Article

Graphene: excursus of the evolution of processes and products

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Abstract

OBJECTIVE: Graphene and derived materials are a new class of fundamental nanomaterials. Thanks to the unique characteristics as excellent electrical conductivity, biocompatibility and thermal properties, this material has aroused a considerable interest in the scientific community from its discovery. The purpose of this work is to pursue the various steps chronologically that led from discovery to single production and in series of graphene, focusing on the advantages and disadvantages of the techniques going to underline the validity and importance of each, on the basis of the searches to be carried out and the products finished to be obtained. In the second section of this work, however, we focused on the evolution of the applications of this super material over time. In fact, graphene and bidimensional materials in graphene have been significantly used in various areas of biomedical research such as fabric engineering, regenerative medicine, bioimaging with application in neural engineering and in the clinic, with different future perspectives still to be completed.

Key words: Graphene, Graphene oxide, Bioimaging, Regenerative medicine, Quantum points of graphene.

Introduction

In 2004 two physicists A. Gem and K. Novoselov of the University of Manchester areolated for the first time a material with a thickness of a single atom: the graphene. Scientists successfully separated graphene from graphite through micromechanical stripping and this discovery led them to win the Nobel Prize for Physics 2010 together. The graphene, a basic constitutive element of graphic materials, is a two -dimensional material consisting of 6 tied sp2 carbon atoms that are strictly packed in a bee honeycomb network with an entire distance of 1.42 Å[1]. Among all sp2 to carbon allotropes, the graphene shows the most remarkable and interesting chemical and physical properties such as an ultraelected electronic mobility (~ 2 × 10.5 cm^2/vs), high thermal conductivity with an exceptional value of 3,500–5,000 W/MK (superior to any other material) and an electric conductivity with a critical current density of 108 A/cm^2. In summary, it can therefore be said that this material has high chemical and mechanical resistance, an excellent thermal and electrical transport and a high transparency[2].

Process Evolution: Production Techniques

Graphene was isolated for the first time in 2004 using a very simple and inexpensive technique, namely, the mechanical exfoliation of graphite by means of a scotch tape. Since that time, other production techniques have been studied and tested, each aimed at producing samples suitable for different applications and uses. Currently, various methods for producing graphene have been developed, with different approaches based on size, shape of the flakes, quality and quantity. Although, in addition to their possible applications, the techniques are limited to: mechanical exfoliation, liquid phase exfoliation suitable for mass production, chemical vapour deposition (CVD), chemical reduction of graphene oxide. One of the main challenges currently appears to be the mass production of high quality graphene with few or no contaminants and / or defects and large particle size at an almost low cost. Commonly, the methods used for the synthesis of graphene from different sources can be divided into top-down (exfoliation and reduction) and bottom-up (chemical vapour deposition) approaches.

Mechanical Exfoliation

The first preparation method used, whose first attempts were made in 1998, is mechanical exfoliation. It is the simplest method that made it possible for the first time to synthesize graphene. In this technique, a piece of graphite was subjected to repeated exfoliation and then transferred to a substrate; specifically, there is the application of a force to the surface of highly oriented graphite crystals to detach and divide the crystalline layers to obtain a single one. Initially, the interaction of AFM (atomic force microscope) and STM (tunnelling microscope) analysis tips with the graphite surface was exploited to provide sufficient energy to overcome inter-plane attraction forces and lead to removal and isolation. of the crystalline monoatomic layer. Later, a much simpler method was developed, called the scotch-tape method, which used simple masking tape to exfoliate graphite. The technique consisted of placing the surface of a graphite crystal on the adhesive tape, peeling off the tape and thus peeling a few layers of material. The tape with the graphite imprint was then folded back on itself and this action was carried out several times. At the end of the process, the thin adhered flakes were easily transferred to an insulating substrate. Mechanical exfoliation is the simplest and most accessible method of isolating graphene flakes of the size of a few square microns, useful for basic research and laboratory scale experiments producing very high quality crystals, but unfortunately, it is not suitable for a production industrial as it is not possible to increase the process[3].

Chemical Reduction of Graphene Oxide (GO).

