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Do biomedical engineers dream of graphene sheets?

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ABSTRACT

During the past few years, graphene has outstandingly emerged as a key nanomaterial for boosting the performance of commercial, industrial and scientific related technologies. The popularity of this novel nanomaterial in biomedical engineering is due to its excellent biological, electronic, optical and thermal properties that, as a whole, surpasses the features of commonly used biomaterials and consequently open a wide range of applications so far within the reach of science fiction. In this minireview, the potential of graphene and its based materials in the expanding biomedical field is highlighted with focus on groundbreaking diagnostic, monitoring and therapeutic strategies. Some of the major challenges related to the synthesis and safety of graphene-based materials are also briefly discussed because of their critical importance in bringing this class of carbon materials closer to the clinic.

INTRODUCTION

Since it was successfully isolated for the first time in 2004[1], graphene has proved to be a frontrunner nanomaterial for a wide range of biomedical engineering applications[2] by narrowing the gap among biology, electronics and nanoscience towards the development of more efficient diagnostic[3] and therapeutic[4] strategies. The vertiginous uprising of graphene in the biomedical field is intrinsically related to its set of amazing features, combining enhanced electrical, thermal, optical and mechanical properties with promising levels of biocompatibility. Briefly, graphene is a 2D flat monolayer of sp^2 hybridized carbon atoms arranged in a honeycomb fashion that can work as a basic building block for other carbon related materials: graphene can be wrapped up into fullerenes (0D), rolled into carbon nanotubes (1D) or piled up into 3D graphite crystals[5]. From a functional point of view, the 2D arrangement guarantees a notably high specific surface area that can be used to establish suitable cell-material interactions[6] and to provide multiple attachment spots for biomolecules[7]. The remarkable electronic and optical properties of graphene are deeply linked with its singular electronic band which, by combining both metallic and semiconducting characteristics, allows the π electrons to behave like ultrarelativistic particles able to move with a speed close to the speed of light. Indeed, this nanomaterial is a bioactive and transparent zero-gap semiconductor capable of significantly upgrading the selectivity and sensitivity of both electrochemical, field-effect transistor (FET) and optical biosensors[8, 9] in the road to more efficient enzymatic biosensing, DNA sensing, and immunosensing. For example, Xu et al.[10] have successfully patterned six parallel ultrasensitive FETs onto a graphene single crystal domain in order to perform reliable and reproducible multiplex analysis of DNA. Relatively to 1D carbon nanomaterials, the

advantages brought by graphene included not only an optimal performance by measuring the kinetics of DNA hybridization and minutely distinguishing single-base mutations in real time, but also the implementation of a more cost- and time-efficient fabrication technique and a simpler functionalization process. In a similar way, the fabrication of nanopores in a graphene sheet proved to be an auspicious strategy to unveil the exact DNA sequences that encode the genetic mechanisms of tumors and hereditary diseases. Actually, according to many experimental and theoretical studies[11, 12], due to the capability of graphene nanopores to detect minimal fluctuations in the ionic flow that moves within, it is possible to precisely associate the passage of each nucleobase through the nanopore with a particular blockage and magnitude variation of the induced ionic electrical current. Concerning the efficiency of this process, both the customization of the graphene nanopores with biological markers like layers of DNA-origami[13] and the combination of graphene with other 2D nanomaterials such as molybdenum disulfide[14] are suitable options to reduce the velocity of the DNA passage through the nanopore and therefore to boost a more accurate sequencing process. Additionally, biomedical researchers are currently exploring the capability of graphene to act as an exceptional reinforcing filler for biomedical platforms due to the mechanical integrity and intrinsic lightness of its 2D honeycomb structure, where each carbon atom is covalently bonded with its three nearest carbon neighbors[4]. As the graphene-based biocomposites are easily compatible with a wide range of nanofabrication techniques, it is often possible to shape their morphology to match different cellular microenvironments such as fibrous[15] and porous[16] structures. In these lines, the presence of graphene was a critical factor to enhance the performance of a polyacrylamide hydrogel proposed as a 3D neural tissue engineering (TE) scaffold as

neurons were only able to generate neuronal networks capable of supporting synaptic activity when cultured in those hybrid hydrogels containing graphene[17].

