Graphene Oxide Thin Films with Drug Delivery Function

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Graphene oxide has been used in different fields of nanomedicine as a manager of drug delivery due to its inherent physical and chemical properties that allow its use in thin films with biomedical applications. Several studies demonstrated its efficacy in the control of the amount and the timely delivery of drugs when it is incorporated in multilayer films. It has been demonstrated that graphene oxide layers incorporated in drug delivery systems are able to work either as a nanocarrier, transporting the drugs to their targets or as a barrier delaying the release of drugs to accommodate the treatment schedules. This allows for the development of structured ,sophisticated and time-controlled systems.

graphene oxide drug delivery polyelectrolyte multilayer films

1. Introduction

Graphene-based materials have an important role in the development of drug delivery systems (DDS) due to their biocompatibility properties and the easiness with which they can be functionalized with biomolecules. Moreover, it is simple to integrate them in multilayer systems with applications in which controlled release of bioactive molecules is necessary. Graphene is a two-dimensional compound, consisting in a monolayer of aromatic carbon atoms (sp2-bound)/covalently bound, organized in a hexagonal lattice structure [1], forming sheets with the thickness of a single atom [2]. It has interesting mechanical properties, large surface area, and high electrical and thermal conductivities [3], and it can exist in other forms, such as graphene oxide (GO) and reduced graphene oxide (rGO), which are more hydrophilic, making them easier to solubilize and disperse in aqueous or polyelectrolyte solutions (such as PBS—phosphate buffered saline) and improving, therefore, their self-assembling properties [4][5]. The high content of groups with reactive oxygen in GO enhances its ability to be functionalized with various materials [1]. It is composed of carboxyl, hydroxyl, and ether groups, allowing it to absorb polar polymers or polar molecules with ease, and, therefore, it is an excellent candidate to form GO/polymer composites [4]. These active groups are ideal for molecule immobilization on the GO surface and make it hydrophilic and a powerful candidate to be used as drug carrier [6]. For instance, carboxyl groups are the main group responsible for GO colloidal stability [7][8][9]. Furthermore, GO has good water and biomedium dispersibility as well as good optical (absorption) and photothermal (conversion) properties [10]. Several functionalization strategies can be used to enhance GO's characteristics and promote its stability and bioavailability. This functionalization can be achieved, for example, by PEGylation (PEG—polyethylene glycol) to improve biocompatibility, solubility, and stability in physiological conditions, by double oxidation of the graphene to provide electrostatic stabilization, by using a

copolymer (e.g., Pluronic F127) to provide steric stabilization, by non-covalent interaction (π-π interactions) with aromatic organic molecules (such as 1-pyrenebutyrate (PB)) to improve aqueous stability, or by using synthetic peptides such as Poly L-Lysine (PLL) to improve its biological activity [11][12][13][14]. The versatility in the functionalization of GO allows its application in several devices. An example is the ability of functionalized GO to cross the blood–brain barrier (BBB), which widens its use in biomedical research, as it overcomes the inability of unstable chemicals or peptides to reach the brain [15][16]. Mendonça et al. [17] also demonstrated the ability of rGO to cause a transitory BBB breakdown and, therefore, cross this BBB into the brain.

2. Graphene Oxide Multilayer Films

Layer-by-layer (LbL) is a versatile technique which allows the incorporation of multiple components culminating in a smart and functional system. It is a simple and cost-effective process that can be used in large-scale production and is eco-friendly, due to the fact that the solutions used are aqueous [9]. LbL is the preferred technique to develop DDS with graphene, and several methods can be used to produce thin films, such as microfluidics [18], perfusion [18], Langmuir–Blodgett assembly [19], spin coating [5], drop coating [20], spray coating [21], or dip coating [4], which is the most common one.

The major driving forces in LbL multilayered films are electrostatic forces (more common), hydrogen bonding, charge transfer interaction, and covalent bonding [3][22]. LbL films can incorporate functional polymers [23], enzymes [24], small molecules [25], polysaccharides [26], carbon nanotubes (CNT) [27], nucleic acids [28][29], or inorganic nanoparticles (NPs) [30] and can be assembled in a wide range of substrates independently of their size or shape, allowing for the creation of three-dimensional objects covered in these films [3][31]. The fact that LbL films can be deposited on different substrates without altering the properties of the films is very useful, because it allows the testing of different film characteristics, which may require different substrates [9][32], for example, the quartz crystal microbalance (QCM), which requires the films to be assembled in a QCM crystal sensor/lamella [33][34], or the transmission electron microscopy, for which the films need to be deposited onto copper grids [35][36]. This also allows for their application in multiple biomedical devices without fear that their properties will change. Fox example, they can be applied in flexible substrates that can adhere to tissue and deliver different types of drugs [7] or odd-shaped substrates as coatings for biomedical implants, such as cardiovascular stents [37] [9][32]. Furthermore, LbL films containing GO can be used, for example, in tissue engineering [11][38], as carriers for vaccines [39], as antibacterials [40], in cancer therapy [41], or in biomedical imaging [42][43][44].

The incorporation of GO in a multilayered film is a chemistry game; it is necessary to have stable chemical interactions in the interfaces of the layers to avoid interdiffusion of components and to ensure a correct growth of film. Usually, to avoid aggregation or agglomerates on the surface of GO layers, bilayers of charged GO are added. GO has a natural negative charge and can act as a negatively charged component in LbL without further functionalization, and the positively charged GO can be obtained by amine functionalization [9][45][46][47][48]. As a result, there is obtained a thin and smooth bilayer of GO that can be incorporated between polyesters or polymeric thin layers. GO can interact with other molecules via hydrophobic or electrostatic interactions, hydrogen bonding, or π-π stacking. GO can be non-covalently functionalized using LbL to improve dispersibility and avoid

aggregation, using, for example, chitosan (Chi)/dextran, Chi/sodium alginate (SA), or protamine sulfate/SA [6][32]. Small molecules, non-polymeric in nature, are more difficult to incorporate into LbL assemblies, because they can penetrate the graphene multilayers, and that can affect the growth of the multilayered system [6].

