The main properties of graphene derivatives facilitating optical and electrical biosensing platforms are discussed, along with how the integration of graphene derivatives, plastic, and paper can lead to innovative devices in order to simplify biosensing technology and manufacture easy-to-use, yet powerful electrical or optical biosensors. Some crucial issues to be overcome in order to bring graphene-based biosensors to the market are also underscored.

1. Introduction

Technologies facilitating diagnostics, drug discovery, food safety, defense, security, and environmental monitoring are of great interest to industry, government initiatives, and research scientists. Biosensing has become an important approach within these technologies, particularly those biosensors integrating highly desirable characteristics such as specificity (suitably identifying the target analyte), high sensitivity (determining low levels or the lack of the analyte), fast results (from minutes to hours), extended shelf life, and easy-to-operate capabilities. However, when it comes to cost-efficient devices these features are often conflicting. Interestingly, nanomaterials enable us to engineer and manipulate unprecedented biosensing systems based on transducing phenomena occurring at the nanoscale. Thus, the outstanding features and new possibilities offered by nanomaterials are opening the way to exciting opportunities to overcome this challenge. In addition, we believe that flexible, abundant and low-cost substrates, that is, plastic and paper can boost such opportunities and their simplicity.

Given their advantageous properties and functionalities, graphene derivatives are two-dimensional nanoscale materials that are under active research, and the research community has devoted great effort and many resources to the development of graphene-based biosensors. In fact, a myriad of analytes including bacteria, viruses, proteins, oligonucleotides, small molecules, and metal ions have been successfully detected using graphene-based biosensors at the cutting edge.\(^1,2\) Due to the excellent properties of graphene derivatives – such as excellent capabilities for direct wiring with biomolecules, a heterogeneous chemical and electronic structure, the possibility to be processed in aqueous suspension, and the ability to be tuned as insulator, semiconductor, or semi-metal; graphene-based biosensors possess a plethora of advantages over other nanomaterial-based sensors, facilitating unique biosensing approaches, which will be discussed throughout this essay.

One should not be misled into thinking that graphene is a single material enjoying always the same properties and performance. On the contrary, graphene has a wide family of materials that offers a myriad of behaviors. In general, graphene derivatives possess high specific surface area (\(>470 \text{ m}^2 \text{ g}^{-1}\)) and broad chemical-modification capabilities. However, it should be remarked that their specific mechanical, electrical, and optical properties depend on different parameters such as their purity degree, structural defects, lateral size, number of layers, and oxidation level, which are strongly related to their production method. Hence, graphene derivatives exploited in biosensing are expected to be judiciously selected according to the targeted application and different questions may arise; for instance, what are the most common graphene derivatives in electrical/optical biosensing; and how do the aforementioned parameters affect biosensing behavior? We will critically address these questions and expose our perspective on the integration of graphene derivatives into plastic- and paper-based substrates (Figure 1).

2. Graphene Derivatives and their Properties facilitating Biosensing Platforms

Pristine graphene, polycrystalline graphene, graphene quantum dots (GQDs), and (reduced) graphene oxide are the most common graphene derivatives used in biosensing. Pristine graphene is a flawless lattice of sp\(^2\)-bonded carbon atoms, that is, without defects throughout its honeycomb-like pattern, and it is a single-crystalline grain.\(^6\) Polycrystalline graphene is composed of single-crystalline graphene grains of distinct orientations with a mosaic-like organization denoted by grain boundaries, which leads to topological defects such as dislocations.\(^7\) GQDs are nanocrystals of graphene that are tiny, sufficient to trigger exciton confinement and the quantum size effect (generally GQDs show diameters of less than 20 nm). As a result, GQDs have photoluminescent properties that can be tuned according to their size and surface chemistry. Graphene oxide (GO) is a lattice of sp\(^2\)-bonded carbon atoms incorporating defects since it is disrupted by sp\(^3\) carbon bonds.
and possesses oxygen-containing groups – carboxyl groups exposed on the edges and hydroxyl and epoxy groups on the basal plane. Hence, GO often has some hydrophilic islands on its basal plane according to its oxidation degree. Reduced graphene oxide (rGO) is composed of GO after being reduced through either a chemical or physical method. Depending on the predominance of the aforementioned hydrophilic islands, GO and rGO can be endowed with water-dispersibility properties. Therefore, GO and rGO micro-/nanosheets can be manipulated and applied in the form of a water-based suspension, which is often crucial in biosensing. Importantly, binding interactions between graphene derivatives and biomolecules such as single-stranded DNA are influenced by the C/O ratio of the employed graphene derivative – high C/O ratios promote stronger binding to biomolecules than low C/O ratios. Moreover, the lateral size of the graphene derivatives modulates the kinetics and the capacity of biomolecules adsorption.

