Graphene for implantable biosensors

Grafeno para biosensores implantables

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Abstract

In this review, we will provide a comprehensive insight on the current research situations and future challenges for the graphene-based implantable biosensors. We introduce the use of graphene derivatives for *in vivo* sensing implants, discussing their synthesis and key properties for this final application. Then the most promising recent examples in the field were critically revised, with special attention to electrochemical and transistor-based biosensors. Although there are still many challenges to overcome, we can anticipate that the latest developments are paving the way for the next generation of this kind of implants. Finally, the emerging 2D materials are also presented, which are following the graphene pathway for the *in vivo* sensing field, with a broad future to explore.

Introduction

In vivo sensing is an emerging field with the potential to change health care in the coming years through personalized medicine and highly precise treatments [1]. Sensing devices implanted into patients could regularly provide appropriate health information. Continuous or periodically health monitoring can assess health conditions that can help diagnosis, treatments, or rehabilitation, increasing the survival ratio of an at-risk population, improving life quality, and reducing medical costs. Since these sensors are able to collect a large amount of data, novel data management and analysis methods, such as Big Data and machine learning, can be also applied to extract highly valuable information [2].

In vivo biosensors can specifically detect biological-related analytes or other biological parameters of interest with high sensitivity operating inside the human body or implanted into a living system. Conventional *in vivo* sensing devices present important mechanical issues, since they are metal- or silicon-based systems that are too rigid for soft biological tissues. Other general challenges need to be overcome, such as long-term biocompatibility and stability, miniaturization, reliability, and costs, to achieve an outstanding sensing performance.

New materials, such as metal nanoparticles, organic polymers and carbon-based nanomaterials, have achieved considerable progress in this field [1,3]. In particular, graphene, one of the most explored carbon-based nanomaterials, and its family can accomplish the above-mentioned requirements. Form its discovery in 2004, has captured the interest of the research community for application in many different fields, ranging from optoelectronics to composites, and supercapacitors, among others [4–6]. Thanks to the great effort spent in the last decades in graphene research field, the most promising applications to reach their final use in our society are being established. Indeed, its use in sensing is emerging as one of these fields in which graphene have gained prominence as main component of the next-generation sensors [7].

The use of graphene in implantable sensing is unquestionable, since its large surface area, remarkable optoelectronic, thermal, and mechanical properties, this carbon material can potentially dominate in all features needed: sensitivity, specificity, linear range, reversibility, response time, long-term stability, and biocompatibility. Herein, we will critically review the most promising recent sensing approaches applied *in vivo* models to be transfer to a clinical level. In addition, we will discuss the key parameters to accomplish all the analytical requirements. Finally, we will consider current limitations in translating the revised technologies into final clinical devices. We have also commented the new related two-dimensional (2D) materials recently discovered that could tackle the key challenges in the field. It is worth noting that *in vivo* graphene-based sensors that perform health monitoring includes wearable sensors, and implantable devices. However, in this review we have focused on implantable sensors *in vivo*, as their development is more challenging due to their invasiveness in living systems. In addition, comprehensive overviews of wearable sensors have previously been examined [7–11].

Production of CVD-G and GO

Many graphene derivatives have been defined, including exfoliated graphene, graphene oxide (GO), reduced GO (rGO), graphene dots, chemical vapour deposition (CVD) graphene, etc. They can be produced by two main methodologies: bottom-up and top-down approaches[12]. Particularly, the bottom-up fabrication by CVD and the top-down oxidative processes of graphite are the preferred techniques for *in vivo* sensing purposes since they are mainly based on electrodes and transistor devices.