Complementary to graphene, graphene oxide (GO) presents a highly reactive surface, with hydrophobic sp^2 carbon regions intercalated by sp^3 regions where the carbons are linked with oxygen functional groups (carboxyl, epoxy and hydroxyl) that guarantee the presence of hydrophilic zones able to promote good water dispensability, a near infrared (NIR) to visible fluorescence and also covalent and/or non-covalent attachment points for biomolecules, metals and polymers[18]. This singular mix of features is receiving increased attention from biomedical engineers, who look to GO as the central building block for versatile strategies capable of combining imaging, sensing and therapy[19, 20]. For instance, GO can be simultaneously used for drug delivery and live cellular imaging by diffusing its oxygen moieties via a mild thermal annealing procedure able to maintain their availability to be conjugated with cancer drugs while inducing blue fluorescence[21]. In other example of cancer nanotheranostics[22], GO was successfully combined with Bi_2Se_3 nanoparticles and polyvinylpyrrolidone with the purpose of synthetizing a multifaceted nanocomposite able to match an excellent performance as X-ray computer tomography and photoacoustic contrast agent for tumor visualization *in vivo* with an enhanced capability to induce a permanent removal of cancer cells via photothermal therapy (Figure 1). Moreover, depending on the biomedical application, the GO functionality can be successfully tuned by adapting the size of the nanosheets[23, 24] or by partially removing its oxygen functional groups[25, 26] via either chemical or thermal methodologies, leading to reduced graphene oxide (rGO), which displays closer properties to those of pristine graphene. However, rGO is conceptually a different nanomaterial relatively to graphene since both the removal of

oxygen functional groups and the restoration of the π-π conjugation that occur during the reduction process are not able to induce a uniform sp^3 – sp^2 hybridization throughout all the extension of the carbon network[27]. In this way, by controlling the degree of reduction, it is possible to modulate the quantity of topological defects and residual oxygen moieties that are capable of influencing the biological, chemical, electrical, mechanical, optical and thermal properties of the final nanomaterial[28-30]. For example, Chen et al.[31] have studied the capacity of three different chitosan derivatives to work simultaneously as reducing and stabilizing agents for GO with the final purpose of selecting the composite with less oxygen content and therefore with a more integrated conjugated carbon network for further drug delivery testing. Results showed that the presence of such composite into the final hydrogel beads was crucial to ensure an efficient π-π stacking with the drug and subsequently guaranteed a better encapsulation capacity and an enhanced drug release profile comparatively to its oxidized counterpart.

In this mini-review, the impact of graphene-based materials (GBM) in biomedical engineering is highlighted by discussing their potential inclusion in advanced diagnostic, monitoring and therapeutic approaches. Also, the viability of this set of carbon materials in the healthcare field is presented by focusing in essential issues such as the development of new synthesis methodologies and the necessity of conclusive studies regarding their short- and long-term toxicity.

GBM in diagnostics and healthcare monitoring

In order to overcome the limited access to health diagnostic and monitoring services, which are mostly located on hospital and clinical settings, there is a growing need to develop portable and therefore cost-effective biomedical devices capable of comfortably reaching

patients living in remote areas. Additionally, such platforms should ideally be real-time personalized tools capable of guaranteeing an accurate recognition of relevant physiological changes by the patient, avoiding the need for a continuously data analysis by highly skilled healthcare professionals. Possible strategies for reaching this goal and subsequently respond to the guidelines of the World Health Organization for ASSURED devices (Affordable, Sensitive, Specific, User-friendly, Rapid and Robust, Equipment free and Deliverable to end-users)[32] include diagnostic and routine health tests performed with upgraded smartphones[33], health monitoring via either non-invasive flexible biosensors[34] or implantable biophotonic devices[35], ingestible electronics[36] as diagnostic tools and biomedical tattoos[37] able to early detect diseases such as cancer. Some of these revolutionary approaches deeply rely on the multifunctional behaviour of carbon-based nanomaterials[38, 39], especially graphene, due to their singular electrical and optical properties and their ability to be incorporated into advanced composites.

