic functionalization, but warrant further vector optimization to achieve efficient gene silencing.

References

[1] Kostarelos, K, Nature Reviews Materials, 1, (2016).
[2] Vincent, M, de Lázaro, I, and Kostarelos, K, Gene Therapy, 24 (2017) 123-132.

Figures

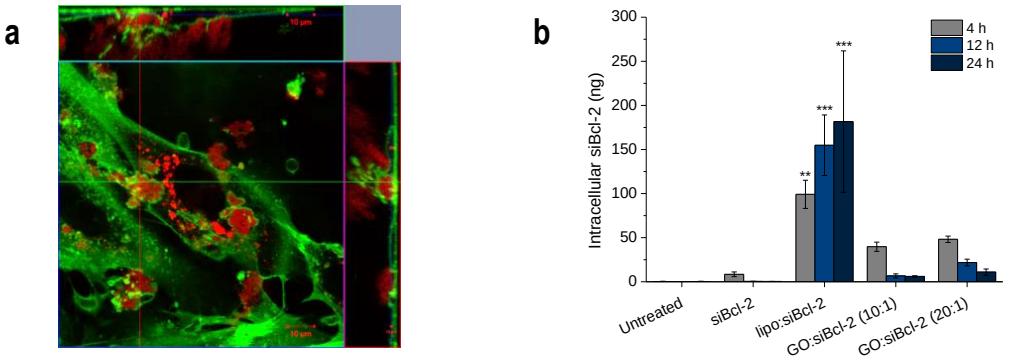

Figure 1: GO as 2D platform for intracellular siRNA delivery. (a) GO internalization in mouse primary fibroblasts was monitored by confocal microscopy (in green, cell membrane; in red, GO intrinsic fluorescence). **(b)** Stem-loop RT-qPCR evidences a rapid decrease in intracellular siRNA levels upon GO-mediated delivery. **p<0.01 and ***p<0.001, one-way ANOVA and Tukey's post-hoc test, n=3.