Applications for graphene and its derivatives in medical devices: Current knowledge and future applications

Jacek Arkowski[1,A,B,D], Marta Obremska[1,E], Kamil Kędzierski[2,B,D], Agnieszka Sławuta[3,D], Magdalena Wawrzyńska[1,A,E,F]

[1] Department of Preclinical Studies, Wroclaw Medical University, Poland
[2] Department of Emergency Medical Services, Wroclaw Medical University, Poland
[3] Department of Internal and Occupational Diseases, Hypertension and Clinical Oncology, Wroclaw Medical University, Poland

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;
D – writing the article; E – critical revision of the article; F – final approval of the article

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Address for correspondence
Magdalena Wawrzyńska
E-mail: magdalena.wawrzynska@umed.wroc.pl

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Abstract

Graphene is a novel carbon-based material with unique crystal nanostructure and extraordinary physical and chemical properties. Several biomedical applications of graphene and graphene-derived materials have been proposed. Its antimicrobial properties might be useful in all areas of medicine where antiseptics are required. On the other hand, the safe limits of graphene concentration for human cells have not been clearly established yet. The possibility to attach various chemically active groups to the basic lattice structure allows researchers to build graphene-based sensors for detecting biochemical molecules (and ultimately – selected cells). Sensors for physical signals, such as cardiac electrical activity, have also been proposed. The unique nanostructure of the material and the resulting physical properties (mechanical strength, elasticity and large surface area) make it a very promising material for scaffolds used in tissue regeneration. Several studies have investigated the potential advantages of a graphene coating for endovascular implants, such as stents or valves. Most of them indicate an advantage of graphene coating over other currently available solutions in terms of better hemocompatibility and facilitating endothelialization. Many of the results published so far are from in vitro studies. Promising as they might be, more data, preferably from experiments on more sophisticated animal models, must be obtained before any valid conclusions as to potential uses of graphene in medicine can be drawn.

Key words: graphene, medical devices, biocompatibility, cardiovascular

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Introduction

Graphene is a novel nanomaterial composed of sp2 carbon atoms arranged in a two-dimensional hexagonal honeycomb crystal lattice. Its unique spatial structure results in extraordinary physical and chemical properties. Some of the most important properties are high mechanical strength, excellent conductivity, a large surface-to-volume ratio, and the potential to bind different types of chemically active groups. As a consequence, several possible uses of graphene in biomedicine have been proposed and tested. The most promising applications of graphene include its use as an antimicrobial agent, a scaffold in regenerative medicine, a material for sensors of both biochemical and biophysical signals, and as a coating for endovascular implants. In this review, we present the biomedical, diagnostic and therapeutic applications of graphene-based nanomaterials in various fields of medicine. The most recent interest is the potential use of graphene coatings in medical devices for cardiovascular applications.

Synthesis, modification and chemical properties

Graphene was created for the first time in 2004 by being stripped from graphite. There are several methods for preparing graphene: mechanical stripping, redox methods, orientation epiphysis, chemical vapor deposition, graphitization, solvothermal methods, organic synthesis, graphite exfoliation, and reduction of graphene oxide (GO).[1]

Graphene-based materials can be classified according to the number and spatial arrangement of the sheets, the oxygen content, and chemical modification. Several sheets stacked on top of each other form graphite, a single rolled sheet forms a carbon nanotube, and spherically wrapped "closed" sheet builds a fullerene. A single layer of pure graphene is hard to synthesize and difficult to suspend in solution. Therefore, GO or reduced GO (rGO) is used more often in biomedical applications. Sheets of GO display several types of chemically active groups (such as carboxylate, epoxid and hydroxyl) which, together with the capacity to build π–π and hydrogen bonds, are responsible for interactions and bonds with other particles. The physical and chemical properties of graphene, GO and rGO can be imagined on a scale with graphene at one end (the best electrical conductivity, hydrophobic, and the least chemically reactive), GO on the other end (reactive, hydrophilic, and poor conductivity) and rGO in between the two.[2]

Many covalent and non-covalent modifications of graphene have been described. In general, the covalent modifications are more stable and offer higher binding capacity – an important distinction for drug carrier applications.

Graphene and graphene-based materials have already found many applications in areas as diverse as fuel cells, electrochemistry and catalysis.