Indeed, the placement of graphene as a central player for developing the new generation of diagnosing and monitoring platforms is being strongly sustained by interdisciplinary inputs from biology, chemistry, electronics and physics, facilitating the upgrading of such devices. One remarkable example is the progress in the conception and fabrication of biosensors, where GBM can efficaciously integrate a wide range of *ex situ* bioanalytical systems[2, 9] that allow an accurate identification of targeted molecules, proteins and cells collected from the patient. In some strategies, these target entities establish a direct electron transfer with graphene, which offers several advantages as electrode material[8, 40] by showing a large specific surface area, a fast electron-transfer kinetics and a notable ability to catalyse the redox reactions that lead respectively to an enhanced sensitivity, a boosted response time

and the detection of biomolecules at low electrochemical potentials. The usefulness of graphene-based electrodes as early diagnostic tools for some common chronic diseases such as cancer[41], diabetes[42] and HIV[43] was recently reported with very promising results. In fact, graphene enabled not only excellent functionalization routes to enhance the selectivity and sensitivity of the biosensors for both small molecules (e.g. glucose) and biomarkers (e.g. for cancer and HIV), but simultaneously broadened the range of available cost-effective design and fabrication techniques concerning electrochemical sensing platforms. Moreover, graphene-based electrodes can also be adapted to integrate real time monitoring modalities. For example, Lee et al.[44] have developed a wearable graphene-hybrid interface capable of detecting glucose above a critical concentration by analysing the sweat on the skin of the patient and, if necessary, counterbalancing the excessive glucose levels with a controlled drug delivery system provided by bioresorbable temperature responsive microneedles (Figure 2). Although multifunctional platforms able to both sense abnormal levels of glucose and induce a negative feedback response are currently presented as an ideal approach for controlling diabetes, a more near-future scenario will include gadgets such as smartphones equipped with screen-printed electrodes (SPE)[45] capable of recoil physiological data and aware the patient through user-friendly applications. A promising route for fabricating SPE was reported recently[46], showing a two-step strategy where, firstly, GO was first combined with a glucose sensitive substance (3-amino phenylboronic acid) and then reduced with the purpose of enhancing the conductivity of the composite. At the end, the rGO composite electrode was able to successfully bind glucose and consequently trigger the smartphone-based cyclic voltammetry detection system, which was displayed on the screen in real-time. Other

groundbreaking sensing strategies are presently focused on biomedical devices able to meticulously detect cancer cells[47, 48]. One example was reported by Wang et al.[49], who fabricated a 3D graphene biointerface capable of using its irregular and conductive surface to enhance the formation of filopodia from the cells due to the established multidimensional cell-material interactions. Then, by combining these topographical features with the potential of graphene to be used as an electrode material, it was possible to upgrade the recognition of the electrical impedance signals coming from cancer cells (relative to the cell capture and sensing efficiencies) with respect to standard 2D gold interfaces.

The impact of graphene is also noticeable in the production of advanced FET biosensors[50] since its presence between the source and the drain contacts of the sensing platform offers advantages comparatively to other materials (e.g. silicon) including high transconductance, stable performance and low working voltage. Also, these graphene-based FET biosensors present highly tuneable chemical[51] and morphological[52] features that can robustiously support a wide range of critical healthcare challenges including the detection of bacteria in contaminated water[53] and the prevention of heart failure[54]. On the other hand, the optical behaviour of GBM is also being addressed as a promising characteristic for efficient sensing and imaging strategies. For instance, changes in the optical features provoked by the adsorption of hemoglobin onto a GO coated fibre grating can easily identify abnormal concentrations of this molecule in the blood for anemia diagnostics[55]. Similarly, graphene is dramatically shifting paradigms in cell imaging by allowing exhaustive real-time monitoring of cellular morphology and physiology either via high resolution optical platforms able to detect even the variations in the refractive index of subcellular components[56] or via quantum dots fluorescence[57, 58].