Until now, all studies had focused on the exfoliation of graphite oxide and subsequent reduction to graphene. From after 2006, however, graphene was synthesized by reduction of graphene oxide. Various chemical and thermal reduction methods have been tested which have produced materials with conductivity in the order of 102 S/cm. The chemical synthesis of graphene, via the reduction of graphene oxide (GO), is a methodology that has the advantage of having high yields and ample opportunity to carry out the process on a large scale. However, the quality of the chemical synthesis product is rather poor, due to a partial reduction of the GO and an abundance of defects in the crystal lattice, which makes the product more suitable for applications that do not strictly require qualitative graphene, such as ‘use in polymeric composites. In fact, the quality of the material produced cannot be considered high since it contains both intrinsic defects (such as edges or deformations) and extrinsic defects (such as groups containing O and H). In general, this process is strongly influenced by the choice of solvent, reducing agent (hydrazine monohydrate, hydroquinone and compounds containing sulfur), and surfactant, which are combined to maintain a stable suspension[4].

Liquid Phase Exfoliation

Subsequently, around 2008, the method of exfoliation in the liquid phase was studied, which is based on the use of the pressure forces that are generated inside a liquid. Currently, it appears to be a widely used method for the production of graphene and involves 3 different steps:

1. dispersion in a solvent or surfactant;
2. exfoliation;
3. purification to separate the exfoliated from the non-exfoliated material and, if supplied in powder form, to completely remove traces of solvent[5].

Powdered graphite is mixed with a solvent with specific physical qualities such as surface tension and / or viscosity or in a mixture of water and surfactant. The suspension is then subjected to mixing through ultrasonic waves, or high shear mixer. These processes create both shear and cavitation forces inside the liquid that cause the graphite crystals to break, reducing them to ever thinner sheets, therefore, single sheets of graphene. The suspension resulting from the process is then purified by ultracentrifugation. Higher spin speeds result in thinner flakes but with a small lateral dimension, which reduce utility for applications such as composites. The number of graphene layers (N), i.e. the thickness of the exfoliated graphite material, is usually determined by transmission electron microscopy (TEM) and atomic force microscopy (AFM). This method is more promising from the point of view of scalability and allows to obtain large quantities of excellent material. In contrast, graphene flakes turn out to be small as side dimensions. In addition, the use of reactive solvents could increase costs and the process itself is not very ecological due to the heavy use of solvents, given the low solubility of graphene[6].

Chemical Vapour Deposition (CVD)

Subsequently, in 2008-2009, the chemical vapour deposition (CVD) method was reported, which is one of the most useful methods for preparing high quality structural monolayer graphene to be used for different devices. To date, several types of CVD methods are available such as plasma enhanced CVD, thermal CVD, hot / cold wall CVD and many others. Large samples can be prepared by exposing a metal to various hydrocarbon precursors at high temperatures. The mechanism of formation of graphene depends, in detail, on the growth substrate and generally begins with the growth of carbon atoms that nucleate on the metal after the decomposition of hydrocarbons and the nuclei subsequently grow in large domains. In addition to gaseous hydrocarbons such as methane, ethylene or acetylene, liquid precursors such as hexane and pentane have also been used[7]. Transferring the graphene from the growth substrate to one of interest can be quite difficult due to the chemical inertia of graphene generating defects in the material, just as thermal variations can affect the stability of the material. CVD, generically, remains one of the most successful methods for producing graphene over a large area even if the process appears complex and a high amount of energy is required for the execution of the method. Since CVD is a valid candidate for the production of high quality graphene in large quantities, several research groups have focused in this direction. Bae et al[8], used a roll-to-roll (RTR) process for the production of 30 inches and subsequently the technique was applied for the continuous production of graphene. One of the latest attempts, carried out in 2015, made it possible to produce high quality monolayer graphene using cold wall CVD with resistive heating, which was also 100 times faster than conventional CVD[9]. In conclusion, it can be stated that CVD allows to synthesize graphene on surfaces of several square centimetres, obtaining high crystalline quality (large crystalline grains, low presence of contaminants and defects and continuity of surfaces), being able to easily transfer the material produced. on a wide range of substrates for different applications.