CVD is the most used methods for industrial-scale fabrication of graphene [13,14]. This procedure allows the synthesis of graphene with reproducibility, high-quality monolayer / few-layer with low number of defects. Thus, the electronic properties of CVD graphene are exceptional to be implemented in

electronic devices as we will discuss below. CVD consists in the synthesis of large-area thin layers on metal surfaces, particularly, Cu or Ni, using a carbon gas source. These metal substrates are not appropriate for *in vivo* sensing applications and graphene must be transferred to more suitable substrates, as for example soft or transparent materials (PDMS, polyimide, PET), which are part of sophisticated systems [15]. To this end, there are several methodologies, such as polymer assisted transfer, dry transfer methods or electrochemical transfer. However, the transfer process may cause damage on its surface, such as wrinkles, and contamination of the sample. To solve these drawbacks, several techniques have recently been developed. However, the complexity of the whole process is still far from an industrial large-scale production.

The top-down oxidative methodology of graphite is generally performed by its chemical or electrochemical oxidation, breaking the Van der Waals interactions between graphite layers [16]. This exfoliation allows to obtain larger quantities of GO which are highly defective but soluble in aqueous media due to the large amount of different oxygenated groups on its surface. This approach is quite adjustable, since there are several methods that permit to tune the oxidation degree, type of oxidized groups and layer dimension [17–19]. However, during this procedure, it is common to use hazard chemicals or solvents that produces oxidation residues which can produced toxic effects even at low concentrations. Although, there are not well-defined agreements to define a medical grade material, particular attention should be paid to the GO composition to be used in *in vivo* applications.

Graphene properties

The properties of graphene (mechanical, transparency, high porosity, thermal conductivity, optoelectronic, etc.) make it ideal in bioelectronics field. In addition, depending on the envisioned bioapplication, some properties are fundamental (**Figure 1**).

As mentioned above, most of the graphene-based *in vivo* implants are electronic devices (*i.e.*, electrodes and transistors); thus, the electronic properties of graphene are crucial. Graphene has unusual conductive properties [20]; it presents a zero-energy band gap with a linear energy dispersion, which permits electrons to travel faster than in other materials. Thus, it exhibits the highest electron mobility (~$2x10^5$ cm^2/Vs) and critical current density of 10^8 A/cm^2. Due to the high quality of graphene produced, CVD graphene is widely used as electrode component in electrochemical sensors [21]. This is also possible thanks to the wide electrochemical window of graphene that makes it suitable to work in biological environments. Regarding the conductivity, unlike graphene, GO shows less conductivity due to the disrupted sp^2 -hybridized carbon network produced by oxidation process and it depends on the degree of oxidation. However, the abundance of oxygen functional groups, such as epoxide, carbonyl derivatives and more groups, provides GO with an inherent redox activity in cathodic and anodic regions. This redox activity is based on the oxidation or reduction of these oxygenated groups, some at mild electrochemical potential conditions for example quinones and epoxides. This allows GO to be also exploited in electrochemical sensing [22].

Besides, CVD graphene can be implemented in field effect transistors (FETs) technology [23]. Its extraordinary charge mobility leads to FETs with a high transconductance, which generates the amplification capacity of the transistor. This fact, together with the low intrinsic noise of this CVD graphene-based FETs, leads to detect electrical signals with high signal-to-noise ratio. Indeed, it has been reported arrays of flexible FETs based on CVD graphene that can detect brain activity in *in vivo* models [24].

Graphene derivatives can also provide extraordinary optical properties, as high transparency, that is a crucial feature for particular *in vivo* sensing performances. For example, CVD graphene permits tissue observation with clear images in a multifunctional bioelectronic device [25].

Mechanical properties have also a fundamental role *in vivo* bioelectronics. The atomic thickness, in addition to the strong covalent bond between the C atoms of the same layer, can provide an improvement in the mechanical properties of implants and protection against stresses and damages due to mechanical perturbations. It is well known that graphene has the highest Young's modulus (1Tpa) and fracture strength. Furthermore, as mentioned before, flexibility of the implant allows to decrease the immune response during implantation, giving biomimetic features to the device. This feature allows the construction of flexible graphene-modified electrodes to recording for instance electrophysiological signals for neuronal and cardiac tissues [26].