Adding to its excellent electrical and optical properties, graphene presents a very interesting set of biological and mechanical properties that allow a solid bridging between *ex situ* sensing/monitoring modalities and *in situ* sensing implants[2, 59, 60]. In fact, the enhanced levels of biocompatibility, flexibility and resistance of graphene-based devices enable a continuous, efficient and long-term signal detection and processing despite the stresses intrinsic to biological environments. Taking this into account, biomedical engineers are currently focusing efforts to expand the functionality of common healthcare accessories such as contact lenses[61] by upgrading them with flexible and transparent electronics (e.g. biosensors and wireless antennas) capable of maintaining their performance independently of the continuous eye blinking. Some advancements boosted by graphene include the recording of electroretinograms[62], diabetes and glaucoma diagnostics[63] and the enhancement of eye protection[64] against dehydration and electromagnetic waves. Likewise, other emerging new class of wearable electronics embraces multifunctional graphene electronic tattoos. A pioneer example was reported recently by Ameri et al.[65], who have used a "wet transfer, dry patterning" methodology to fabricate a device capable of efficiently perform common physiological measurements like electrocardiograms, electromyograms and electroencephalograms (Figure 3). Also, in this case, graphene was indispensable to guarantee a temporal attachment to the skin via van der Waals forces, a mechanical integrity able to resist skin deformations and an optical transmittance adequate to make the electronic tattoo unnoticeable.

GBM in therapeutic strategies

GBM are currently one of the most significant opportunities of modern science to unlock sustainable solutions for some of the major challenges concerning cancer therapeutics and

regenerative medicine[4, 66]. This expectation comes from the possibility of rearranging specific sets of graphene related properties depending of the implemented strategy. For instance, the modulation of solubility, photosensitivity and load capacity can enhance the efficiency of cancer treatment modalities such as drug delivery[67, 68] and phototherapy[69, 70]. Moreover, the chemical, electrical and mechanical tunability of GBM can enhance the features of advanced TE scaffolds[71], leading to a meticulous recreation of specific cellular microenvironments and consequently to a successful reinforcement or replacement of natural regeneration processes. Thus, graphene seems to perfectly fit into the concept of personalized medicine[72, 73], which states that, ideally, a therapeutic agent should be tailored to match the specific requirements of the patient and then delivered/implanted with precision in the target area without toxic effects.

In fact, although the heterogeneity of cancer[74] is presently compromising the fulfilment of personalized medicine, new insights brought by nanotechnology and nanomedicine during the past few years have allowed the growing of nanotheranostics[38, 75] as a near-future promising alternative to fight this devastating disease. This approach requires the development of multifunctional nanomedical systems able to detect and kill cancer cells while the efficiency of their performance is monitored. Biomedical engineers are placing GBM, specifically GO[20, 66], as a cornerstone to build nanotheranostic strategies encouraged by its improved levels of biocompatibility and stability relatively to other common materials like metals and polymers. Additionally, its functionality enables an easy surface modification with anchored ligands (e.g. drugs, peptides and proteins) able to selectively bind to receptors overexpressed by tumours or specific cell types such as endothelial cells, leading to successful active targeting approaches that guarantee a localized deliver of

