Effect of polymer surface adsorption on graphene nanoplatelets biocompatibility

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Introduction: Several biomedical applications have been studied for graphene-based materials (GBMs), including biosensing/bioimaging, drug delivery, cancer photothermal therapy, regenerative medicine, and antibacterial materials. In view of the growing interest in using GBMs in this context, it is paramount to evaluate their biocompatibility. The present work studies a commercially available product, with reduced cost comparing with single layer graphene: graphene nanoplatelets (GNP). GNP has been reported to display good results as biopolymers fillers, improving mechanical and thermal performance, and biocompatibility, namely reducing platelets activation without increasing toxicity [1]. Covalent and non-covalent surface modification with polymers is a strategy to overcome possible toxicity of GBMs powders. Covalent functionalization often implies using toxic solvents as reaction medium, while the procedures for non-covalent surface modification are simple and easily up-scalable. This work aims to study the biocompatibility of GNP, as well as the effect of non-covalent surface modification with several biocompatible polymers.

Materials and Methods: Graphene nanoplatelets C-750 (GNP-C), were acquired from XG Sciences, having thickness <2 nm, length 1-2 µm, surface area 750 m^2g^{-1}. GNP-C were modified by surface adsorption with different polymers, namely: poly(vinyl alcohol) - PVA, hydroxyethyl cellulose - HEC, poly(ethylene glycol), poly(vinyl pirrolidone), chondroitin sulfate potassium, glucosamine sulfate potassium and hyaluronic acid. Polymers and GNP-C (1:1 ratio) were dispersed in water performing sonication with a Hielsher UIP 1000 probe during 5 minutes. The dispersion was then centrifuged at 4000 rpm for 15 min and supernatant discarded, assuring removal of excess polymer. Scanning electron microscopy (SEM) was used to observe morphology. Dynamic Light Scattering (DLS), X-ray photoelectron spectroscopy (XPS), Raman Spectroscopy, and Thermogravimetric analysis (TGA) were used for chemical characterization and quantification of the mass of adsorbed polymers. Hemolysis and Resazurin assays were performed to evaluate GBMs biocompatibility with HFF-1 cells. Also, Live/Dead assay was made, in which dead cells were stained with propidium iodide (PI), live cells with calcein and the total number of cells with hoechst 33342, allowing calculation of cell death (%).Transmission electron microscopy (TEM) images were obtained to evaluate GNP/cell interactions. Reactive oxygen species (ROS) were quantified using the indicator CM-H_2DCFH-DA.

Results and discussion: All materials were characterized but here, results are presented only for GNP-C modified with PVA and HEC, since these materials unveiled the best non-hemolytic properties. SEM images reveal that GNP-C is constituted by individual particles with diameter around 2 µm and small wrinkled flakes (0.5 µm). GNP-C-PVA forms a film on platelets surface, while GNP-C-HEC is in the form of very small particles. DLS results show that surface adsorption of polymers increase GNP-C particle sizes, which has two populations around 0.5 and 2 µm, due to encapsulation of the platelets and induction of inter-platelet interactions that lead to agglomeration. Particle size increase is higher for GNP-C-PVA (around 25 µm) than for GNP-C-HEC (around 8 µm). XPS, Raman and TGA confirm the presence of polymers at GNP-C surface, being of 21 % and 15%, respectively for GNP-C-PVA and HEC. Hemolysis for all GBMs, was below 1.7%, up to concentrations of 500 µg mL^{-1} at 3h. Live/Dead assay shows that cell death is low (<6%) for all materials in concentrations between 1-100 µg mL^{-1}. For 20 and 50 µg mL^{-1}, cell death is significantly lower ($p < 0.05$) for modified GNP-C-PVA, comparing to pristine GNP-C. For 50 µg mL^{-1}, GNP-C increases ROS production by 4.4 fold comparing with negative control (PBS). For GNP-C-PVA and HEC it also increases by 3.3 and 5.2 fold, respectively. These results are in agreement with those obtained in resazurin assay. TEM images show that GNP-C was almost completely exfoliated interacting with plasma membrane, being internalized without causing membrane damages, being found often in cytoplasm and in some cases interacting with mitochondria, which may induce ROS production. GNP-C-PVA was more agglomerated, presenting larger volume than GNP-C, being found more often outside plasma membrane than in cytoplasm. Internalized GNP-C-HEC particles presented smaller length (0.5 - 1.5 µm) than GNP-C-PVA and GNP-C (0.5 - 3 µm), being often found spread in cytoplasm**.** GNP-C-HEC was also observed in contact in mitochondria.

Conclusions: The biocompatibility of GNP modified by surface adsorption of polymers was evaluated *in vitro*. Hemolysis tended to decrease after adsorption of most polymers, however PVA and HEC presented the best results, and these materials were therefore characterized with more detail. Small sized GNP-C enters cells inducing ROS production, and therefore, toxicity. PVA encapsulation of GNP-C increased particle size, decreasing internalization and interaction with HFF-1 cells, therefore avoiding ROS production. HEC favoured internalization of small GNP-C particles, having the opposite effect. This work shows that GNP-C-PVA has potential to be used as coating or filler for medical devices, with the purpose of improving mechanical and/or thermal properties, while reducing acute toxicity, improving biointeractions and reducing infection probability. Also, smaller particles can be separated and used for drug delivery. GNP-C-HEC is more toxic, but its smaller particles present increased internalization, therefore potentially offering advantages in targeted delivery of toxic drugs or cancer photothermal ablation.

References: [1] Pinto AM, Gonçalves IC, Magalhães FD, Colloids and Surf B Biointerfaces, **111** (2013) 188.