Electronic Supplementary Material (ESI) for Nanoscale Advances.

Supporting Information

The impact of processing on the cytotoxicity of graphene oxide

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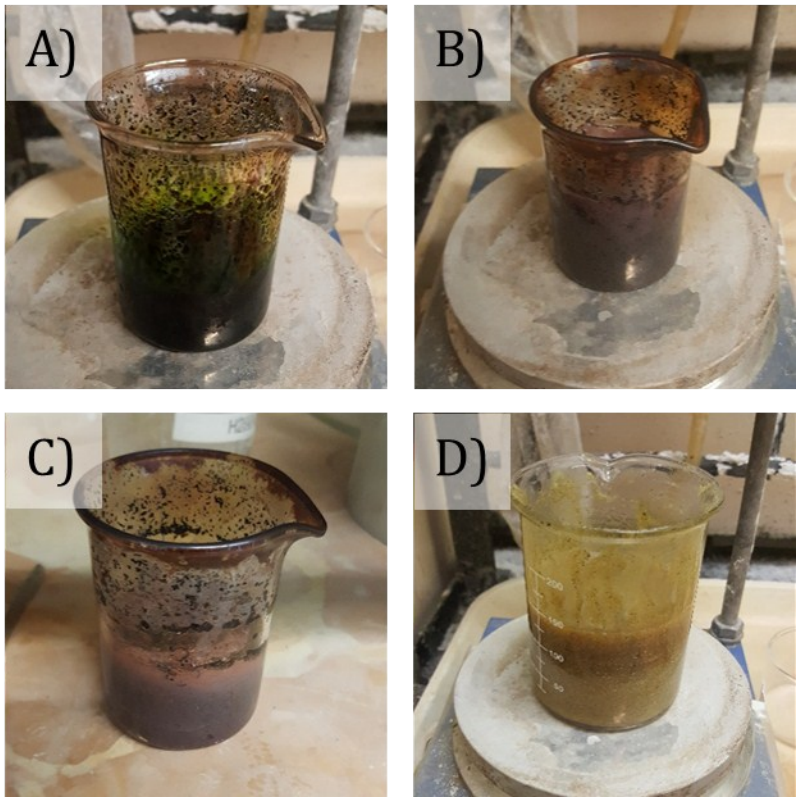
Synthesis of graphene oxide: Observations

With the gradual addition of $KMnO_4$ into the KNO_3, graphite and sulfuric acid solution, the solution became thicker and greener (Fig. S1 A). After 24 h the solution was purple and too thick to stir (Fig. S1 B). There were no drastic changes in the solution's appearance after sitting for 4 days (Fig. S1 C) or following the addition of the dilute sulfuric acid. Upon addition of the 30 wt% H_2O_2 for the purpose of reacting the extra, the solution turned a golden-yellow with considerable effervescence (Fig. S1 D). During the acidic washing steps, the golden suspended solid easily precipitated out of solution. With increasing washes, the solution became less golden and became browner. The last three water washes, the "precipitate" became increasingly gel like.



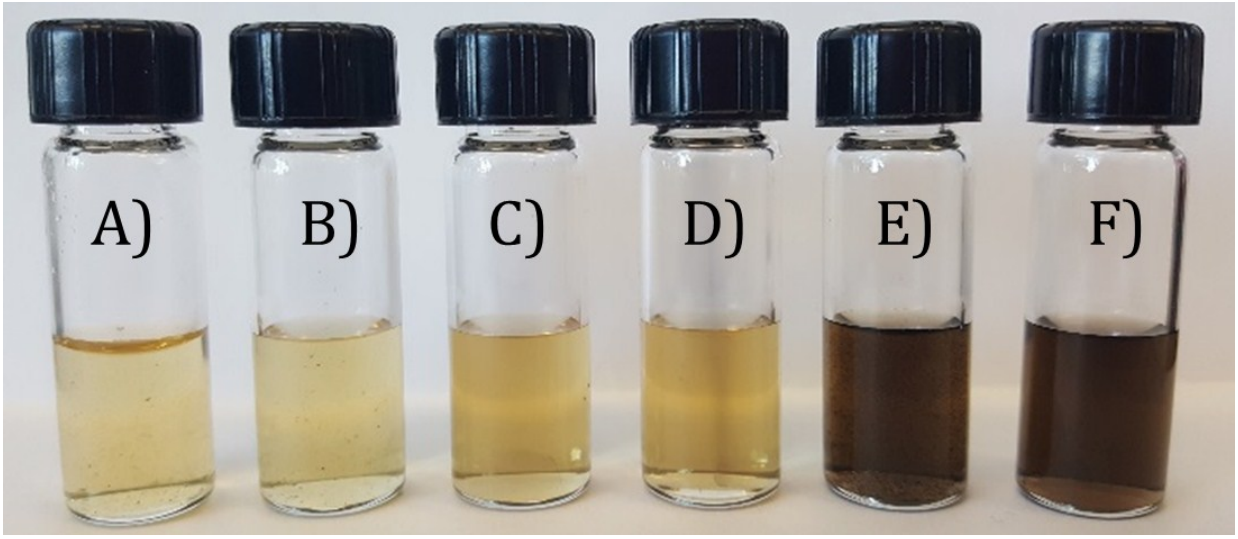
The final product was a thick brown gel.