therapeutic agents (chemotherapy[76] and gene therapy[77]) or phototherapy (e.g. photothermal[78] and photodynamic[79] therapies). For delivery purposes, GO presents a high surface area, aspect ratio and cell internationalization ability that fulfil important requirements for an excellent nanocarrier platform. Indeed, in a combinatorial strategy suggested by Li et al.[80], GO was covalently linked to polyethylenimine with the purpose of anchoring two materials (folate and heparin) able to specifically recognize breast cancer cells. The composite was also loaded with doxorubicin, a well-known chemotherapy medication, via π-π and hydrophobic interactions. Then, the final compound was administered *in vivo* together with an inhibitor of the metastatic process, producing a synergetic effect that suppressed both the tumour growth and the pulmonary metastasis. In a complementary approach[81], the potential of GO as a photosensitive material was explored in the shape of a nanocomposite capable of effectively killing solid tumours by mediating both photothermal and photodynamic therapies while providing *in vivo* multi-colour fluorescence imaging. Other promising modalities include the combination of the properties of GO/rGO with magnetic nanoparticles towards the development of advanced nanotheranostic systems suitable to perform magnetic resonance imaging[82] and/or conduct magnetic targeting[83].

Other offshoot of personalized medicine involves the modulation of stem cell biology in the direction of progressive therapies able to replace the current regenerative medicine approaches (e.g. allografts) by reprogramming patient's mature cells into an immature state – induced pluripotent stem cells (iPSCs) – and control their further differentiation into a selected cell type[84]. In this way, it would be possible to suppress significant limitations like donor availability, immune rejection and ethical issues since the patient will be both the

source and the receiver of the cells[85]. However, before reaching this ideal scenario, it is mandatory to deepen our knowledge on the maintenance of the differentiated phenotype of the cells as well as on their expansion, differentiation, transplantation and protection during and after the treatment. Thus, one of the hottest topics in the field of regenerative medicine is the *in vitro* recreation of cell niches skilled to provide cell-material interactions capable of accurately simulating the effects of specific extracellular matrices on stem cell behaviour. This will necessarily lead to a better understanding of the phenomena that modulate differentiation patterns of these cells[84, 86, 87]. The role of GBM in the development of these TE scaffolds is becoming increasingly prominent,[2, 66, 71] mostly due to their capacity to facilitate the customization of the bulk properties, shape and functionality of composites applied for mimicking different cellular microenvironments such as bone, heart, nerve and skin. For instance, regarding bone regeneration, graphene, GO and rGO have been used as osteoinductive agents[88] due to their ability to promote osteogenic differentiation of stem cells via different mechanisms. One example was recently proposed by Wu et al.[89], who reported that the presence of graphene into a polymer film was able to enhance alkaline phosphatase activity, the formation of a mineralized matrix and the activation of a genetic signalling pathway, responsible for inducing the efficient osteogenic differentiation of rat bone marrow-derived mesenchymal stem cells (MSCs). Complementary to the dynamics of molecular circuits, graphene composites provide an excellent opportunity to fabricate biocompatible 3D microenvironments (e.g. porous networks[90, 91] or electrospun fibres[92]) with features that also enhance both *in vitro* and *in vivo* osteogenic differentiation of stem cells. Some of them include surface chemistry able to potentiate mineralization (e.g. hydroxyapatite formation) and improve wettability,

interconnected porous systems capable of optimizing cell proliferation/migration across the scaffold, suitable biodegradation profiles and reinforced mechanical properties, among others.