To start a multilayered film, it is necessary to charge the substrate to enhance the adsorption of the first layer. The most common assembly process is to start with a negatively charged substrate that is usually obtained by piranha solution (composed of sulfuric acid (H_2SO_4) and hydrogen peroxide (H_2O_2) in the proportion of 7:3, v/v) and oxygen plasma treatment [49] and, therefore, a negatively charged layer. However, it is also possible to invert the order, functionalizing the substrate with positive charges by dipping the substrate in APTES solution ((3-aminopropyl) triethoxysilane) (3 min). Furthermore, substrates should be previously cleaned to remove organic contaminants with piranha solution followed by a rinse with deionized (DI) water [45]. The cleanness and functionalization of substrate is important to ensure the homogeneity of the following layers. Usually, the films exhibit a linear growth; however, this can vary on the first few bilayers, due to interaction with the chosen substrate [9].

The pH of the dipping solutions can greatly affect the resulting film's thickness and roughness, as it can affect the charge balance between the oppositely charged components and the degree of ionization of weak polyelectrolytes. If the pH and pKa of the dipping solution are near each other, the film's thickness is increased [45][50]. The use of different or even cross-linked polymers in LbL films allows for the control of the porosity and rate of degradation and dissociation of the films [50].

3. Graphene Oxide as Carrier in Drug Delivery

Ideally, nanocarriers must have a size between 30 and 200 nm to be retained in blood vessels. Above this size, they are prone to aggregation in the liver or the spleen, and, below that, they can suffer blood renal filtration, being filtered from the plasma to the urine [8]. When encapsulating or bonding a drug to its respective carrier, it is also important to consider the ratio of drug:carrier, as this can affect encapsulation/bonding efficiency [51].

In recent years GO has gained a lot of attention for its potential use in biomedical applications, with several publications reporting its benefits as a drug in cancer therapy [52][53][54][55][56][57][58][59][60][61]. For example, Liu et al. [51] investigated a possible nanocarrier for oral drug administration in cancer treatment, developing a thin film with graphene as nanocarrier and pingyangmycin (PYM) as the anti-cancer drug of choice. Orally administered drugs have reduced side effects when compared with IV administration and are more easily accepted by the patients; however, they have some drawbacks, such as being easily degraded by the gastric acid and pepsin present in the stomach, low bioavailability, rapid clearance, and poor tissue distribution. The developed film was composed of poly(acrylic acid)-cysteine (PAA-cys), poly(allylamine hydrochloride) (PAH), and GO as follows: PAA-cys-PAH-GO-PYM. PAA-cys helps the DDS to adhere to the small intestine mucus layer, improving the drug bioavailability, and PAA-cys and PAH polyelectrolytes are cross-linked in the surface of the GO-PYM conjugate and help protect the nanocarrier and drug from gastric acids, allowing the safe passage of the DDS to the intestine, where it can release

the drug. Drug release was higher at lower pH, further demonstrating the potential of this DDS in improving targeting to the tumor tissues (acidic environment) and reducing effects in normal tissue.

Another study reports the use of GO NPs as nanocarriers to enhance the drug bioavailability and water solubility. GO is subjected to the “piercing effect” and, therefore, is easily internalized by cells, and its sheet-like structure helps to stabilize the capsule layers, avoiding any drug escape before it reaches its target, making it an ideal choice for this kind of application. Here, they coated GO NPs with a single layer of carboxymethylcellulose (CMC) (to increase drug loading capabilities), with curcumin (Cur), a powerful anti-cancer “drug”, encapsulated into CMC. CMC was then cross-linked with poly N-vinylpyrrolidone (PVP) to produce stimuli-responsive NPs (redox-responsive disulfide linkage). The high glutathione concentrations inside the tumor induce breakage on the bridge between CMC and PVP and, therefore, cause the curcumin to be released. Further NP functionalization was obtained by conjugation of PEGylated monoclonal folic acid antibody (FA) onto the NP surface. This is an important step, as FA binds to folate receptors that are highly expressed in cancer cells, thus helping to direct the NPs to these cells and allowing them to be internalized by phagocytosis. PEGylation helps to reduce protein adsorption onto nanocarriers (preventing “protein corona” and, therefore, renal clearance), prolongs NPs blood circulation, and enhances binding efficiency of FA to the receptors. It also helps to prevent the drug from leaking early and protects it against degradation or enzymatic cleavage. The final composition of the NPs was Cur-FA-CMC/PVP GO NPs. Another important piece of information to retain is that highly charged NPs suffer less aggregation due to repulsive electrostatic forces, allowing for more stable dispersion in physiological conditions [8][62].

Associated with cancer treatment, several stimuli-responsive films which can be used in photothermal therapy are also being studied [62][63][64][65][66]. Photothermal therapy relies on the capacity of NPs to convert NIR (near-infrared radiation) to vibrational energy, generating heat and thus killing cancer cells (where the NPs accumulate). The high efficiency of NIR is mainly because it can reach the NPs without damaging the tissues in between, since biological tissues do not possess chromophores absorbing in the NIR region. GO is a great nanomaterial to use in these kinds of films, as it can generate heat sufficient to kill cancer cells when exposed to NIR, being even more effective than CNT [67]. Therefore, by using GO, it is possible to obtain a NIR-responsive capsule/film with drug loading capabilities and in which GO not only serves as a structural component but also as a NIR responsive material, avoiding the use of gold and silver NPs or addition of NIR-dyes or CNT (which were used until now to fabricate NIR-responsive capsules). This allows for a much simpler and cost-effective process [10].