Fig. S1: Images of the reaction of graphite into GO at different reaction stages: A) post addition of $KMnO_4$, B) post 24 h stirring, C) post 4 days undisturbed, D) post addition of H_2O_2.

Material properties: Visible characteristics

IHGO was a translucent brown solution which was similar in color to c-IHGO, s-IHGO and s-c-IHGO. IHGO and c-IHGO had visible brown flakes as seen in Fig. S2 A) and B). Upon sonication, these visible flakes were no longer observed as may be seen in Fig. S2 C)

and D). The two base washed samples, bw-IHGO and bw-s-IHGO were a similar dark brown/black colour. bw-IHGO had visible flakes in solution, but again, the sonicated



sample bw-s-IHGO did not as seen in Fig. S2 E) and F).

Fig. S2: Images of A) IHGO, B) c-IHGO, C) s-IHGO, D) s-c-IHGO, E) bw-IHGO and F) bw-s-IHGO at 50 µg/mL.

Flake morphology: Small features on IHGO flakes

IHGO had small features in the sample observed by AFM which were not observed in c-IHGO. These small features were difficult to see on the scale used in the main manuscript that was needed to demonstrate the large flake size of IHGO. An AFM image of IHGO is shown in Fig. S3 on a smaller scale so that the small features mentioned may be more clearly seen.

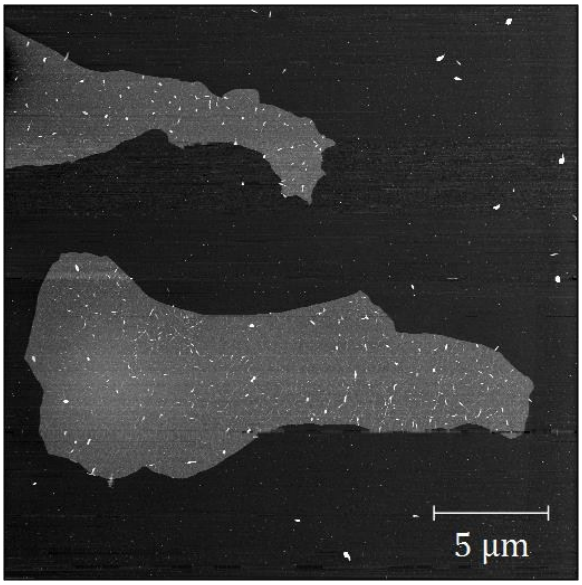


Fig. S3: AFM image of IHGO, note the small raised features present on and off the GO flakes.

Flake morphology: Graphenea

A commercial source of GO, Graphenea - GGO, was base washed. The base washed material, bw-GGO, was observed to have a higher cytotoxicity than GGO. In order to relate the properties of the material to the measured cytotoxicity, AFM images were taken of GGO and bw-GGO and are displayed in Fig. S4. It may be seen from the AFM images that the flakes of GGO are planar and disperse, when the flakes of bw-GGO are wrinkled and more aggregated. These findings mirror the observations from the IHGO and bw-IHGO samples (Fig. 2).

