Splenic capture and in vivo subcellular degradation of thin, biological-grade graphene oxide sheets studied by correlative microscopy

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The development of nanomaterials for biomedical and pharmaceutical application has the potential to provide breakthrough to medical technologies such as improved drug delivery, tissue engineering, diagnostics and a plethora of other biomedical strategies [1]. Graphene-oxide (GO) is a particularly interesting 2D materials in biomedicine because of its dispersibility in aqueous environments and its functionalisation opportunities. Before considering the translation GO materials as drug-delivery vector to pharmaceutical research, it is of critical importance to understand the toxicological implication and *in vivo* biodegradability of GO in living system [2-4]. This requires to visualise and locate GO at the ultrastructural level, which is challenging task using current characterisation techniques.

We developed an experimental protocol based on correlative microscopy combining confocal Raman spectroscopy, TEM imaging and electron diffraction (ED). Unlike other nanomaterials, GO is difficult to differentiate from other subcellular objects because of the similarly of the morphology of GO sheets with other subcellular structures. Electron diffraction provides structural information and can in principle distinguish the crystalline GO sheets from the amorphous cellular tissues. However, our experiments show that this approach doesn't work using standard TEM experimental protocol. We demonstrate that this is because of the small signal to background of the GO sheets in the diffraction pattern and we derive a new experimental protocol to address this issue. For the first time, we identify and locate GO at the ultrastructural level in a consistent and robust manner.

TEM, scanning TEM and ED experiment have been performed on a FEI Talos F200A operating at 80 kV using electron dose rates ranging between 45 $e^{-}.A^{-1}.s^{-1}$ and 84 $e^{-}.A^{-1}.s^{-1}$. Raman scanning was completed using a DXRi Raman Mapping system (Thermo Scientific, UK) using a wavelength of 633 nm, a power of 1 mW and a pixel size of 3 μm^2.

In this work, confocal Raman mapping in combination with immunohistochemistry and fluorescence activated cell sorting have been used, allowing the non-destructive mapping of the exact location of GO within the spleen. Moreover, using Raman spectroscopy, we track the fate of GO in whole tissues, while in parallel we use TEM imaging coupled with electron diffraction to analyse the material within ultrathin tissue sections, as shown in Figure 1. Using such a strategy, we reveal the precise intracellular location of GO and its amorphous trajectory (both visually and spectroscopically) over a 9 month period as catalysed by the cellular machinery.

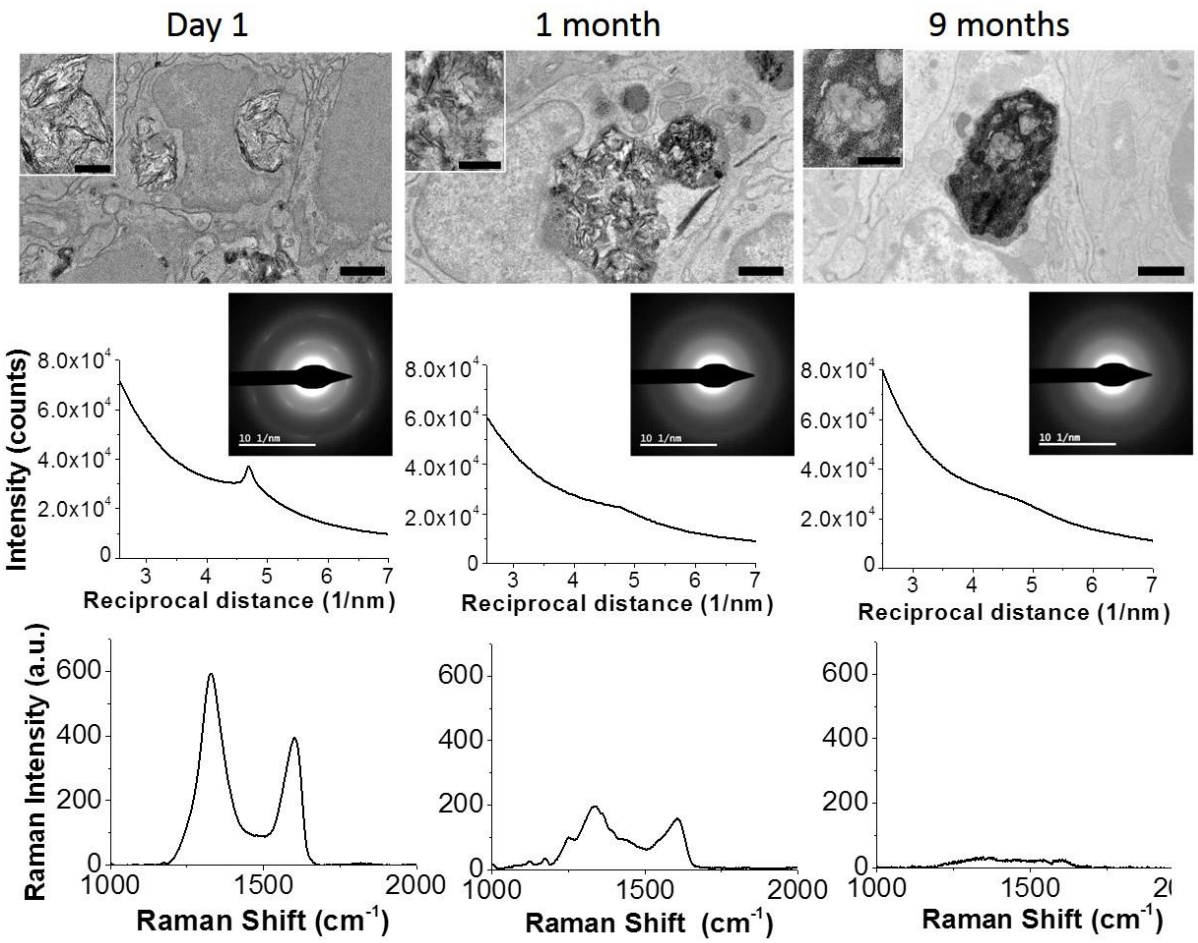

Figure 1. First row: TEM images of spleen tissue for 1 day, 1 month and 9 months (scale bars 1 μm and 0.5 μm in the insets); second row: corresponding electron diffraction patterns (inset) and line profiles demonstrating the identification of GO at the ultrastructural level; third row: corresponding Raman spectra (D and G peaks) showing the degradation of GO over time.

[1] Kostarelos, K. & Novoselov, K. S. Science 344, p. 261 (2014).

[2] Girish, C. M. *et al.* Advanced healthcare materials 2, p. 1489 (2013).

[3] Yang, K. *et al.* ACS nano 5, p. 516 (2011).

[4] Jasim, D. A. *et al.* ACS nano 10, p. 10753 (2016).