Graphene derivatives incorporate inherent features, such as purity degree, defects, and functional groups according to their synthesis route, which are directly related to their performance and production cost. For instance, pristine graphene has remarkable properties based on its perfect crystalline structure. On the other hand, its production is hardly scalable and with high production cost. Hence, a balance between production cost and performance is of major importance for industrial applications and to implement graphene-based devices in daily-life technologies. This serious concern has been deeply discussed in the literature.

### 2.1. Electrical-Based Platforms

Heterogeneous electron transfer (HET) in graphene derivatives is correlated with the oxygen content, the amount of defects, the edge-like architecture, the thickness, and the impurities. Unlike pristine graphene or polycrystalline graphene, GO is highly disordered and contains different functional groups according to its production method. rGO, reduced either chemically, thermally, or electrochemically, can still have oxygen-containing functional groups and can suffer from a higher level of disorder or defect areas due to the reduction process. The increase of the number of defects in the structure increases HET, whereas the presence of oxygen functional groups decreases HET. Hence, effective reduction approaches are an important feature in graphene-based thin films. Laser-scribed reduction, for instance, is highly effective and, in addition, increases the number of edge-like structures available within the produced foam-like structure with high surface area. The reduction process not only can induce different structures, which can increase the surface area, but also the printing methods.

Vacuum-filtration-based thin films are characterized by their well-oriented GO-sheet thin films, whereas drop-casting or ink-jet printing induces a higher number of edge-like structures by the drop spontaneous drying process. On the other hand, in vacuum filtration, the printing of GO-based films over rough substrates tends to change the film structure; furthermore, printing rGO using the same technique results in an increase in the number of edge-like structures possibly due to the rGO suspended sheets in solution that tend to aggregate or have different mechanical and hydrophobic properties, in comparison with GO. In the literature, it is hard to define different graphene-based structures in electrical biosensing platforms due to the lack of surface characterization; however, increased surface areas and number of defects have been widely described in a myriad of graphene-derived sensors to perform better than planar or defect-free structures such as pristine graphene. In the literature, it is hard to define different graphene-based structures in electrical biosensing platforms due to the lack of surface characterization; however, increased surface areas and number of defects have been widely described in a myriad of graphene-derived sensors to perform better than planar or defect-free structures such as pristine graphene. In the literature, it is hard to define different graphene-based structures in electrical biosensing platforms due to the lack of surface characterization; however, increased surface areas and number of defects have been widely described in a myriad of graphene-derived sensors to perform better than planar or defect-free structures such as pristine graphene. Electrochemical sensors can be read-out using voltammetric, impedimetric, or potentiometric techniques. In voltammetric measurements, the electrode potential is controlled to drive an electron-transfer reaction with a measured current associated to it. This can be done taking advantage, for example, of [Fe(CN)₆]³⁻ or metals where the shift is associated to a quantitative perturbation. Impedimetric measurements monitor changes in the interfacial properties of the electrode surface and do not require labels generating signals to detect biorecognition events. The potentiometric technique also
is an important tool in graphene biosensors due to the high mobility of either electrons and holes in graphene. The semimetallic behavior of graphene enables charge carriers to be tuned from electrons to holes using external electric fields enabling the Dirac point and the doping type of graphene field-effect transistors (FET) to be determined. This is very important in biosensors for DNA analysis or protein detection, where the attachment of DNA or proteins can lead to a shift in the Dirac point enabling quantitative detection of loaded molecules. This is attributed to a change in the surface charge, which can be related to the presence of a protein at a certain pH or to the length of the targeted DNA, inducing a higher or lower charge density resulting from the doping of these charged compounds to graphene. This methodology has been used for pH sensors, cell-based sensors, or cancer-biomarker sensing platforms. Solution-gated FETs have been reported using pristine or polycrystalline graphene, and recently RGO has been tested, reporting characteristics similar to polycrystalline or pristine graphene. This could be a step-forward in the development of low-cost graphene-based devices.