Alternatively, the impact of graphene can be extended to cardiac differentiation pathways. For example, the simple inclusion of a graphene dispersion in the mouse embryoid bodies (EBs) structure induced an augmentation of their electrical conductivity and Young modulus, leading consequently to a viable cardiac differentiation process that could be successfully enhanced via electrical stimulation (ES) (Figure 4)[93]. Another group[94] has recently reported that graphene substrates can be used as electrically active platforms able to efficiently promote the differentiation of human iPSCs into cardiomyocytes and then improve their maturation into functional cardiac cells. Analogous to its impact in directing cardiac differentiation, GBM are being combined with other biomaterials for treating cardiovascular diseases by acting as anticoagulants[95], inflammatory modulators[96] and conductive scaffolds[97-99] with electromechanical properties that encourage the seeded cardiomyocytes to match the behaviour of the native cardiac tissue. Additionally, such scaffolds can usually respond to external ES, leading to an accurate regulation of important features such as cell alignment and maturation. In other applications, graphene-based scaffolds are combined with ES in order to improve the proliferation and differentiation of stem cells into neural lineages[100, 101]. As a matter of fact, Aznar-Cervantes et al.[102] have reported that the application of electrical stimulation onto a silk fibroin electrospun scaffold coated with rGO induced excellent levels of differentiation of PC-12 cells into neural phenotypes even without the presence of more traditionally pursued biochemical cues (e.g. neural growth factors). The need for growth factors to provoke differentiation was also

suppressed by applying ES into an inkjet-printed graphene interdigitated electrode, leading to an efficient transdifferentiation of MSCs into Schwann cells[103]. Strategies involving ES can also establish a preferential differentiation pathway, for example, a conductive 3D rolled GO foam was able to conduct electrical currents that favoured the differentiation of human neural stem cells into neurons rather than glia[104].

A further advantage brought by the inclusion of GBM in TE applications is their ability to kill bacteria via complex mechanisms such as oxidative stress and removal of phospholipids from the bacteria membrane[105, 106]. Although these mechanisms are not yet fully understood since they depend on the particular characteristics of both the nanosheets used (e.g. size, hydrophilicity) and the targeted microbial entity, their positive effects are already being explored in TE strategies, especially in skin regeneration[107, 108]. Additional benefits of graphene-based scaffolds in wound healing approaches include not only an extraordinary capacity to enhance stem cell responses both *in vitro* and *in vivo* but also degradability and mechanical features that match the ideal period for a subcutaneous implant (4 weeks),[109] as well as the successful promotion of collagen deposition and angiogenesis[110].

Challenges and perspectives

Although their impact is real and unmistakable, especially regarding the production of the next-generation of biomedical platforms, GBM still hold the status of dream materials for biomedical engineers since their full potential has not been either discovered or achieved yet. In fact, the feasibility of this class of nanomaterials in the healthcare system is not only related to the understanding/modulation of their properties, but also to the development of

new synthesis methodologies and manipulation regulations and the necessity of conclusive studies regarding short- and long-term toxicity.

Nowadays, one major issue, common to the other fields where the graphene influence is growing, is the lack of a production methodology able to simultaneously guarantee quality, scalability and cost effectiveness[2, 40]. Indeed, neither the bottom-up synthesis strategies such as chemical vapor deposition nor the top-down approaches like the mechanical exfoliation of graphite and the modified Hummer's methods are currently capable of producing high-quality GBM without costly and complex manufacturing processes, low yield and toxic reagents. Therefore, the development of scalable and sustainable methodologies based on green chemistry principles[66, 111, 112] can be considered a major milestone to place graphene-based biomedical devices as strong candidates for real world applications and commercialization. In this context, a promising strategy was recently reported by González et al.[113], who have successfully developed a mechanochemical treatment with carbohydrates to exfoliate graphite and subsequently generate graphene in an environmentally friendly approach. Another imperative progress before pondering the medical use of such devices must be done by regulatory authorities, who should narrow the large spectrum of experimental conditions around a defined safety level that could lead, consequently, to standardized characterization, nomenclature and results concerning GBM[114].