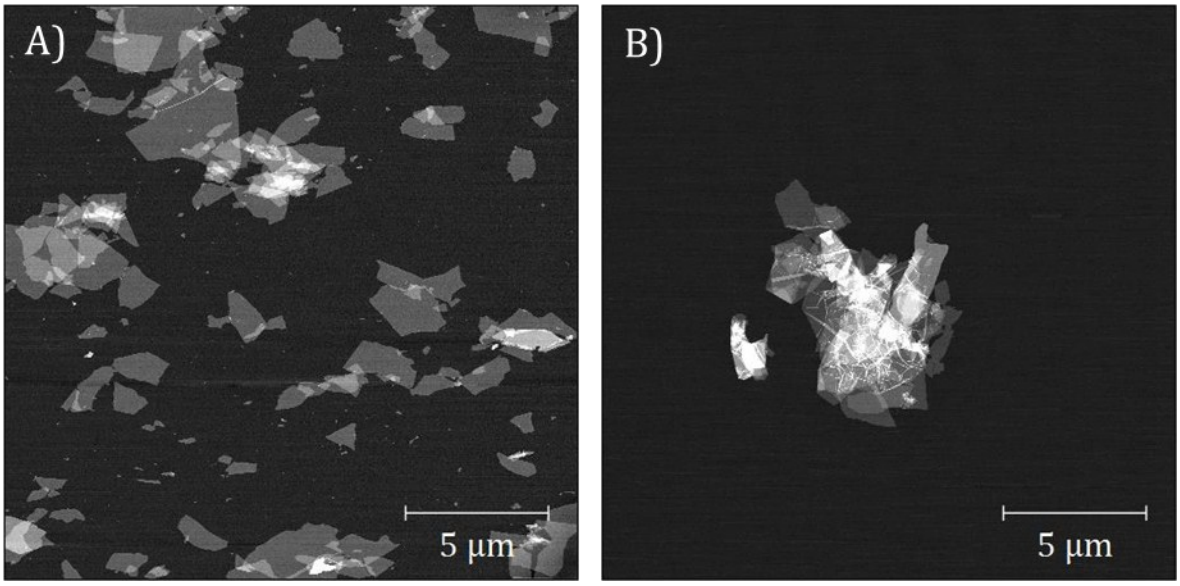


Fig. S4: AFM images of A) GGO, B) bw-GGO. The z-scale for A) is 5 nm, and B), 7 nm.

Cytotoxicity: Impact of cleaning

The process of cleaning IHGO and s-IHGO decreased the amounts of nitrogen and sulfur impurities from ca. 2% to ca. 1%. Cleaning also was observed to remove small features from IHGO via AFM images. Cleaning did not however impact the cytotoxicity of IHGO and s-IHGO as seen in Fig. S5. Looking at individual panels of Fig. S5, there may appear to be a difference between the cleaned and un-cleaned samples, however, when considering the two sets of materials on the four cell lines, no trends in the difference in toxicity between the two samples exist. From this, it was concluded that the levels of sulfur and nitrogen which were removed do not impact the cytotoxicity of GO. The IHGO and s-IHGO data were reported in the main manuscript in Fig. 3 and 4 respectively for the purpose of facilitating comparison between them and the cleaned materials.