A study led by Xie et al. [32] successfully developed a magnetic DDS based on GO sheets, with both magnetic and photothermal response. This kind of particle can be used in targeted therapy and has been greatly studied in cancer applications. Xie et al. [32] developed a graphene oxide-based nanocomposite loaded with doxorubicin (DOX—an antitumor antibiotic). GO has a large DOX loading capacity, making it the ideal nanocarrier. To magnetize the GO sheets, researchers used the thermal reaction method to deposit Fe_3O_4 onto the GO sheets. However, mGO (magnetic GO) is even more prone to aggregation than GO, and, therefore, it requires further functionalization. For that purpose, mGO sheets were coated with Chi and SA through the LbL technique. The final composite composition was mGO-Chi/SA-DOX. A ratio of mGO sheets:Chi:SA of 1:4:4 was needed in order to achieve a stable dispersion. DOX was loaded via π-π stacking and electrostatic attraction. They successfully

developed a nanocarrier that not only could be directed to the target cells with magnetism, as it had strong photothermal response, causing the cancer cells to die upon NIR irradiation, but had a pH-dependent release of DOX, ideal to use with cancer cells that have a pH lower than normal cells.

Another example is GO-iron oxide (IO) PEGylated nanocomposites (GO-IONP-PEG) that can be used as a drug carrier triggered by a magnetic field, being used in photothermal therapy. GO functionalization with PEG greatly increases its stability in physiological solutions. In these cases, GO-IONP-PEG particles can be loaded, for example, with DOX by π-π stacking and then be used in cancer treatment. However, this composite can also be used as a t2-weighted magnetic resonance contrast in tumors, as demonstrated by Ma et al. [68].

Another potential application for GO-based compounds is as a carrier and/or enhancer for antibacterial or antimicrobial agents [6][56][69][70][71].

As an example, the work carried out by Cao et al. [6], in which they developed a system for sustained release of an antibacterial peptide ($G(IIKK)_4I\text{-}NH_2$) using GO as a nanocarrier and assembling it into a thin film that can be used, for example, as coating for surfaces or devices. $G(IIKK)_4I\text{-}NH_2$ cannot be efficiently loaded into LbL by itself due to its low charge number, hence the need for a GO. The films were composed of $G(IIKK)_4I\text{-}NH_2$ (positively charged), poly(acrylic acid) (PAA, Polyanion), and poly(β-amino ester) (PBAE, polycation), and it was possible to confirm that $G(IIKK)_4I\text{-}NH_2$ retained its antibacterial properties even when incorporated in the films. The release speed can be tuned by varying the number of layers of PBAE.

GO's ability to reach the brain makes it also a good candidate for neurological applications [15][72][73], as reported, for example, by Xiong et al. [72], who developed a DDS to treat Parkinson's disease (PD) using lactoferrin (Lf)-functionalized GO as a nanocarrier for the natural drug puerarin (Pue), which presents anti-PD properties (Lf-GO-Pue). Lf was used for its ability to bind to the vascular endothelial receptor in the BBB and, therefore, promote the transport of this DDS across this barrier through a receptor-mediated transport. Their work showed promising results, with the in vivo test showing that, in PD-induced mice, there were significantly less neuronal damage and compartmental deficits.

Furthermore, it is possible to obtain hollow capsules of rGO that can be used to incorporate particles or bioactive molecules. These capsules were obtained by the sequential adsorption (due to electrostatic interactions) of positively charged ($rGO\text{-}NH_3^+$) and negatively charged ($rGO\text{-}COO^-$)) onto a sacrificial PS (polystyrene) substrate, which can then be removed by tetrahydrofuran (THF) solvent exposure [3].

Besides the already mentioned examples, there are several others that report using GO as a nanocarrier, for example, for proteins, protecting them against proteolysis and helping retain their activity [74]; as a carrier for pirfenidone (a drug used to treat subarachnoid hemorrhage) [75]; as a carrier for phytomedicines, augmenting their biocompatibility and reducing their toxicity [76]; as a carrier for famotidine (an anti-ulcerous medicine), reducing its side effects through a controlled and sustained release [77]; as a carrier for quercetin (a bioactive flavonoid with powerful antioxidant characteristics), helping circumvent its low bioavailability, extensive first passage metabolism,

and instability in aqueous intestinal fluids [78]; as a carrier for transdermal delivery (hydrogel) of ondansetron, a medicine to help control vertigo that has low bioavailability and short half-life [79]; as a carrier for growth factors to enhance cell differentiation [80]; and as a carrier for pain management medicines such as flurbiprofen or buprenorphine, helping reduce its side effects and promoting a sustained release without constant need for dosing [81][82].

4. Graphene Oxide for Controlled Drug Release

In a DDS, it is of the utmost importance to control the release of the bioactive compounds. Recently, graphene-based materials, such as graphene oxide, have been gaining a lot of attention for their ability to act as a barrier or capping layer, delaying and controlling the release of biomolecules across time [20][83][84][85][86][87][88].

There are several studies using ovalbumin (OVA—the main protein found in egg white, largely used as a nutrient supplement) as model drug; some researchers were able to use GO either as a nanocarrier or a capping layer to build thin films, preventing the early release of the biomacromolecule and obtaining, therefore, a long-term delivery that lasted over 70 days [34]. This mechanism was also applied in films containing OVA as the model protein/drug and PBAE as a hydrolytically degradable polymer. They were able to delay the release of OVA from less than an hour to several weeks by using GO as a capping layer in films with $(PBAE/OVA)_{20}(GO^{+}/GO^{-})_{n}$, or as a barrier layer in films with $(PBAE/OVA\text{-}AF555)_{20}(GO^{+}/GO^{-})_{5}(PBAE/OVA\text{-}TR)_{20}(GO^{+}/GO^{-})_{2}(PBAE/OVA\text{-}FL)_{20}$ (where AF555—Alexa fluor; FL—fluorescein; and TR—Texas red were used as labels to differentiate between OVAs). When GO was used as a barrier layer, a sequential and controlled release of OVA was possible, with the possibility to control the release gaps by varying the number of GO bilayers. Furthermore, GO's low permeability helps to reduce interlayer diffusion. This opens up a world of possibilities for DDS with multiple drugs which can be release in a sequential and ordered manner [83].