Even though the classification of GBM according to chemical (e.g. C/O ratio) and morphological (e.g. average lateral size, number of graphene layers) criteria must be extended to non-invasive diagnostic and monitoring systems to avoid dangerous long-time exposures of cells and/or tissues, this issue becomes even more relevant for engineering

implantable platforms with complete *in vitro* and *in vivo* toxicological profiles, including the maximum concentration and the mechanisms of delivery/degradation/elimination of the nanomaterials from the body[2, 6, 7, 115]. In fact, the evaluation of the risk-benefit balance is very complex and must be specific for each application due to the enormous differences that GBM can present in their atomic composition. For example, a recent study evaluated the effects of GO, chemical rGO and thermal rGO in human lung cells[116], showing that although the higher oxygen content of GO enabled more affinity with the cell membrane, the smaller lateral dimensions and sharp edges of the thermal rGO were able to boost the cellular uptake and therefore lead to more severe consequences regarding cellular viability, oxidative stress, genotoxicity and cell death. Similar results were obtained by Contreras-Torres et al.[117], who reported that myocardial cells were able to efficiently internalize the smaller low-rGO (37% content of oxygen) sheets relatively to the original GO (54% of oxygen), leading to a more acute generation of oxidative stress and consequently to a significant lower half maximal inhibitory concentration (129.4 ± 1.2 μg mL^{-1} and 652.1 ± 1.2 μg mL^{-1}, respectively).

Opposing to these properties, there is a small number of studies concerning the effects of the number of layers in the toxicity potential of GBM, notwithstanding the relevance of this parameter to correctly distinguish their different categories – for example graphene (i.e. until 10 layers of graphene) from ultrafine graphite (i.e. between 10 graphene sheets and 100 nm of thickness)[114] - and to determine other characteristics such as absorptive capacity, bending stiffness and specific surface area[115]. However, in a noticeable exception, Cho et al.[118] have analysed the dose and size dependence toxicity *in vitro* and *in vivo* of GO samples with different number of layers, revealing interesting results. Specifically, the

binding and phagocytic uptake processes that were activated by the single-layered GO proved to be more influential in inducing cell damage and inflammatory responses than the necrotic and apoptotic mechanisms triggered by the multi-layered GO. Additionally, the tests *in vivo* showed that, independently of the number of layers, the intravenous injection of GO provoked inflammation in both lungs and kidneys, being the more severe results reported for the multi-layered GO due to its higher volume and thickness.

Based on this, one priority of biomedical engineers should be the development of a standard characterization methodology for the reported GBM, which should include normative experimental conditions for common characterization techniques such as X-ray photoelectron spectroscopy (for chemical analysis) and transmission electron microscopy (for morphological analysis). In fact, the collection and organization of this data together with the parameters regarding both short- and long-term *in vitro* and *in vivo* toxicity should allow the construction of predictive models capable of forecasting and interpreting the molecular mechanisms affected by the interactions between GBM and the different organizational levels of livings systems (i.e. organelles, cells, tissues and organs)[119-121] with the final purpose of consistently enhancing the biological response of the new graphene-based biomedical platforms. For instance, in an influential theoretical study[122], it was suggested that, after cellular uptake, the hydrophobic character and the flatness of the graphene nanosheets leaded to a disruption in the protein-protein hydrophobic interactions and consequently to functional deficiencies at the metabolic level that could result in cell death. This report combined with other theoretical models have reinforced the paradigm of functionalizing GBM with suitable biomaterials/biomolecules in order to enhance their biocompatibility by preventing the triggering of undesirable processes such as oxidative

stress, cell membrane damage and mutations capable of compromising cell survival and proliferation. In fact, for the immediate future, this topic should be an integral part of graphene research since traditional coating strategies such as PEGylation[123] have presented unsuccessful results on improving the pharmacokinetic behaviour of GBM[124-126].

In summary, despite the innovative and extensive results provided by GBM in biomedical applications, including revolutionary diagnosis, monitoring and therapeutic approaches, there is still a long road ahead until some important concerns related to production processes and biological interactions including toxicity are irrefutably solved. However, it is quite possible that this road will be covered more quickly than expected based on the vertiginously rapid growth of the field and the enthusiasm of the scientific community, which are helping to overcome the mentioned challenges, leading to real and decisive breakthroughs in a near future.

Conflicts of interest

There are no conflicts to declare.

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