Additional Techniques

There are also graphite thermal exfoliation techniques that almost completely provide single-layer graphene as a product. These are methods that have many advantages over mechanical exfoliation: first of all the time required to complete the process. In fact, high temperature exfoliation processes can last even a few seconds. In addition, most thermal exfoliation methods produce graphene in a gaseous environment, thus avoiding the use of liquids. When graphite oxide is used as a precursor, thermal exfoliation simultaneously leads to the reduction of graphene. During heating, the functional groups bonded to the graphite layers decompose and produce gases that generate pressure between adjacent layers. Exfoliation takes place when the pressure exceeds the attractive forces between the layers. For this reason, graphite oxide, expanded graphite and graphite intercalation compounds are preferably used as raw materials for thermal exfoliation instead of simple graphite. In addition to the mechanical, chemical and thermal methods described above, there are other more recently developed methods which are equally promising. The electrochemical method, for example, consists in applying a potential to graphite bars used as electrodes in a conductive solution, which gradually flake off and generate graphene nanosheets in solution. The most significant advantage of this method is that it allows to produce graphene with modulable properties simply by changing the starting material and the process conditions. Consequently, graphene can be tailor-made, depending on the specific application[10].
Below in Table I the summary of the advantages and disadvantages of graphene production techniques.

Product Evolution: Application of Graphene

The extraordinary properties of graphene, including its conductivity, transmittance, flexibility and strength, allow it to be used in various applications such as electronics, composites, membranes and next generation renewable energy technologies (e.g. solar cells). Specifically, however, among the emerging nanomaterials used for biomedical applications, graphene-based biomaterials have attracted a lot of scientific and technological interest in recent years, showing great future prospects as antibacterial agents, biosensors, bioimaging tools and as components of engineering. of stem cells and tissues.

Table I. Advantages and disadvantages of graphene synthesis and production techniques

Synthesis techniques	**Advantages**	**Disadvantages**
Top down		
1. Mechanical exfoliation	- Simple method - Easily accessible - Suitable for graphene flake insulation - It allows to produce graphene crystals of the highest quality - Suitable for basic and laboratory research	- Not suitable for business and industrial production - Inability to increase the process.
2. Chemical reduction of graphene oxide (GO)	- Very high yield - Possibility of carrying out the process on a large scale	- Very poor quality of the product - Synthetic product suitable only for applications that do not require a qualitative graphene.
3. Liquid phase exfoliation	- Synthesis of excellent material	- Very small side dimensions of the product - High costs for the use of reactive solvents - Not very ecological process
4. Thermal exfoliation	- Very fast processing times - Gaseous and non-liquid environment	- Product not of excellent quality
5. Electrochemical exfoliation	- High quality product - Considerably higher yield - Ecological choice - Production of graphene with modulating properties.	
Bottom-up		
1. Chemical vapour deposition (CVD)	- Useful for preparing high quality structural graphene - Easy material transfer on a wide range of substrates.	- Very complex process - High energy quantity

Antibacterial Agents

Around 2016, an all Italian research obtained promising results aimed at designing nanomaterials for new antimicrobial therapies. This research analyzed the antimicrobial effects of graphene oxide on various human pathogens such as: *E. coli*, *C.albicans*, *E.faecalis* and *S.aureus.* The scholars have evaluated how coating medical instruments with this material can help reduce infections, especially after surgery, also allowing to reduce the use of antibiotics. Initially, the studies were very contradictory in this regard, but the Italian team analyzed how the size and concentration of graphene oxide sheets affect its antimicrobial action on important human pathogens. This effectiveness depends on its size, the agent's exposure to the material and other parameters; examining the graphene oxide in sheets of about 200 nanometers it was studied how, in aqueous solution it is able to eliminate about 90% of *S. aureus* and *E. faecalis*, and about 50% of *E. coli* in one time equal to about two hours. It was explained how graphene oxide sheets can cut bacterial membranes acting almost like a "nano-knife", they can envelop bacteria and block their growth or oxidize the cellular components of bacteria. The action against the *C. albicans* fungus was also discovered, with an efficacy similar to *E. Coli.* In addition, the possibility of mixing graphene oxide with different biocompatible polymers was evaluated to obtain an antibacterial coating suitable for medical devices susceptible to bacterial colonization[11]. Subsequently in 2019 Elia et al[12], studied a nanotechnology strategy consisting of graphene oxide or carbon nanofibers (CNF) combined with the irradiation of light-emitting diodes (LEDs) as novel nano-weapons against two Gram-positive pathogens. multidrug-resistant, clinically relevant: methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-resistant *Staphylococcus epidermidis* (MRSE). Several tests and studies on other pathogens such as viruses, fungi and other resistant bacteria are still ongoing.