In an electro-responsive film, GO was also used as a barrier layer. OVA was used as model drug, and rGO was also incorporated in the films as an electroconductive material (because GO has poor conductivity). $(PBAE/rGO^{-}/GO^{+}/OVA/GO^{+}/rGO^{-})_{n}$ (n stands for the number of repeated layers) showed negligible OVA release when there were no stimuli present and 50 times more OVA release upon electric stimulation, further confirming GO barrier capabilities [89].

rGO can also be used as a barrier layer in order to prevent spontaneous release of cDNA, as demonstrated by Jeong et al. [20], who developed a method to activate DNA nanodevices based on electro-responsive films containing rGO with the following composition: $(PBAE/cDNA/rGO^{+}/cDNA/rGO^{+}/PEDOT{:}PSS)_{n}/(rGO^{+}/rGO^{-})_{n}$, where PEDOT:PSS stands for poly(3,4-ethylenedioxythiophene) polystyrene sulfonate.

GO is also able to prevent the release of drugs in multilayered systems. A recent work related with LbL films composed of brimonidine (Brim), an alpha-2 adrenergic agonist used normally to lower intraocular pressure, encapsulated in polycyclodextrins (PolyCD+Brim) intercalated with layers of PBAE and bilayers of charged GO showed that GO can delay the drug release for several days. The study compares films with and without GO (the

film $(PBAE/PolyCD+Brim)_6/GO^+/GO^-/(PBAE/PolyCD+Brim)_4GO^+/GO^-/(PBAE/PolyCD+Brim)_4$ and the film $(PBAE/PolyCD+Brim)_4$), and it was observed that the GO has an important role in the management of drug behavior. The number of GO bilayers is proportional to the delay time, suggesting that this system is a good model to fine-tune a DDS able to deliver a precise drug concentration at a specific period of time [33].

GO can also be used to coat a siRNA-loaded porous silicon (Si) NP in order to delay siRNA release, being able not only to slow down the release time but also the enzymatic degradation of the siRNA and the dissolution of the porous Si NP [90].

References

1. Huang, X.; Qi, X.; Boey, F.; Zhang, H. Graphene-Based Composites. Chem. Soc. Rev. 2012, 41, 666–686.
2. Si, Y.; Samulski, E.T. Synthesis of Water Soluble Graphene. Nano Lett. 2008, 8, 1679–1682.
3. Hong, J.; Char, K.; Kim, B.-S. Hollow Capsules of Reduced Graphene Oxide Nanosheets Assembled on a Sacrificial Colloidal Particle. J. Phys. Chem. Lett. 2010, 1, 3442–3445.
4. Lian, Y.; Liu, Y.; Jiang, T.; Shu, J.; Lian, H.; Cao, M. Enhanced Electromechanical Performance of Graphite Oxide-Nafion Nanocomposite Actuator. J. Phys. Chem. C 2010, 114, 9659–9663.
5. Hong, W.; Xu, Y.; Lu, G.; Li, C.; Shi, G. Transparent Graphene/PEDOT–PSS Composite Films as Counter Electrodes of Dye-sensitized Solar Cells. Electrochem. Commun. 2008, 10, 1555–1558.
6. Cao, M.; Zhao, W.; Wang, L.; Li, R.; Gong, H.; Zhang, Y.; Xu, H.; Lu, J.R. Graphene Oxide-Assisted Accumulation and Layer-by-Layer Assembly of Antibacterial Peptide for Sustained Release Applications. ACS Appl Mater. Interfaces 2018, 10, 24937–24946.
7. Gao, F.; Hu, Y.; Li, G.; Liu, S.; Quan, L.; Yang, Z.; Wei, Y.; Pan, C. Layer-by-layer Deposition of Bioactive Layers on Magnesium Alloy Stent Materials to Improve Corrosion Resistance and Biocompatibility. Bioact. Mater. 2020, 5, 611–623.
8. Sahne, F.; Mohammadi, M.; Najafpour, G.D. Single-Layer Assembly of Multifunctional Carboxymethylcellulose on Graphene Oxide Nanoparticles for Improving In Vivo Curcumin Delivery into Tumor Cells. ACS Biomater. Sci. Eng. 2019, 5, 2595–2609.
9. Assunção, I.C.C.; Sério, S.; Ferreira, Q.; Jones, N.C.; Hoffmann, S.V.; Ribeiro, P.A.; Raposo, M. Graphene Oxide Layer-by-Layer Films for Sensors and Devices. Nanomaterials 2021, 11, 1556.
10. Kurapati, R.; Raichur, A.M. Near-Infrared Light-Responsive Graphene Oxide Composite Multilayer Capsules: A Novel Route for Remote Controlled Drug Delivery. Chem. Commun. 2013, 49, 734–736.