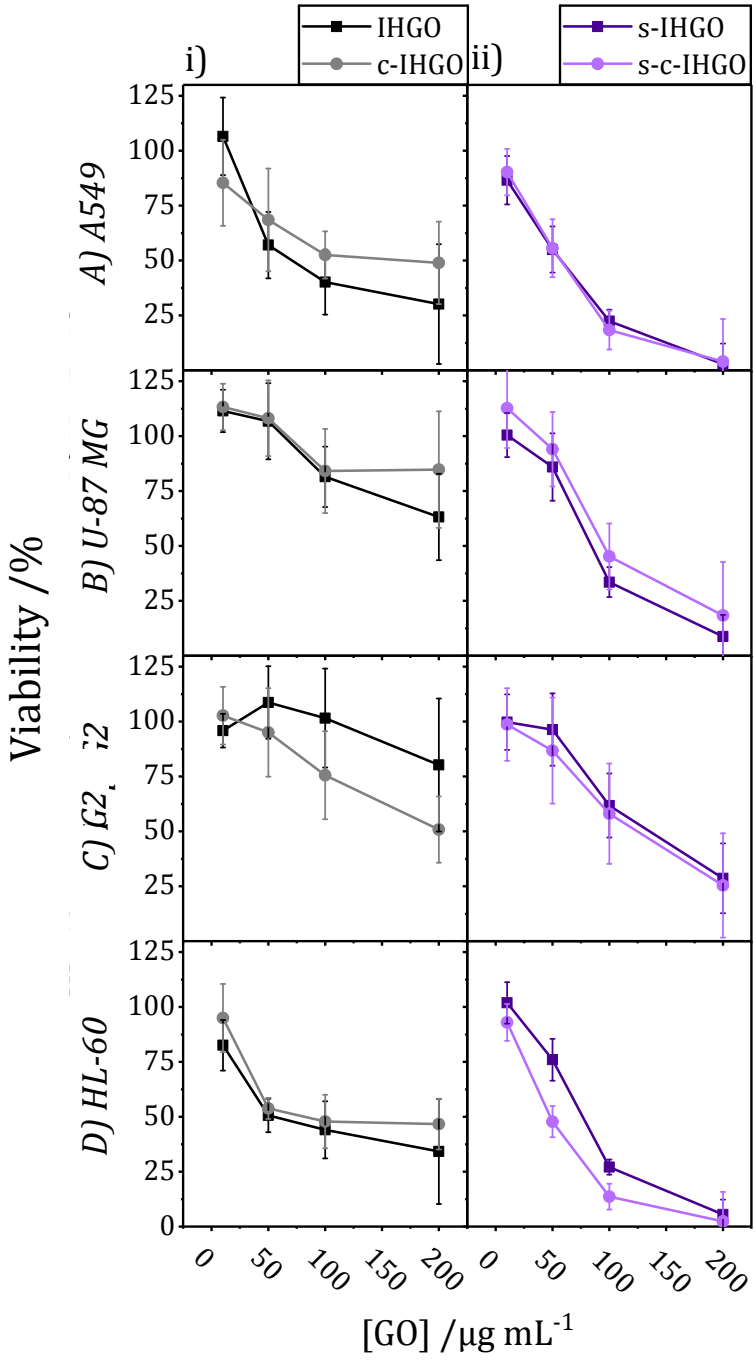


Fig. S5: Cytotoxicity of i) IHGO and c-IHGO and ii) s-IHGO and s-c-IHGO on A) A549, b) U-87 MG, C) HepG2 and D) HL-60 24 + 4 h post exposure as determined by the WST-8 assay.

Cytotoxicity: A separate analyst and a separately prepared material

Through a series of experiments performed by one analyst on a sample of IHGO prepared in duplicate, it was concluded that base washing results in a material with the highest cytotoxicity and that sonication and a combination of base washing and sonication result in materials with a cytotoxicity intermediate to IHGO and bw-IHGO. In order to provide further evidence to support these findings, two additional series of experiments were performed; a second analyst using the same source of IHGO (called IHGO A) was used to prepared bw-IHGO, and bw-s-IHGO and s-IHGO, and IHGO was prepared separately (called IHGO B) to prepare the same samples. The cytotoxicity of all materials was evaluated using the A549 cell line and the WST-8 assay. The results are shown in Fig. S6. The only difference that arose from these additional experiments was the observation than s-IHGO was somewhat less toxic than bw-s-IHGO for IHGO B. This could be a real observation, or a product of only evaluating a single cell line, or a result of the different starting material. Most importantly, to support the findings of the first series of experiments, it was again concluded than bw-IHGO is the most cytotoxic and s-IHGO and bw-IHGO have a cytotoxicity intermediate to IHGO and bw-IHGO.