The plethora of scientific literature on graphene electrochemistry relies on GO- or rGO-based suspensions which are drop-cast or electrodeposited onto underlying electrodes, where basically the oxidized graphene derivatives act in synergy with the underlying electrodes, producing summative electrochemical effects. This has also been achieved for a myriad of electrochemical (bio)sensors that are graphene-modified using screen-printed carbon electrodes (SPCE), gold, or indium tin oxide (ITO). For plastic-based biosensors, our research group has also studied this kind of sensor, reaching advantageous tunable electrochemical properties in an SPCE modified with GO and rGO for enzyme immobilization (e.g., tyrosinase) and drug detection, respectively, (Figure 2A,B). Similarly, in paper-based electrochemical biosensors, rGO has been used for tuning the electrochemical properties of microfluidic paper-based analytical devices for the detection of four different cancer biomarkers. This was achieved using photoresist-patterned paper followed by screen printing of carbon and Ag/AgCl, and additionally the drop-casting of graphene and nanoparticles. Despite the low cost described by the authors, the fabrication process can be seen as a time and labor-consuming method.

Electrical biosensors exploiting rGO as a transducer are also able to exhibit an advantageous performance, either in plastic or paper architectures. In fact, rGO-based flexible FETs printed on plastic exhibit a performance comparable with those based on pristine graphene, which represents a good alternative for scalable bioapplications of rGO thin-film transistors. In general, electrical biosensors based on the combination of GO or rGO and plastic are making an evolution in performance and production cost. However, polycrystalline-graphene-based biosensors are making one step forward for portable and wearable biosensors that, until now, could not be observed in GO or rGO electrochemical biodevices with implemented human–machine interfaces and enabling theranostics.

In the paper-based field, ink-jet printing is playing an important role for device production, which could be related to the opportunity to pattern GO- or rGO-based inks without changing the features of the paper in a simple fashion and enabling the improvement of the conductivity via UV-pulsed-laser irradiation. Other deposition techniques, involving pressure, are prone to changing the paper thickness and its fluidics. It is worth mentioning that, in spite of the large surface roughness of conventional printing paper, graphene-based (opto)electronic devices can also be fabricated using this substrate, which could be of interest for innovative biosensing. In fact, multilayer graphene (growth via chemical vapor deposition) has been demonstrated to be transferred to conventional paper, taking advantage of the hydrophobic character of multilayer polycrystalline graphene.

The production of graphene-based thin films is of major importance to a wide variety of applications, such as electrical and electrochemical sensors. Their low cost and simplicity could improve the fast integration and abundance of graphene-based devices. Recently our research team reported a simple way to
transfer GO patterned structures to target substrates by simple water activation and pressure using wax printed membranes, (Figure 2C). Since wax printing is a cheap and versatile technique, one can produce unlimited shapes and structures, and the control over the thickness provided by the vacuum filtration vertically nanometer-sized films to be printed. This represents an interesting alternative to existing patterning technologies such as screen-, gravure-, and inkjet-printing, microcontact stamps, and lithography. In fact, we are able to print over paper, textile, or plastics, opening venues for low-cost and flexible all-graphene-based biosensors, overall in cases where the size of the sample to be analyzed is not a major issue.

2.2. Optical-Based Platforms

Graphene derivatives are optically omnivorous, that is, their fluorescence-quenching capabilities are virtually universal, and they are able to quench any fluorescence wavelength via energy transfer. It is noteworthy that not only does their number of layers determine the efficiency of their fluorescence-quenching ability, but also their oxidation degree affects their quenching factor – the more the utilized GO is oxidized, the less efficient it becomes in terms of fluorescence-quenching capability. In addition, long-range energy transfer is also known to occur in graphene derivatives, which is observable up to 30 nm. Oxidized graphene derivatives undergo a recombination of electron–hole pairs localized within an sp² carbon domain embedded within an sp³ matrix, resulting in photoluminescent properties. Their maximum emission wavelength can be modulated from the blue region to the near-infrared region in accordance with their oxygen-containing moieties, lateral size, and oxidation degree. Although the synthesis of photoluminescent graphene derivatives endowed with high quantum yield (e.g., >70%) has been scarcely developed, nitrogen doping has been reported to be an effective route to address this paucity and improve such an important parameter.

The most common graphene-based optical biosensing principle relies on the interaction between single-stranded DNA (ssDNA) and graphene derivatives, which is facilitated by hydrophobic interactions and the strong π–π stacking interaction between the hexagonal cells of the graphene derivatives and the ring structures in nucleotides. This leads to a simple, yet sensitive and versatile optical biosensing mechanism: in the absence of an analyte, a fluorescent-dye-labeled ssDNA is complexed and quenched via the interaction with the graphene derivative, whereas the complex dye–ssDNA/graphene is detached in the presence of an analyte. Consequently, the dye-labeled ssDNA recovers its fluorescence as the graphene derivative weakly interacts with the rigid structure of double-stranded DNA or aptamer–target complexes. GQDs, and r/GO micro- and nanosheets are the most common graphene derivatives in optical biosensing, whereas graphene-based optical biosensing mainly capitalizes on the aforementioned photonic phenomenon.