11. Shin, S.R.; Aghaei-Ghareh-Bolagh, B.; Gao, X.; Nikkhah, M.; Jung, S.M.; Dolatshahi-Pirouz, A.; Kim, S.B.; Kim, S.M.; Dokmeci, M.R.; Tang, X.S.; et al. Layer-by-Layer Assembly of 3D Tissue Constructs with Functionalized Graphene. Adv. Funct. Mater. 2014, 24, 6136–6144.
12. Hong, B.J.; Compton, O.C.; An, Z.; Eryazici, I.; Nguyen, S.T. Successful Stabilization of Graphene Oxide in Electrolyte Solutions: Enhancement of Biofunctionalization and Cellular Uptake. ACS Nano 2012, 6, 63–73.
13. Xu, Y.; Bai, H.; Lu, G.; Li, C.; Shi, G. Flexible Graphene Films via the Filtration of Water-Soluble Noncovalent Functionalized Graphene Sheets. J. Am. Chem. Soc. 2008, 130, 5856–5857.
14. Demirel, E.; Karaca, E.; Yuksel Durmaz, Y. Effective PEGylation Method to Improve Biocompatibility of Graphene Derivatives. Eur. Polym. J. 2020, 124, 109504.
15. Li, M.; Yang, X.; Ren, J.; Qu, K.; Qu, X. Using Graphene Oxide High Near-Infrared Absorbance for Photothermal Treatment of Alzheimer's Disease. Adv. Mater. 2012, 24, 1722–1728.
16. Su, S.; Wang, J.; Qiu, J.; Martinez-Zaguilan, R.; Sennoune, S.R.; Wang, S. In Vitro Study of Transportation of Porphyrin Immobilized Graphene Oxide Through Blood Brain Barrier. Mater. Sci. Eng. C Mater. Biol. Appl. 2020, 107, 110313.
17. Mendonça, M.C.; Soares, E.S.; de Jesus, M.B.; Ceragioli, H.J.; Ferreira, M.S.; Catharino, R.R.; da Cruz-Höfling, M.A. Reduced Graphene Oxide Induces Transient Blood-Brain Barrier Opening: An In Vivo Study. J. Nanobiotechnol. 2015, 13, 78.
18. Costa, R.R.; Alatorre-Meda, M.; Mano, J.F. Drug Nano-Reservoirs Synthesized using Layer-by-Layer Technologies. Biotechnol. Adv. 2015, 33, 1310–1326.
19. Li, X.; Zhang, G.; Bai, X.; Sun, X.; Wang, X.; Wang, E.; Dai, H. Highly Conducting Graphene Sheets and Langmuir–Blodgett Films. Nat. Nanotechnol. 2008, 3, 538–542.
20. Jeong, H.; Ranallo, S.; Rossetti, M.; Heo, J.; Shin, J.; Park, K.; Ricci, F.; Hong, J. Electronic Activation of a DNA Nanodevice using a Multilayer Nanofilm. Small 2016, 12, 5572–5578.
21. Hernandez, Y.; Nicolosi, V.; Lotya, M.; Blighe, F.M.; Sun, Z.; De, S.; McGovern, I.T.; Holland, B.; Byrne, M.; Gun'Ko, Y.K.; et al. High-Yield Production of Graphene by Liquid-Phase Exfoliation of Graphite. Nat. Nanotechnol. 2008, 3, 563–568.
22. Zhang, X.; Liang, T.; Ma, Q. Layer-by-Layer Assembled Nano-Drug Delivery Systems for Cancer Treatment. Drug Deliv. 2021, 28, 655–669.
23. Yang, W.J.; Pranantyo, D.; Neoh, K.-G.; Kang, E.-T.; Teo, S.L.-M.; Rittschof, D. Layer-by-Layer Click Deposition of Functional Polymer Coatings for Combating Marine Biofouling. Biomacromolecules 2012, 13, 2769–2780.
24. Sieber, S.; Siegrist, S.; Schwarz, S.; Porta, F.; Schenk, S.H.; Huwyler, J. Immobilization of Enzymes on PLGA Sub-Micrometer Particles by Crosslinked Layer-by-Layer Deposition.

Macromol. Biosci. 2017, 17, 1700015.

25. Smith, R.; Riollano, M.; Leung, A.; Hammond, P. Layer-by-Layer Platform Technology for Small-Molecule Delivery. Angew. Chem. Int. Ed. 2009, 48, 8974–8977.
26. Shutava, T.G.; Livanovich, K.S.; Sharamet, A.A. Layer-by-Layer Films of Polysaccharides Modified with Polyethylene Glycol and Dextran. Colloids Surf. B Biointerfaces 2019, 173, 412–420.
27. Silva, D.P.B.; Miyazaki, C.M.; Mascagni, D.B.T.; Ferreira, M. Layer-by-Layer Films of Gold Nanoparticles and Carbon Nanotubes for Improved Amperometric Detection of Cholesterol. J. Nanosci. Nanotechnol. 2019, 19, 5483–5488.
28. Blacklock, J.; Mao, G.; Oupický, D.; Möhwald, H. DNA Release Dynamics from Bioreducible Layer-by-Layer Films. Langmuir 2010, 26, 8597–8605.
29. Hong, C.A.; Son, H.Y.; Nam, Y.S. Layer-by-Layer SiRNA/Poly(L-Lysine) Multilayers on Polydopamine-Coated Surface for Efficient Cell Adhesion and Gene Silencing. Sci. Rep. 2018, 8, 7738.
30. Srivastava, S.; Kotov, N.A. Composite Layer-by-Layer (LBL) Assembly with Inorganic Nanoparticles and Nanowires. Acc. Chem. Res. 2008, 41, 1831–1841.
31. Smith, R.C.; Leung, A.; Kim, B.-S.; Hammond, P.T. Hydrophobic Effects in the Critical Destabilization and Release Dynamics of Degradable Multilayer Films. Chem. Mater. 2009, 21, 1108–1115.
32. Xie, M.; Zhang, F.; Peng, H.; Zhang, Y.; Li, Y.; Xu, Y.; Xie, J. Layer-by-Layer Modification of Magnetic Graphene Oxide by Chitosan and Sodium Alginate with Enhanced Dispersibility for Targeted Drug Delivery and Photothermal Therapy. Colloids Surf. B Biointerfaces 2019, 176, 462–470.
33. Machado, M.; Silva, G.A.; Bitoque, D.B.; Ferreira, J.; Pinto, L.A.; Morgado, J.; Ferreira, Q. Self-Assembled Multilayer Films for Time-Controlled Ocular Drug Delivery. ACS Appl. Bio Mater. 2019, 2, 4173–4180.
34. Tanum, J.; Heo, J.; Hong, J. Spontaneous Biomacromolecule Absorption and Long-Term Release by Graphene Oxide. ACS Omega 2018, 3, 5903–5909.
35. Wang, F.; Li, J.; Tang, X.; Huang, K.; Chen, L. Polyelectrolyte Three Layer Nanoparticles of Chitosan/Dextran Sulfate/Chitosan for Dual Drug Delivery. Colloids Surf. B Biointerfaces 2020, 190, 110925.
36. Guo, H.; Guo, Q.; Chu, T.; Zhang, X.; Wu, Z.; Yu, D. Glucose-Sensitive Polyelectrolyte Nanocapsules Based on Layer-by-Layer Technique for Protein Drug Delivery. J. Mater. Sci. Mater. Med. 2014, 25, 121–129.