Biosensors

In biomedical applications, graphene-based nanostructures with highly sensitive and selective performance as biosensors have recently been reported. The studies concerning this specific applications date back to the period 2015-2017 up to 2021 with studies on graphene-based biosensors for the detection of COVID-19. Graphene is a semi-metal with ultra-high charge mobility capable of offering excellent electronic properties and of being functionalized on its surface, and it is for these reasons that graphene-based materials have been exploited in bio-sensing applications. The functionalized area of graphene is able to directly detect the biomolecules from its oxide components due to the synthesis in which many epoxide, hydroxyl and carboxyl groups are formed on the edges and on the surface sites. In addition, functionalized graphene allows the binding of heteroatoms, antibodies, enzymes, DNA, proteins and several specific molecules. Graphene provides a high possibility of active sites for charge-biomolecule interactions thanks to the large specific surface area that leads to improved detection and supports the desired functionalization to target biomolecules and improve selectivity[13]. In 2017, a new biosensor was reported, specific for the detection of human papillomavirus (HPV), where the graphene-polyanalinia (G-PANI) electrode is modified using a pyrrolidinyl nucleic acid probe (anthraquinone-labeled peptide) and printed with the inkjet printing method. And it is through electrostatic attraction that the response of the electrochemical signal on the synthetic oligonucleotide target is measured[14]. Promising results were shown in the same year by studying and creating graphene microelectrodes integrated with bilayer lipid membranes. Under these conditions the biosensor achieves good reproducibility, reusability, high selectivity, fast response times, long life and high sensitivity. In addition, the use of graphene microelectrodes in the detection of various toxic substances such as toxins, diagnosis of D-dimers, urea and cholesterol has been reported[15]. As a consequence of these promising results, in 2020 a biosensor made on field effect transistors was presented, that is, a graphene-FET used to detect clinical samples of SARS-CoV2 through the spike protein, with promising results. These devices exploit the change in surface potential induced by binding to biomolecules. When some charged molecules bind to the gate they change the charge distribution in the semiconductor and this results in a change in the conductivity of the transistor. Typically, these Bio-FETs include the classic FET structure with source, drain and gate to which the sensitive biological element is added, often a film with binding sites for the analyte. In this new technology the basic operation is the same as the classic FET but a thin graphene channel, tens of microns thick, has been added between the electrodes of the two main components. Typically, graphene behaves as an inert material but under certain conditions it can absorb different molecules and bind easily, this allows the graphene-FET to be functionalized with biologically sensitive systems such as enzymes, DNA, RNA or antigens[16].

Bioimaging

Around 2014, graphene quantum dots (GQDs) were first defined as a new class of fluorophores[17]. The GQDs showed great photophysical properties and good biocompatibility, with characteristics very similar to a molecule compared to other quantum dots, therefore to be used in various applications of life science and in biomedicine. Until before, all the fluorophores used in bioimaging were organic dyes with not very relevant characteristics such as the tendency to photo-bleaching. On the contrary, GQDs have unique optical properties directly linked to their shape, size and surface chemistry, so that they are considered suitable for bioimaging. In addition, even with femtosecond laser (NIR) excitation in the near infrared, GQDs show very little photo bleaching. Their small size allows them to easily cross biological barriers, targeting specific areas and anatomical regions that are difficult to access[18]. Over time, several *in vivo* and *in vitro* studies have made it possible to know the different characteristics and capabilities of GQDs. In fact, in 2015, Kumar et al[19], synthesized functionalized green GQDs, highly biocompatible with dimensions between 3 and 14 nm through the acid treatment of graphite powder. *In vivo* studies, on the other hand, date back to very recent times thanks to the fact that many researchers had carried out studies on nanoparticles of 40 nm and therefore of the same size as GQDs. In recent times, however, Fan et al[20], have exploited the lower Ph of solid tumors compared to that of normal tumors, therefore a pH of 6.5 compared to a pH of 7.0-7.4. pH -sensitive GQDs were prepared capable of changing their emission from green (pH <6.8) to blue (pH> 6.8). These GQDs were injected subcutaneously into tumors and adjacent muscles in mice carrying various tumors, such as glioblastoma multiform. Evaluated the images under the microscope, after 24 hours it was shown how the tumors emitted green light and the muscles blue light. The ability of GQDs to reach tumor regions by measuring fluorescence was tested by intravenous injection. It was possible to see how quantum dots successfully crossed the blood brain barrier, being able to classify them as potential probes for fluorescence-guided cancer surgery but also for diagnosis.