Our research group has explored the photoluminescence-quenching capabilities and the oxygenated two-dimensional network of GO in order to exploit it as a pathogen-revealing agent. To this aim, we used a conventional glass-slide-based microarray system, whose limit of detection is 760 times more sensitive than a conventional immunoassay, though expensive, relatively time-consuming, and not particularly amenable to portability (Figure 3A). We then demonstrated an innovative

![Figure 3](https://www.advancedsciencenews.com/wileyonlinelibrary.com)

**Figure 3.** Graphene oxide as a pathogen-revealing agent. A) Microarray immunoassay revealed by GO on a glass slide. Adapted with permission. Copyright 2013, Wiley-VCH. B) Upper part: lateral flow immunoassay revealed by GO on paper. Adapted with permission. Copyright 2015, American Chemical Society. Lower part: immunoassay revealed by GO on bacterial cellulose nanopaper. Adapted with permission. Copyright 2015, American Chemical Society.
3. Simple Biosensors based on the Integration of Graphene Derivatives into Low-Cost, Lightweight, and Flexible Substrates: Discussion and Future Outlook

Graphene derivatives compete with other well-known materials (e.g., silicon, germanium, and ITO). However, this novel family of materials is likely to find its best opportunities in innovative areas, that is, functions, applications, products, or technologies not dominated by other conventional materials. Aside from this, currently, the graphene market is mainly focused on production of graphene derivatives, whereas some opto-/electronic devices such as FETs, magnetic sensors, and photodetectors have also been launched. In contrast, the development of final products involving graphene-based biosensors is an emergent area. Thus, in the mid-term, graphene-based biosensors are likely to find excellent opportunities and a great market niche within this context.

Well-established technologies allowing diagnostics, drug discovery, food safety, defense, security, and environmental monitoring often require bulky infrastructure, conventional electrical supply, time-consuming and labor-intensive procedures, and qualified personnel. Aiming at simplifying such technologies and manufacturing easy-to-use, yet powerful and innovative biosensing devices, we envisage a new generation of biosensing devices based on the synergy between flexible, lightweight, easy-to-use, versatile, and cost-effective materials (paper and plastic) and the outstanding properties of graphene derivatives.

When graphene derivatives are combined with cheap, lightweight, and flexible substrates, interesting opportunities arise. Flexibility facilitates advantages in terms of size and weight of the engineered biosensor. Moreover, flexibility obviates bulky architectures, facilitates assembly processes, and allows the generation of wearable and implantable devices. Aside from their flexibility, abundance, and low-cost, plastic and paper can be readily modified to create breakthrough devices using suspensions of graphene derivatives and a myriad of deposition techniques, including drop-casting, dip-coating, spraying, spin-coating, inkjet printing, screen printing, high-volume roll-to-roll gravure printing, pressure-based mechanisms, etc. Among these innovative biosensors created through the combination of graphene derivatives and flexible substrates, we could find advantageous theranostic platforms, point-of-care devices, wearable devices, and omnipresent biosensors targeting different tasks involved with diverse applications like healthcare, therapy, diagnostics, agriculture, environment, food safety, and security. The tuning with new electronic and photonics functions in flexible substrates might be a future in new technologies based on graphene derivatives for autonomous biosensing devices with integrated photonics, electronics, and microfluidics. On the other hand, we believe there is still much to discover and develop within the field of this kind of biosensor. For instance, GO or rGO membranes, also termed as GO or rGO papers, are making a step forward in the supercapacitor field. These membranes have also been tested for molecular sieving, which could be applied in the generation of breakthrough biosensing architectures. Although analytical lab-on-a-chip (LOC) applications capitalizing on the properties of graphene derivatives are not widely reported in the literature, one can find encouraging approaches facilitating electrochemical amplification mechanisms, multiplexed optical-detection strategies, and a novel crystallographic analysis which provide excellent examples of how the thickness, conductivity, and transparency offered by graphene derivatives represent a powerful tool for lab-on-a-chip development.