37. Yang, M.-C.; Tsou, H.-M.; Hsiao, Y.-S.; Cheng, Y.-W.; Liu, C.-C.; Huang, L.-Y.; Peng, X.-Y.; Liu, T.-Y.; Yung, M.-C.; Hsu, C.-C. Electrochemical Polymerization of PEDOT–Graphene Oxide–Heparin Composite Coating for Anti-Fouling and Anti-Clotting of Cardiovascular Stents. Polymers 2019, 11, 1520.
38. Qi, W.; Xue, Z.; Yuan, W.; Wang, H. Layer-by-Layer Assembled Graphene Oxide Composite Films for Enhanced Mechanical Properties and Fibroblast Cell Affinity. J. Mater. Chem. B 2014, 2, 325–331.
39. Cao, W.; He, L.; Cao, W.; Huang, X.; Jia, K.; Dai, J. Recent Progress of Graphene Oxide as a Potential Vaccine Carrier and Adjuvant. Acta Biomater. 2020, 112, 14–28.
40. Mangadlao, J.D.; Santos, C.M.; Felipe, M.J.L.; de Leon, A.C.C.; Rodrigues, D.F.; Advincula, R.C. On the Antibacterial Mechanism of Graphene Oxide (GO) Langmuir–Blodgett Films. Chem. Commun. 2015, 51, 2886–2889.
41. Sharker, S.M.; Lee, J.E.; Kim, S.H.; Jeong, J.H.; In, I.; Lee, H.; Park, S.Y. PH Triggered in Vivo Photothermal Therapy and Fluorescence Nanoplatform of Cancer Based on Responsive Polymer-Indocyanine Green Integrated Reduced Graphene Oxide. Biomaterials 2015, 61, 229–238.
42. Gollavelli, G.; Ling, Y.-C. Multi-Functional Graphene as an In Vitro and In Vivo Imaging Probe. Biomaterials 2012, 33, 2532–2545.
43. Sheng, Z.; Song, L.; Zheng, J.; Hu, D.; He, M.; Zheng, M.; Gao, G.; Gong, P.; Zhang, P.; Ma, Y.; et al. Protein-Assisted Fabrication of Nano-Reduced Graphene Oxide for Combined In Vivo Photoacoustic Imaging and Photothermal Therapy. Biomaterials 2013, 34, 5236–5243.
44. Shi, S.; Yang, K.; Hong, H.; Valdovinos, H.F.; Nayak, T.R.; Zhang, Y.; Theuer, C.P.; Barnhart, T.E.; Liu, Z.; Cai, W. Tumor Vasculature Targeting and Imaging in Living Mice with Reduced Graphene Oxide. Biomaterials 2013, 34, 3002–3009.
45. Ahn, E.; Lee, T.; Gu, M.; Park, M.; Min, S.H.; Kim, B.-S. Layer-by-Layer Assembly for Graphene-Based Multilayer Nanocomposites: The Field Manual. Chem. Mater. 2017, 29, 69–79.
46. Araújo, M. Nanostructured Films for Controlled Release of Drugs for Glaucoma Treatment. Master’s Thesis, Universidade de Lisboa, Lisbon, Portugal, 2016.
47. Morais, H. Nanostructured Films of Graphene for Controlled Ocular Drug Delivery. Master’s Thesis, Instituto Politécnico de Lisboa, Lisbon, Portugal, 2017.
48. Pereira, T. Development of a Liquid Cell to Study the Release of Brimonidine in Real Time. Master’s Thesis, Instituto Pólitécnico de Lisboa, Lisbon, Portugal, 2019.
49. Raposo, M.; Oliveira, O. Adsorption Mechanisms in Layer-by-Layer Films. Braz. J. Phys. 1998, 28.