Stem Cell and Tissue Engineering

In the field of regenerative medicine and tissue engineering, graphene has been regarded as a versatile “nano-platform”. The application of graphene in this context of biomedicine is to be associated with modern times, starting from 2019 onwards. In fact, in 2019, Jagiello et al[21], demonstrated the impact of graphene-based substrates (reduced graphene oxide and oxide) on the biological properties of mesenchymal / stromal stem cells. Their results underline that both graphene oxide and reduced graphene oxide-based scaffolds show potential applicability as novel, biocompatible, safe materials for use in biomedicine. In 2020, however, other studies made it possible to combine graphene with bioceramics to recreate a 3D printable scaffold with specific characteristics for bone regeneration, also studying how carbon affects cell proliferation and differentiation *in vitro*. Through this study it was proposed to add carbon-based material for a new biocompatible 3D scaffold that could become the key structural material for bone regeneration[22]. Also in the same year it was shown how a graphene oxide scaffold stimulated the proliferation of myogenic progenitor cells and endocrine functions of differentiating cells, thus actively participating in the construction of muscle tissue[23]. In addition, in the same year, studies were carried out on the potential application of graphene oxide in the manufacture of electrodes for retinal prostheses, used for retinopathies. Graphene was chosen as the material for the electrodes due to its superior chemical-physical characteristics compared to other materials. These prostheses provide excellent assistance in the treatment of age-related macular degeneration (AMD) and retinitis pigmentosa. From here we can understand how this specific material can be applied in the field of cell and tissue regeneration associated with nanotechnologies and electrical and electronic engineering[24].

Applications in the Neural Field

Health services require more and more innovative solutions to meet the growing demands and graphene in 2018 paved the way in the treatment and management of diseases of the nervous system with the help of special neural implants. The first studies were conducted at the University of Manchester and the Catalan Institute of Nanosciences and Nanotechnology in Spain, where graphene and related materials were examined for the design of devices for neural implants to record and stimulate electrical activity in conjunction with a targeted administration of certain drugs. These neural implants appear to be a very promising approach for the detection, monitoring and treatment of a range of different sensory and motor disorders of the central and peripheral nervous systems.

These implants have the function of creating a connection and interaction between neural tissue, nerve fibers or individual neurons and external devices, used to record, monitor and stimulate brain activity to intervene in the functions of the central nervous system. Starting from this, it can be said that graphene is a versatile substrate that can take different shapes with different properties and proves to be excellent for generating 2D materials used for stimulation and recording devices. Monolayer graphene nanosheets facilitate the recording of electrical activity in neuronal tissue, while reduced graphene oxide has been selected for electrical stimulation of the nervous system, capable of providing stimulation for long periods. This research group also looked at the technology to develop a retinal implant for people who have lost their lives due to retinal diseases. In addition, graphene can be modulated and functionalized with oxygen allowing the administration of anti-inflammatory drugs and neurotransmitters released at different rates after implantation, thanks to the hydrogel coating of the graphene sheets. Hence the research for the biomedical applications of graphene and 2D materials has expanded into various fields, even reaching the diagnosis of cancer[25].

Conclusions

In conclusion, graphene thanks to the structure made of carbon atoms has extraordinary properties hence the name “material of wonders” or even “miraculous material”. Graphene has a higher resistance than steel and a somewhat unique electronic and thermal conduction capacity. Looking towards the future, research and industry need to collaborate to drive innovations and direct research towards the most promising applications also from a commercial point of view, evaluating the significant progress that has been made in the last 8-9 years. The most promising discoveries as seen in the previous sections were those in the field of engineering and regenerative medicine thanks to the physical, chemical and biological properties of graphene, paying particular attention to its use as a vehicle for the delivery of genes / drugs. It is also expected that, thanks to the combination of graphene (but also derivatives) with other compositions, the future possibility of generating and manufacturing new intelligent and multifunctional materials. It is obvious that this biomaterial is not only positive but also problems related, above all, to cytotoxic and genotoxic effects. At the moment there is no gold standard to overcome the problems associated with graphene, it is thought, in fact, to continue with more in-depth investigations on this material and its derivatives by developing new experimental models on which to base before proceeding with further clinical applications.

Informed Consent Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

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