Integration of graphene into LOC applications, lateral flow (LF), and general point-of-care (POC) devices, including wearable or implantables, is a critical issue. As already mentioned, electrical, optical (fluorescent, colorimetric etc.), or other transduction techniques, thanks to the advantageous graphene properties, can be integrated. Future development of the different systems/devices are strongly related to the analyte (e.g., chemical or biological molecules) to be detected, as well as the objective of the device: disposable (one use only) or for
multimodal/continuous monitoring. While disposable devices, such as LF, would require a certain “shelf life” (variably influenced by storage conditions, such as exposure to heat, light, and moisture). LOC and other POC devices for continuous monitoring would definitively require long-term stability of graphene while working in a real environment. All the mentioned issues should be carefully considered case by case, depending also on the application (health or environment monitoring, safety and security, or any other industrial quality monitoring), requested analytical performance (reproducibility, detection limit, sensitivity, selectivity, operation range, etc.) or threshold values (ON-OFF sensing applications), beside others.

Solving real-world problems through simple – albeit powerful, safe, and robust – biosensors based on the integration of graphene derivatives into cheap and flexible substrates will enforce the successful transition of these innovative devices to the market. Moreover, the novel insulating, semiconducting, and optoelectronic properties provided by other exciting 2D materials (hexagonal boron nitride, metal dichalcogenides, black phosphorus) and their combination is also expected to bring breakthrough approaches solving real-world problems through biosensing. However, overall commercialization of graphene-based biosensors will imply overcoming diverse technical, production, and market issues. Here we identify some of these crucial issues:

i) Relevant stakeholders in the biosensing field demand different challenging characteristics, including but not limited to miniaturization potential, low power-budget (μW to mW), robust shelf and operational lifetime stability, recalibration capabilities (as they may suffer from temperature or relative-humidity drifts), and mechanical shock resistance.

ii) The performance reproducibility of graphene-based biosensors strongly depends on the homogeneity of the quality of the involved graphene derivatives, whose manufacturing scalability is not particularly easy to control, above all when it comes to devices relying on a single flake, such as bio-FETs. Moreover, sensitivity and specificity (overall in clinical diagnostics), which are seldom reported in the literature in relation to biosensors, should be systematically determined in terms of percentage of false negatives and positives, respectively.[43]

iii) Graphene-deposition techniques yield different coating qualities in terms of surface coverage and interconnection between graphene flakes, which should be judiciously considered along with substrate roughness and morphology of the individual flakes; especially in electrical approaches, where conductivity is heavily affected by these features. Firstly, single layers undergo a folding and wrinkling effect to prevent collapse into multilayers.[18,19] Secondly, some graphene-deposition techniques tend to promote an extreme degree of such a folding and wrinkling effect (e.g., drop-casting and spin-coating, which reduce surface coverage and increase the roughness, deteriorating the electrical performance). In contrast, other graphene-deposition techniques are amenable to performing a large-area monolayer of flakes with much higher surface coverage, leading to conducting thin films.[44]

iv) Although thoroughly systematic and well-designed experiments reveal encouraging guidance for the use of graphene in biological environments,[45,46] the toxicity of graphene derivatives is still under debate overall in the case of wearable platforms.

v) Corresponding/right validation and evaluation in real settings and ongoing proficiency-testing of graphene-based biosensors are crucial to demonstrate advantages over well-known sensing technologies, in terms of simplicity, sensitivity, specificity, operating time, or cost.

All in all, graphene-based biosensing performance heavily depends on purity degree, lateral size, number of layers, oxidation level, distribution of oxygen-containing moieties, and/or defects of the employed graphene derivatives, since these features often modulate electrical conductivity, fluorescence-quenching capabilities, photoluminescence, binding interactions (between graphene derivatives and biomolecules) and kinetics, and capacity of biomolecules adsorption. Hence, scientists and technologists working with graphene-based biosensors are strongly encouraged to consider and characterize all these features judiciously with the aim to standardize the quality of graphene derivatives in biosensing and ensure reproducibility of this novel type of biosensors.

Getting their best opportunities in applications not dominated by conventional materials, simple graphene-based biosensors will lead to breakthrough solutions for the real world.

Acknowledgements

The authors acknowledge the support from H2020-EU (INTCATCH Project, Ref. 689341), MINECO (Spain, MAT2014-52485-P, RTC-2014-2619-7 and Severo Ochoa Program, Grant SEV-2013-0295) and Secretaria d’Universitats i Recerca del Departament d’Economia i Coneixement de la Generalitat de Catalunya (2014 SGR 260).

Received: September 12, 2016
Revised: October 26, 2016
Published online: November 29, 2016

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