50. Han, U.; Seo, Y.; Hong, J. Effect of pH on the Structure and Drug Release Profiles of Layer-by-Layer Assembled Films Containing Polyelectrolyte, Micelles, and Graphene Oxide. Sci. Rep. 2016, 6, 24158.
51. Liu, Y.; Han, J.; Pan, H.; Jia, D.; Chen, L.; Yang, X. Oral Delivery of Pingyangmycin by Layer-by-Layer (LbL) Self-Assembly Polyelectrolyte-Grafted Nano Graphene Oxide. J. Nanosci. Nanotechnol. 2019, 19, 2260–2268.
52. Kavinkumar, T.; Varunkumar, K.; Ravikumar, V.; Manivannan, S. Anticancer Activity of Graphene Oxide-Reduced Graphene Oxide-Silver Nanoparticle Composites. J. Colloid Interface Sci. 2017, 505, 1125–1133.
53. Angelopoulou, A.; Voulgari, E.; Diamanti, E.K.; Gournis, D.; Avgoustakis, K. Graphene Oxide Stabilized by PLA-PEG Copolymers for the Controlled Delivery of Paclitaxel. Eur. J. Pharm. Biopharm. 2015, 93, 18–26.
54. Wang, L.H.; Liu, J.Y.; Sui, L.; Zhao, P.H.; Ma, H.D.; Wei, Z.; Wang, Y.L. Folate-Modified Graphene Oxide as the Drug Delivery System to Load Temozolomide. Curr. Pharm. Biotechnol. 2020, 21, 1088–1098.
55. Barahuie, F.; Saifullah, B.; Dorniani, D.; Fakurazi, S.; Karthivashan, G.; Hussein, M.Z.; Elfghi, F.M. Graphene Oxide as a Nanocarrier for Controlled Release and Targeted Delivery of an Anticancer Active Agent, Chlorogenic Acid. Mater. Sci. Eng. C Mater. Biol. Appl. 2017, 74, 177–185.
56. Hussein-Al-Ali, S.H.; Abudoleh, S.M.; Hussein, M.Z.; Bullo, S.; Palanisamy, A. Graphene Oxide-Ellagic Acid Nanocomposite as Effective Anticancer and Antimicrobial Agent. IET Nanobiotechnol. 2021, 15, 79–89.
57. Nasrollahi, F.; Varshosaz, J.; Khodadadi, A.A.; Lim, S.; Jahanian-Najafabadi, A. Targeted Delivery of Docetaxel by Use of Transferrin/Poly(allylamine hydrochloride)-Functionalized Graphene Oxide Nanocarrier. ACS Appl. Mater. Interfaces 2016, 8, 13282–13293.
58. Gong, P.; Zhao, Q.; Dai, D.; Zhang, S.; Tian, Z.; Sun, L.; Ren, J.; Liu, Z. Functionalized Ultrasmall Fluorinated Graphene with High NIR Absorbance for Controlled Delivery of Mixed Anticancer Drugs. Chem.–A Eur. J. 2017, 23, 17531–17541.
59. Wang, X.; Hao, L.; Zhang, C.; Chen, J.; Zhang, P. High Efficient Anti-Cancer Drug Delivery Systems using Tea Polyphenols Reduced and Functionalized Graphene Oxide. J. Biomater. Appl. 2017, 31, 1108–1122.
60. Zhang, B.; Yang, X.; Wang, Y.; Zhai, G. Heparin Modified Graphene Oxide for pH-Sensitive Sustained Release of Doxorubicin Hydrochloride. Mater. Sci. Eng. C 2017, 75, 198–206.
61. Farnaz, R.; Maryam, S.; Masoumeh, J.; Parvaneh, S. Colloidal HSA-Graphene Oxide Nanosheets for Sustained Release of Oxaliplatin: Preparation, Release Mechanism, Cytotoxicity and Electrochemical Approaches. Colloids Surf. B Biointerfaces 2018, 171, 10–16.

62. Deng, L.; Li, Q.; Al-Rehili, S.; Omar, H.; Almalik, A.; Alshamsan, A.; Zhang, J.; Khashab, N.M. Hybrid Iron Oxide-Graphene Oxide-Polysaccharides Microcapsule: A Micro-Matryoshka for On-Demand Drug Release and Antitumor Therapy In Vivo. ACS Appl. Mater. Interfaces 2016, 8, 6859–6868.
63. Han, L.; Hao, Y.-N.; Wei, X.; Chen, X.-W.; Shu, Y.; Wang, J.-H. Hollow Copper Sulfide Nanosphere–Doxorubicin/Graphene Oxide Core–Shell Nanocomposite for Photothermo-Chemotherapy. ACS Biomater. Sci. Eng. 2017, 3, 3230–3235.
64. Patil, T.V.; Patel, D.K.; Dutta, S.D.; Ganguly, K.; Lim, K.-T. Graphene Oxide-Based Stimuli-Responsive Platforms for Biomedical Applications. Molecules 2021, 26, 2797.
65. Lima-Sousa, R.; de Melo-Diogo, D.; Alves, C.G.; Cabral, C.S.D.; Miguel, S.P.; Mendonça, A.G.; Correia, I.J. Injectable In Situ Forming Thermo-Responsive Graphene Based Hydrogels for Cancer Chemo-Photothermal Therapy and NIR Light-Enhanced Antibacterial Applications. Mater. Sci. Eng. C Mater. Biol. Appl. 2020, 117, 111294.
66. Dembereldorj, U.; Choi, S.Y.; Ganbold, E.O.; Song, N.W.; Kim, D.; Choo, J.; Lee, S.Y.; Kim, S.; Joo, S.W. Gold Nanorod-Assembled PEGylated Graphene-Oxide Nanocomposites for Photothermal Cancer Therapy. Photochem. Photobiol. 2014, 90, 659–666.
67. Markovic, Z.M.; Harhaji-Trajkovic, L.M.; Todorovic-Markovic, B.M.; Kepić, D.P.; Arsikin, K.M.; Jovanović, S.P.; Pantovic, A.C.; Dramićanin, M.D.; Trajkovic, V.S. In Vitro Comparison of the Photothermal Anticancer Activity of Graphene Nanoparticles and Carbon Nanotubes. Biomaterials 2011, 32, 1121–1129.
68. Ma, X.; Tao, H.; Yang, K.; Feng, L.; Cheng, L.; Shi, X.; Li, Y.; Guo, L.; Liu, Z. A Functionalized Graphene Oxide-Iron Oxide Nanocomposite for Magnetically Targeted Drug Delivery, Photothermal Therapy, and Magnetic Resonance Imaging. Nano Res. 2012, 5, 199–212.
69. Pan, N.; Wang, Y.; Ren, X.; Huang, T.-S.; Kim, I.S. Graphene Oxide as a Polymeric N-halamine Carrier and Release Platform: Highly-Efficient, Sustained-Release Antibacterial Property and Great Storage Stability. Mater. Sci. Eng. C 2019, 103, 109877.
70. Kumar, G.; Chaudhary, K.; Mogha, N.K.; Kant, A.; Masram, D.T. Extended Release of Metronidazole Drug using Chitosan/Graphene Oxide Bionanocomposite Beads as the Drug Carrier. ACS Omega 2021, 6, 20433–20444.
71. Tran, L.; Phuong, L.; Hoang Thi, T.T.; Park, K. Graphene Oxide Immobilized Surfaces Facilitate the Sustained Release of Doxycycline for the Prevention of Implant Related Infection. Colloids Surf. B Biointerfaces 2019, 181.
72. Xiong, S.; Luo, J.; Wang, Q.; Li, Z.; Li, J.; Liu, Q.; Gao, L.; Fang, S.; Li, Y.; Pan, H.; et al. Targeted Graphene Oxide for Drug Delivery as a Therapeutic Nanoplatform Against Parkinson’s Disease. Biomater. Sci. 2021, 9, 1705–1715.