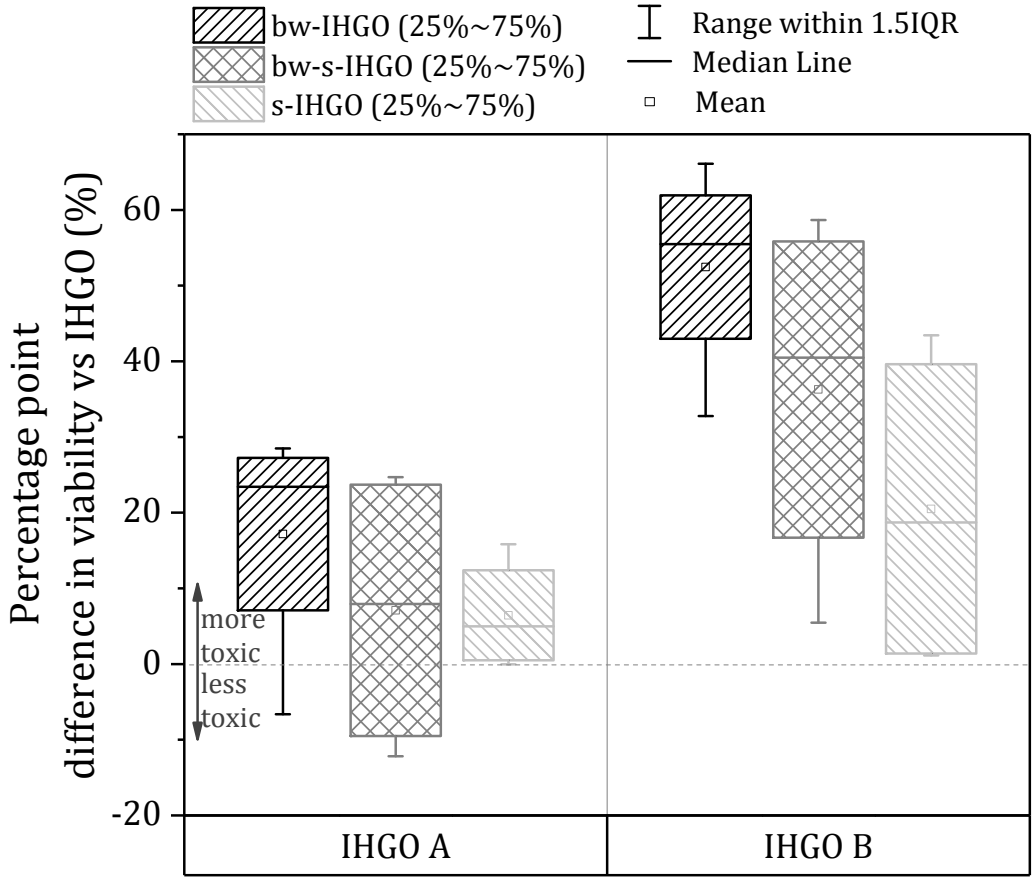


Fig. S6: Average percentage point difference in cytotoxicity between bw-IHGO, s-IHGO or bw-s-IHGO and IHGO across the four concentrations studied on A549, U-87 MG, HepG2 and HL60 cells as determined by the WST-8 assay for 24 h treatment.

Cytotoxicity: A base washed material intermediate to bw-IHGO

In an attempt to prepare bw-IHGO, a material was obtained that was intermediate to IHGO and bw-IHGO. An AFM image of intermediate material is shown in the ESI Fig. S7. It can be seen that the flakes have more creases than IHGO, but not the wrinkles observed in bw-IHGO. The cytotoxicity of the material was evaluated and compared to the starting IHGO and fully bw-IHGO as shown in ESI figure 7 A), it may be observed that the cytotoxicity of the intermediate material is more cytotoxic than IHGO, but not to the level of the bw-IHGO.

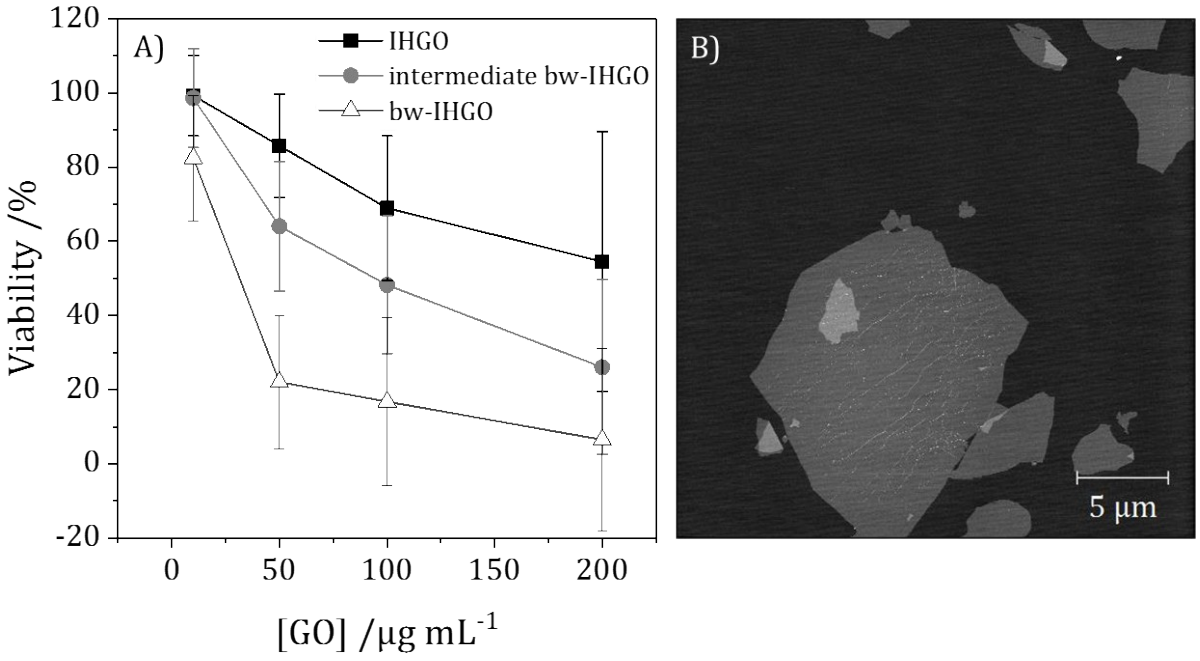


Fig. S7: A) Cytotoxicity of IHGO, an intermediate bw-IHGO and bw-IHGO sample on the A549 cell line 24 + 4 h post exposure as determined by the WST-8 assay. B) AFM image of the intermediate bw-IHGO sample with a z-scale of 5 nm.