Graphene surfaces can be chemically modified by controlled chemical functionalization using either covalent or noncovalent methods [27]. Through chemical modification, selectivity capabilities can be implemented to the graphene sensing devices that requires receptors (*e.g.*, antibodies, enzymes, nucleic acids, etc) for the specific recognition of the biological analyte.

But why, despite the large number of studies focused on graphene biomedical devices developed, only few architectures reach an *in vivo* application? Most of the times, the answer to this question resides in one key factor: biocompatibility. Although there are many publications in the literature evidencing the biocompatibility of graphene-based materials *in vitro* [11], translating them into a clinical use is not generalizable, and an extensive *ad hoc* biocompatibility evaluation needs to be performed [28]. If during the *in vitro* trials is crucial assessing the cytotoxicity of the materials employed, the *in vivo* biocompatibility estimation includes the evaluation of

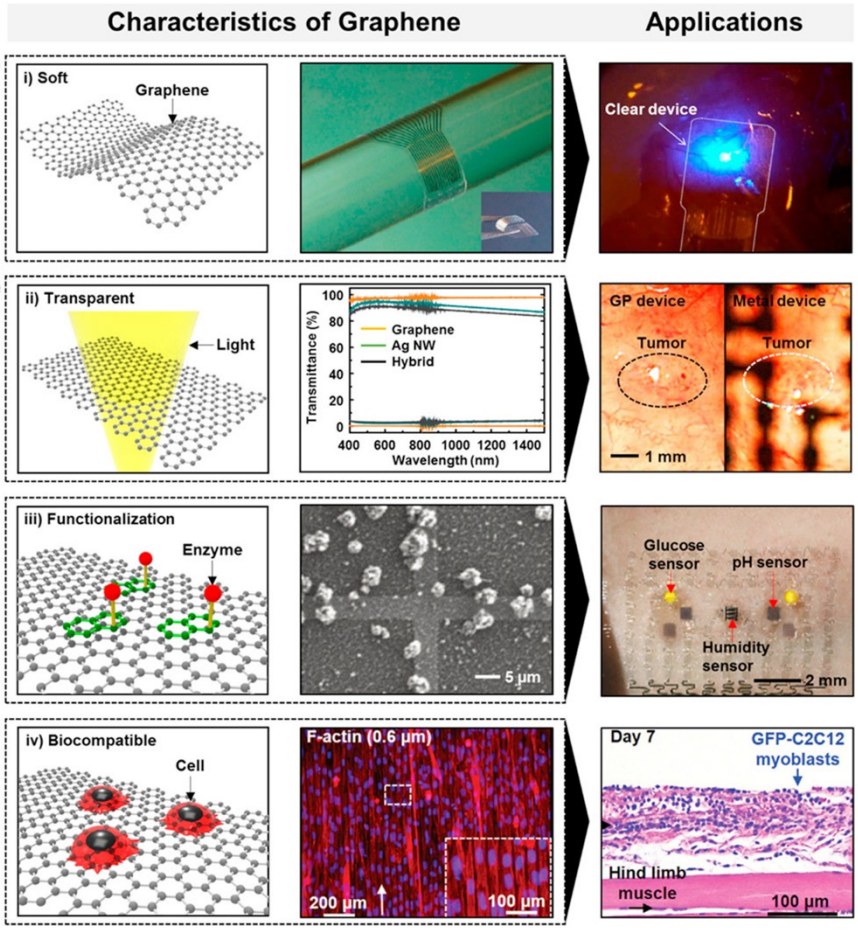

Figure 1. Properties of Graphene and bioapplications. Reproduced from ref. [10], Copyright 2019 American Chemical Society.

more complex immune responses that are not easily modelled for systematic studies. When talking about implants for sensing, the main factor to be evaluated are wound healing response to implantation, chronic inflammation, or foreign body response [29], and long-term stability and functionality of the device [30]. Although is not easy to assess the *in vivo* biocompatibility of a device, many different strategies are nowadays employed to reduce its inflammation process and promote its long-term durability [31]. Among others, tailoring the mechanical and chemical-physical properties of the device are of crucial importance for the success of an implant. As an example, the introduction of biomimetic coatings (proteins, biocompatible polymers etc.) [32,33] and the use of biocompatible flexible support materials (*e.g.*, polyimide)[34–36], are two successful strategies to reduce the immune response and ensure the implant durability.