73. Bramini, M.; Alberini, G.; Colombo, E.; Chiacchiaretta, M.; DiFrancesco, M.L.; Maya-Vetencourt, J.F.; Maragliano, L.; Benfenati, F.; Cesca, F. Interfacing Graphene-Based Materials with Neural Cells. Front. Syst. Neurosci. 2018, 12.
74. Emadi, F.; Amini, A.; Gholami, A.; Ghasemi, Y. Functionalized Graphene Oxide with Chitosan for Protein Nanocarriers to Protect against Enzymatic Cleavage and Retain Collagenase Activity. Sci Rep. 2017, 7, 42258.
75. Yang, L.; Wang, F.; Han, H.; Yang, L.; Zhang, G.; Fan, Z. Functionalized Graphene Oxide as a Drug Carrier for Loading Pirfenidone in Treatment of Subarachnoid Hemorrhage. Colloids Surf. B Biointerfaces 2015, 129, 21–29.
76. Figueroa, T.; Aguayo, C.; Fernández, K. Design and Characterization of Chitosan-Graphene Oxide Nanocomposites for the Delivery of Proanthocyanidins. Int. J. Nanomed. 2020, 15, 1229–1238.
77. Mahajan, C.R.; Joshi, L.B.; Varma, U.; Naik, J.B.; Chaudhari, V.R.; Mishra, S. Sustainable Drug Delivery of Famotidine using Chitosan-Functionalized Graphene Oxide as Nanocarrier. Glob. Chall. 2019, 3, 1900002.
78. Rahmanian, N.; Hamishehkar, H.; Dolatabadi, J.E.; Arsalani, N. Nano Graphene Oxide: A Novel Carrier for Oral Delivery of Flavonoids. Colloids Surf. B Biointerfaces 2014, 123, 331–338.
79. Li, H.; Jia, Y.; Liu, C. Pluronic® F127 Stabilized Reduced Graphene Oxide Hydrogel for Transdermal Delivery of Ondansetron: Ex Vivo and Animal Studies. Colloids Surf. B Biointerfaces 2020, 195, 111259.
80. Zhou, M.; Lozano, N.; Wychowaniec, J.K.; Hodgkinson, T.; Richardson, S.M.; Kostarelos, K.; Hoyland, J.A. Graphene Oxide: A growth Factor Delivery Carrier to Enhance Chondrogenic Differentiation of Human Mesenchymal Stem Cells in 3D Hydrogels. Acta Biomater 2019, 96, 271–280.
81. Yang, C.; Li, T. Transdermal Delivery of Flurbiprofen from Polyoxypropylene-Polyoxyethylene Block Copolymer Stabilized Reduced Graphene Oxide to Manage Pain in Spondylitis: In Vitro and In Vivo Studies. Eur. J. Pharm. Sci. Off. J. Eur. Fed. Pharm. Sci. 2021, 165, 105929.
82. Zhang, Z.; Wang, X.; Li, P.; Bai, M.; Qi, W. Transdermal Delivery of Buprenorphine from Reduced Graphene Oxide Laden Hydrogel to Treat Osteoarthritis. J. Biomater. Sci. Polym. Ed. 2021, 32, 874–885.
83. Hong, J.; Shah, N.J.; Drake, A.C.; DeMuth, P.C.; Lee, J.B.; Chen, J.; Hammond, P.T. Graphene Multilayers as Gates for Multi-Week Sequential Release of Proteins from Surfaces. ACS Nano 2012, 6, 81–88.
84. Li, W.; Zhang, G.; Wei, X. Lidocaine-Loaded Reduced Graphene Oxide Hydrogel for Prolongation of Effects of Local Anesthesia: In Vitro and In Vivo Analyses. J. Biomater. Appl. 2021, 35, 1034–

1042.

85. Luo, S.; Jin, S.; Yang, T.; Wu, B.; Xu, C.; Luo, L.; Chen, Y. Sustained Release of Tulobuterol from Graphene Oxide Laden Hydrogel to Manage Asthma. J. Biomater. Sci. Polym. Ed. 2021, 32, 524–535.
86. Choi, M.; Chung, J.-H.; Cho, Y.; Hong, B.Y.; Hong, J. Nano-Film Modification of Collagen Hydrogels for Controlled Growth Factor Release. Chem. Eng. Sci. 2015, 137, 626–630.
87. Maulvi, F.A.; Soni, P.D.; Patel, P.J.; Desai, A.R.; Desai, D.T.; Shukla, M.R.; Shah, S.A.; Shah, D.O.; Willcox, M.D.P. Controlled Bimatoprost Release from Graphene Oxide Laden Contact Lenses: In Vitro and In Vivo Studies. Colloids Surf. B Biointerfaces 2021, 208, 112096.
88. Laurenti, M.; Lamberti, A.; Genchi, G.G.; Roppolo, I.; Canavese, G.; Vitale-Brovarone, C.; Ciofani, G.; Cauda, V. Graphene Oxide Finely Tunes the Bioactivity and Drug Delivery of Mesoporous ZnO Scaffolds. ACS Appl. Mater. Interfaces 2019, 11, 449–456.
89. Choi, M.; Kim, K.-G.; Heo, J.; Jeong, H.; Kim, S.Y.; Hong, J. Multilayered Graphene Nano-Film for Controlled Protein Delivery by Desired Electro-Stimuli. Sci. Rep. 2015, 5, 17631.
90. Joo, J.; Kwon, E.J.; Kang, J.; Skalak, M.; Anglin, E.J.; Mann, A.P.; Ruoslahti, E.; Bhatia, S.N.; Sailor, M.J. Porous Silicon–Graphene Oxide Core–Shell Nanoparticles for Targeted Delivery of siRNA to the Injured Brain. Nanoscale Horiz. 2016, 1, 407–414.