Cytotoxicity and biocompatibility of highly water-soluble graphene nanoribbons derivitized with p-carboxyphenyldiazonium salt.

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The unique thermal[1], electronic[2], and mechanical[3] features of nano carbon-based materials such as nanotubes, graphene and nanoribbons, are currently under investigation for applications in biotechnology. Although there has been a great deal of work undertaken in regards to biocompatibility and toxicology of carbon nanotubes[4], and graphene[5-7], little or no work has been done on graphene nanoribbons, which can be thought of as unzipped carbon nanotubes.

In this work we evaluated, for the first time, the cytotoxicity of multi-layer graphene nanoribbons (GNRs) which have been made highly water soluble (4.7 mg/ml) by repetitious derivatization with p-carboxyphenyldiazonium salt[8]. Cytotoxicity was evaluated for both pancreatic (PANC-1, MIAPaCa-2) and Hepatic (Hep3B, HepG2) cancer cell lines.

Assays used to evaluate toxicity include; the standard 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) and WST-1 viability assays; lactate dehydrogenase (LDH) cell permeability assay; and cell flow cytometry with FITC Annexin-V and PI staining to assess cell death mechanism (apoptosis, necrosis etc.). The effect of GNRs on cellular DNA cycle was also looked at using PI staining and RNase A. Internalization dynamics were analyzed using scanning electron and transmission electron microscopy (SEM/TEM) and optical bright-field microscopy.

At the time of abstract submission, our initial results indicate that these GNRs are completely soluble in phosphate buffered saline (PBS) and are inherently non-toxic for concentrations 0.1 - 10 mg/L as shown by MTT, WST-1, LDH and cell flow cytometry data. There is mild toxicity for concentrations of 100 mg/L however, which we believe is due to the GNRs disrupting the cell adhesion properties, causing the cells to disattach from the cell plate and be removed via cell media aspiration.

Finally, there is strong evidence to suggest that upon internalization, GNRs translocate to the nuclear membrane as can be seen in Fig. 1. Although further work must be done to verify, this would allow GNRs to be used as nuclear delivery vectors for drugs and small molecules such as siRNA or as effective thermal actuators in non-invasive radiofrequency cancer therapy, currently under development within our laboratories[9-12]

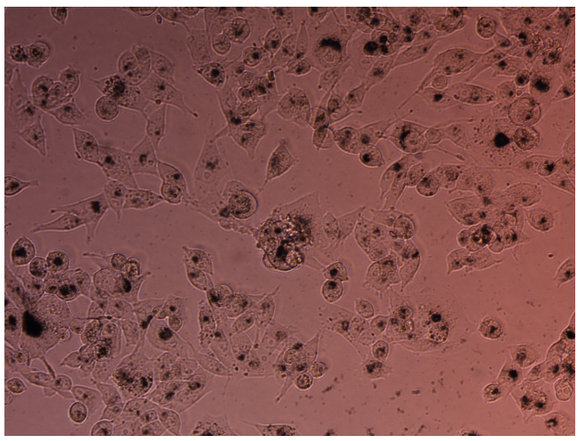

Fig 1: Bright-field optical microscopy image of pancreatic cells (MIAPaCa-1) exposed to GNRs of concentration 10 mg/L after a period of 24 hrs. The black spots in the middle are indicative of GNR clusters around the nuclear membrane.

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