DCE-MRI to study vascular dependency of radiolabelled nano-graphene oxide nanoparticle delivery

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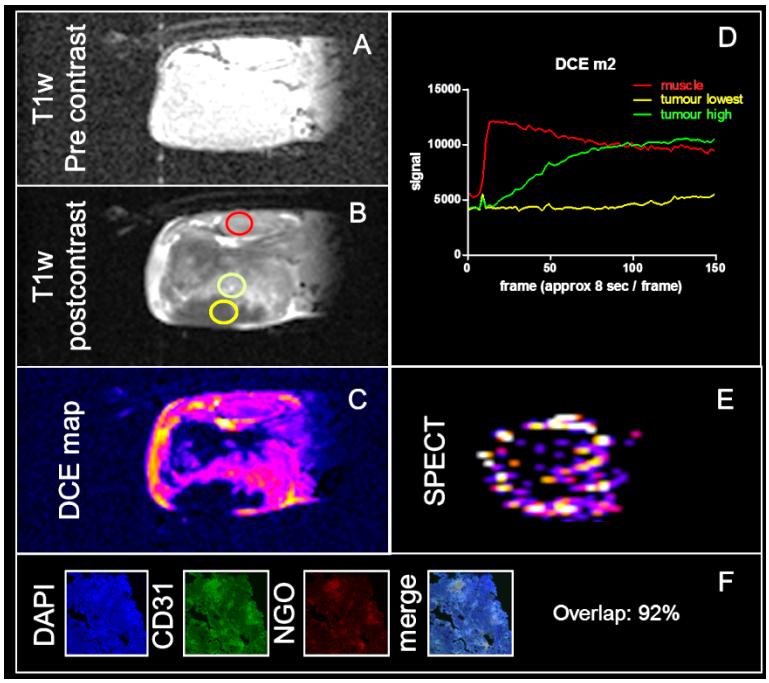
Introduction: Graphene, a single layer of carbon atoms, has been demonstrated to possess remarkable mechanical and electrical properties, and has therefore received much attention in the past years for many different applications. The oxidized form of graphene, graphene oxide is soluble in water, and is easily chemically modified. There are many potential applications of graphene oxide in the life sciences. The large surface area of graphene oxide allows adsorption of large quantities of compounds such as cytotoxic drugs for cancer therapy, plasmids for gene delivery, etc. A poly-ethylene-glycol conjugated (PEGylated) form of nano-graphene oxide (NGO) was shown to target tumour xenografts, and has been successfully used for photodynamic therapy in a mouse model. Our group has synthesised an ^{111}In radiolabelled variant of PEGylated NGO that allows the study of the biodistribution and tumour delivery of NGO in vivo using SPECT/CT imaging, and we have shown tumour accumulation of ^{111}In-NGO-PEG. However, the mechanism of this apparent tumour specificity has not yet been elucidated. The size of the PEGylated NGO particle is such that it would not be expected to extravasate from tumour vessels. Dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) is a method for investigating microvascular structure and function by tracking the pharmacokinetics of intravenously injected low-molecular weight contrast agents as they pass through the tumour vasculature. It is sensitive to changes in tumour blood perfusion and vascular permeability and has been applied for cancer detection, characterisation, staging and therapy monitoring. Here, we demonstrate the feasibility of the use of DCE-MRI to study the vascular dependency of ^{111}In-labelled NGO-PEG.

Methods: Nano-graphene oxide (NGO) was produced by oxidizing graphite using a modified Hummers' method followed by exfoliation using hard sonication and filtration through a 0.2 µm filter. Quality of NGO was confirmed using FTIR and fluorescence spectroscopy. Size was determined by AFM and DLS. PEGylatation with an 8-arm PEGamine (MW = 10,000) was achieved via EDC/NHS activation of base-rinsed NGO, resulting in NGO-PEG. Amino-benzylDTPA, a metal ion chelator, was adsorbed onto NGO using Vanderwaals interactions (ππ-stacking), to allow ^{111}In radiolabelling. CaNT breast carcinoma-bearing CBA mice were injected intravenously with 1 mg ^{111}In-NGO-PEG and were imaged using DCE-MRI and SPECT/CT 24 h later. DCE-MRI data were collected using a T1-weighted, gradient and RF spoiled, respiration triggered, 3-D gradient-echo sequence. Imaging parameters were: flip angle = 5º; TR = 1.3 ms; TE = 0.65 ms; FOV = 54 x 27 x 27 mm^3; isotropic 420 µm resolution; 8 s temporal resolution. After 10 images, 30 µl of Omniscan™ (0.5 M gadodiamide, GE Healthcare) was injected over 5 s via a tail vein. Semi-quantitative Gd-uptake parameters on a voxel-by-voxel basis were extracted for the whole tumour using Matlab. SPECT/CT images were acquired sequentially on the same mouse using the nanoSPECT/CT system (Bioscan). Images were co-registered using the T1w image after contrast and the CT image. After SPECT imaging, mice were sacrificed and the tumours were removed and stained for CD31 to visualise blood vessels. Samples were counterstained with DAPI to show cell nuclei. Confocal fluorescence microscopy, using the innate fluorescence of NGO was used to study colocalisation of NGO with tumour vessels.



Results: Tumour uptake of ^{111}In-NGO-PEG was modest at 2 percent of the injected dose per gram of tissue. Conversely, tumour-to-muscle contrast was exellent at 250:1 (Figure panel E). High temporal resolution DCE imaging allowed building detailed parametric maps of Gd delivery (Figure panel C, D). Marked heterogeneity was observed by both imaging modalities. More importantly, areas of fast Gd uptake on DCE-MRI correlated well with areas of high ^{111}In-NGO-PEG uptake on SPECT, indicating high vascular dependency of the latter. This was confirmed by confocal microscopy (figure panel F), which showed an overlap coefficient of 0.92 between blood vessels (CD31, green) and NGO-PEG (red).

Discussion and conclusions: DCE-MRI proved a valuable tool to study the biodistribution and tumour delivery characteristics of radiolabelled nano-graphene oxide. Taken together, these results suggest that the tumour uptake of NGO-PEG depends heavily on tumour vascular density and permeability.