Graphene-based *in vivo* biosensors

A biosensor could be define as device able to selectively detect and/or quantify biological-related analytes [37]. By moving from *in vitro* to *in vivo* sensing, the device must deal with complex matrix interferences, low and transient concentrations of the target analytes and immune response from the living organism [31,38,39]. Thus, just few device architectures survive to the first *in vivo* trials, and these are those which have fast response, low limit of detection, biocompatibility and operate in a point-of-care manner [1].

Considering the aforementioned properties such as electrical conductivity, biocompatibility and flexibility, graphene-related materials are promising candidates to build bioelectronic devices for *in vivo* biosensing applications [40,41].

Within the pool of the wide number of different systems, two main families of graphene-based devices were recently applied to *in vivo* biosensing: electrochemical biosensors and transistor-based biosensors. As mentioned above, it is worth clarifying that these kinds of devices can be classified also in wearable devices, considered non-invasive; and implanted devices, invasive. Herein, the discussion will be focused only on implanted devices. More information on wearable devices can be find in other reviews [7–11].

Electrochemical biosensors

In an electrochemical biosensor, the analyte recognition is transduced into an electrochemical signal [42]. These devices are usually composed of a chemically modified electrode including a biospecific receptor on its surface. Graphene-based materials were successfully applied in the development of

electrochemical biosensor [22,27], as they work as transducing platform and give the opportunity of immobilizing the recognition element through different functionalization routes [43,44].

An example of real-time monitoring of analytes was shown by Taylor *et al*. who employed carbon fibre microelectrodes modified with electropolymerized PEDOT/graphene oxide composite to sense dopamine with high sensitivity and response rate [45].

To achieve high spatial and temporal resolution, the latest device architectures employ multielectrode arrays instead of single electrode probes. As an example, in the work published by Liu *at al.* a rGO/Au_2O_3 nanocomposite multielectrode array was used as a neural probe to sense H_2O_2 in hyperacute stroke model [46]. The inclusion of the graphene-based material allows to enhance both the sensitivity and the limit of detection of the sensor, while the multichannel device make it possible to simultaneously monitoring electrophysiological and chemical signal.

Multielectrode arrays were successfully applied also in electrophysiological signal monitoring, as electrical signal from neurons activation [47]. Further than only recording electrical signal from the brain, following works achieved simultaneous recording and stimulation through graphene-based electrodes [34,48,49]. As an example, the device based on porous graphene developed by Lu and co-authors allows both the recording of physiological oscillation and the electrical microstimulation of knee and ankle flexion [50]. This last application is promising in the high-resolution brain mapping and in the treatment of neurodegenerative diseases.

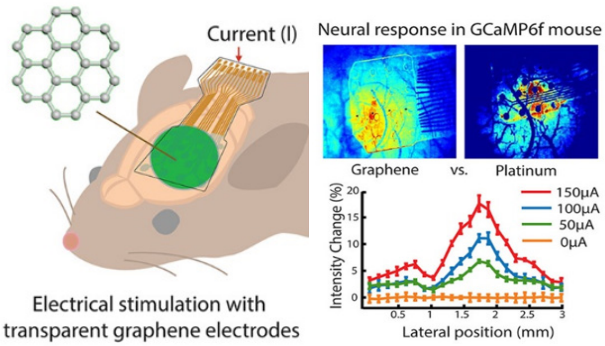

Figure 2. Transparent graphene-based electrode array for brain stimulation and recording. Reproduced from Ref. [48], Copyright 2018 American Chemical Society.

