Graphene-based films interaction with proteins, bacteria, mammalian cells, and blood constituents: the impact of surface features

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Abstract

Graphene-based materials (GBMs) availability has increased and so has the exploitation of their applications [1]. However, interaction between GBMs-integrating surfaces and the major biological systems in our body is still poorly understood [2]. The aim of this study was to systematically explore the features of these nanomaterials that most strongly impact the interactions of GBMs films with plasma proteins, bacteria, mammalian cells, and blood components [3].
GBMs films were produced by vacuum filtration using GBMs with different layer thickness and oxidation degree (graphene oxide (GO) and reduced GO (rGO) – 0.34 nm thick; few-layer graphene (FLG) and few-layer GO (FLGO) – 6-8 nm thick), which caused films to depict different surface features. After characterization by XPS, XRD, SEM, AFM, and water contact angle measurements, GO and FLGO films reveal a more oxidized (~32%), smoother (nano-roughness), and hydrophilic surface, while rGO and FLG are less or non-oxidized (13.5% and 3.5%, respectively), rougher (micro-roughness), and more hydrophobic. All films promote glutathione oxidation (Ellman's assay), indicating their potential to induce oxidative stress in biological systems: after 2 h, GO films showed the highest ability to oxidize glutathione (86.9%), followed by FLGO (50.5%) and FLG (44.0%), while rGO has reduced capacity; after 24 h, all materials reach 100% oxidation, with exception of rGO. Human plasma proteins, which mediate most of the biological interactions, were identified and quantified by mass spectrometry (nanoLC-MS/MS), adsorbing in smaller amounts to oxidized surfaces (8.3% on GO and 7.6% on FLGO) than to reduced or non-oxidized films (26.5% on rGO and 49.3% on FLG), highlighting the impact of GBMs oxidation degree on protein adsorption. Similarly, and as demonstrated quantitatively through LIVE/DEAD assay, clinically relevant bacteria – *Staphylococcus epidermidis, Staphylococcus aureus, Pseudomonas aeruginosa,* and *Escherichia coli* – adhere less to GO and FLGO films, while rGO and FLG favour bacterial adhesion and viability. Surface features caused by the oxidation degree and thickness of the GBMs powders within the films have less influence toward human foreskin fibroblasts as unravelled by Ethidium-1/Calcein/Hoechst fluorescence staining: all materials allow cell adhesion, proliferation and viability up to 14 days, despite less on rGO surfaces. Blood cells (leukocytes, erythrocytes and platelets) adhere to all films, with higher numbers in less or non-oxidized surfaces, despite none having caused hemolysis (<5%).
Unlike thickness, oxidation degree of GBMs sheets strongly impact surface morphology/topography/chemistry of the films, consequently affecting protein adsorption and thus bacteria, fibroblasts and blood cells response. Overall, this study provides useful guidelines regarding the choice of the GBMs to use in the development of surfaces depending on the envisioned application. Oxidized materials appear as the most promising for biomedical applications that require low bacterial adhesion without being cytotoxic to mammalian cells.

Funding: SFRH/BD/120154/2016, PTDC/CTM-COM/32431/2017, UID/BIM/04293/2019.

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