Similarly, Park *et al*. developed an innovative approach which employs a transparent graphene multielectrode arrays to perform optogenetic brain stimulation and recording [48]. In this work the benefit of working with transparent electrodes that enables light-based stimulation is emphasized, application not feasible with previous used platinum electrodes (**Figure 2**).

Transistor-based biosensors

As an alternative to the widely developed electrodes electronics, in the recent years the recording of electrophysiological and biochemical signal was performed through the FET technology [51]. This type of devices shows several advantages like amplification capability, low sensitivity to environmental interferences and ease miniaturization that coupled with the high electronic performances and flexibility of graphene represent the perfect match for *in vivo* biosensing.

In graphene-based FETs, usually solution-gated FETs (SGFETs), graphene is deposited as a channel material between two metallic conductors [41]. With this configuration, a variation of the electric field in proximity of graphene surface can be finely detected as a change in graphene electrical conductance [52]. This corresponds to the so-called field effect and these variations can be correlated with alterations in biochemical environment or electrophysiological inputs [53].

One of the first graphene transistors implanted *in vivo* was showed by Mannoor et al in 2012 [54]. Therein, the authors developed a device composed by graphene on silk transferred on a gold electrodes coil (**Figure 3**). This last component allows the wireless monitoring of graphene resistance, while the presence of water-soluble silk makes the device easily implantable. In addition, the immobilization of a bacteria selective peptides on graphene surface permits the recognition of bacteria at a single cell level in saliva through a tooth implantation of the device.

As already highlighted for microelectrodes, the trend with transistors is also to pass from single device to multidevice arrays. The work reported by Blaschke *et al*., a 16 SGFETs array based on graphene on polyimide flexible substrate was used to recording *in vivo* brain activity [55]. In a following study of the same authors, a similar microtransistor array was employed to recording of infraslow cortical brain activity, usually associated with stroke and brain injury [56]. This innovative application opens the possibility of mapping brain activity with high resolution and sensitivity, this was possible by the designed electronics and graphene biocompatibility and electrochemical stability.

Conclusions and Perspective

The advantages of graphene for implantable biosensors have been revised above. In addition, significant progress in the foreseeable future for applications in healthcare, personalized medicine, disease treatment, human and machine interfaces have been examined. In this last section, the challenges of graphene-based implantable sensors *in vivo* will be discussed.

In the last decade, researchers have widely explored the use of graphene for analytical and *in vitro* sensors [27,57]. Although several promising works could revolutionize the medicine field in the next years, the contribution of the research community to implantable sensing is more limited since it must satisfy the highly strict regulations for medical devices [58]. Indeed, the

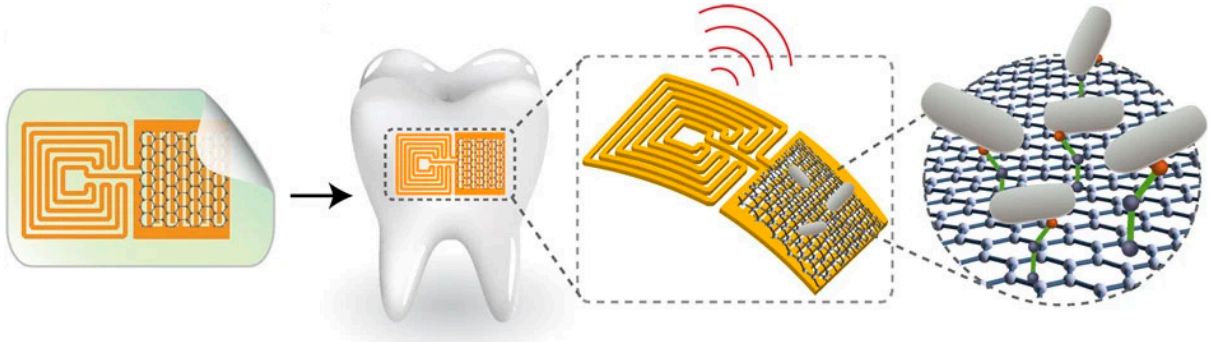

Figure 3. Schematic representation of peptide–graphene nanosensor. Reproduced from ref. [26], Copyright 2017 American Chemical Society.

most challenging issue is related to human health risks. The biocompatibility and biological toxicity, of graphene must be further assessed, especially in long-term *in vivo* studies. In addition, graphene *in vivo* sensors, must satisfy the strict regulations on sterilization to avoid the inflammatory response after implantation because are invasive. Long-term stability and mechanical durability are also necessary since implantation can require tedious surgical interventions and then the device can be stressed in some tissues.

One of the main current issues is related to the synthesis of graphene. CVD graphene is the most used derivative for in *vivo* sensing. CVD is a promising method to prepare high quality, homogeneous, reproducible graphene at industrial level. However, uniform large areas of monolayer graphene are harsh to be synthesized. In addition, the mandatory transfer process can decrease the quality of the material obtained and thus the proper function of the final device. Therefore, new developments in CVD technology are necessary. The direct growth of high-quality graphene on flexible and biocompatible surfaces at large scale should be the optimum solution.

Another main challenge in most biosensors is to implement specificity to the target analyte. As mentioned above, this is achieved by a controlled chemical modification of graphene to link specific receptors. But the lack of selectivity is still burdening *in vivo* sensors in some types of tissues. For example, the development of graphene implants in the nervous system with real time detection of biomarkers, such as neurotransmitters, neuromodulators, etc. are yet to be achieved [23]. Furthermore, integrated multifunctional sensors that are able to monitor different biological parameters using different receptors are highly desirable for disease treatments.

Although exciting implantable sensors have been developed, the scenario to produce fully integrated implantable sensors that can replace the current technology under the same performance conditions are still far. In addition to outstanding sensors, it also requires the development of other components, such as graphene compatible integrated circuits, miniaturized wireless hardware, and efficient power sources. Hence, although graphene provides a variety of distinctive characteristics to *in vivo* sensing, limitations are also present.

In recent years, other 2D materials have been demonstrated to be promising sensing device components [40]. Beyond graphene, within the world of 2D materials, it is possible to find different families according to their composition and electronic properties (**Figure 4**): as a typical 2D insulator, h-BN is a graphene-like layered material which has great resistance to charge transport and a great in-plane thermal conductivity. Examples of semiconducting 2D materials can be black phosphorus and transition metal dichalcogenides. An interesting feature of these semiconductor materials is that their electronic properties can be tuned with different approaches, for instance number of layers or changing their composition. This wide range of band gaps and carrier mobilities make them suitable candidates for FETs. Finally, as typical 2D metals, MXenes have been recognized as a good promising biosensing platform due to the high metallic conductivity and excellent electrical properties. But, to the best of our knowledge, there are just a few reported examples of *in vivo* sensing with such new 2D materials [59].

Similar to the current graphene “infancy”, despite the number of publications about newer 2D materials has been increasing in recent years, there are still many issues to address. The first challenge is related to their large-scale synthesis with desirable size and thickness. To this regard, it is usual to use toxic reagents and solvents in the preparation process. Another main challenge is their long-term stability in *in vivo* experiments. A firm understanding of their toxicology / biocompatibility will be necessary for each material depending on the synthesis procedure. During *in vivo* experiments, 2D materials encounter complex biological environments and these conditions could degrade the device over a short time depending on the material. Cheng *et al.* have reported MoS_2-based bioresorbable and multi-functional sensor for intracranial monitoring of pressure, temperature, strain, and motion in animal models [59]. Preliminary studies suggest that monolayer MoS_2 is a biocompatible semiconductor, which can be completely dissolve in biofluids after more than 2 months. However, more exhaustive studies of their biocompatibility, degradation process and environmental stability are need it. At this point, this field of *in vivo* researching on 2D materials is by now following the footsteps left by graphene during the last decade.

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