Safety and potential cytotoxicity

As with every new material, concerns have been raised about its biocompatibility and potential interactions with living cells. Obviously, safety issues must be clarified before any serious biomedical applications of a substance can be considered.

It has been found that graphene interacts with proteins and nucleic acids – molecules essential for key cell functions.[3–5] There have been a number of studies where the influence of graphene and graphene-related materials on cell viability have been tested. At this point, it is somewhat difficult to draw any general conclusions from these experiments. Different cell lines (both human and non-human) were used with graphene-related materials modified in various ways. It may tentatively be concluded that GO in concentrations on the order of 100 μg/mL might exert negative effects (reduced viability) on cell lines (both murine and human). Most researchers have concluded that the effect is dose-dependent, with some evidence pointing towards a higher toxicity for pristine graphene than for GO.[6] It was found that articulate state, particle size and surface charge/oxygen content of the graphene all had a strong impact on the toxicological and biological responses to human red blood cells.[7] Interestingly, Sasidharan et al. showed that pristine graphene accumulated in the cell membrane, which leads to apoptosis due to the high oxidative stress, whereas its carboxyl-functionalized derivative internalized in the cells without any cytotoxicity.[8] Additionally, the method of administration might play an important role, as shown by 1 study, where orally administered PEG-functionalized GO sheets led to no tissue uptake while intraperitoneal injection resulted in accumulation in the spleen and liver.[9] Several mechanisms of these effects have been proposed. In a study by Luan et al., it was found that hydrophobic protein–protein interactions can be interrupted by graphene. The researchers attributed this effect to the separation of 2-deoxyribose-5-phosphate aldolase and phosphoglucose isomerase.[10] Another study showed that GO provokes oxidative stress in A549 cells, which may decrease cell viability.[11]

There have been some attempts to investigate the effect of graphene on a whole multicellular organism. In a systematic evaluation of graphene quantum dots in mice, toxicity was found to be low overall in terms of the influence on reproduction and cell viability.[12]

It seems that especially low concentrations of graphene do not provoke any measurable noxious effects. For instance, in a study by Jiang et al., a low concentration of graphene quantum dots showed a relatively weak influence on the morphology, viability, membrane integrity, internal cellular reactive oxygen species level, and mortality of HeLa cells. Similarly, small amounts of graphene quantum dots brought little harm to the cardiovascular system of zebrafish embryos.[13] The authors concluded that at least this particular graphene derivative demonstrates

good biocompatibility and that, thanks to its high quantum yield and strong photoluminescence, it might be a promising material for cell imaging, biolabeling and other biomedical applications.

Antibacterial activity

The potential antibacterial activity of graphene materials has been proposed as another, more favorable aspect of graphene–cell interactions. The researchers who studied the issue found that the effect depends on the lattice size and shape and the number of layers and surface modifications.[14–16] In particular, a reduction in cell viability was reported for *Escherichia coli* and *Staphylococcus aureus* in contact with GO and rGO. Cell membrane damage was proposed as the underlying mechanism.[17] At the molecular level, the antimicrobial effects were associated with protein disruption, lipid extraction, and reactive oxygen species (ROS) production.[10,18–20]

Even more convincing results were observed when graphene was functionalized with known antimicrobial agents, such as silver nanoparticles, other metals or metal ions/oxides, polymers and enzymes.[21]

On the one hand, these findings present an opportunity of using graphene-based materials as antibacterial agents. On the other hand, great care must be taken to investigate and quantify any potential undesirable effects on cells and the human body before these materials can find widespread application that puts them in contact with living human cells.

Detecting biomolecules

Due to its high surface-to-volume ratio (allowing the attachment of a large amount of the required ligand) and very good conductivity, graphene may be very useful for building sensitive electrical and electrochemical sensors. A receptor (such as an antibody or enzyme) bound to graphene can interact with ions, organic molecules or even whole cells. The chemical signal of interaction between the receptor and target molecule may be converted to electricity.

As an example, at least 2 types of graphene-based glucose sensors have been developed. One of them uses glucose oxidase attached to graphene,[22] and the other is based on competitive binding of glucose or a graphene-based molecule to the sensor.[23] The high prevalence and often asymptomatic onset of type II diabetes and the serious consequences of inadequate treatment make any improvements in early detection and accurate monitoring very important for the large number of patients suffering from this disease.

An accurate and reliable detection of biomarker molecules is similarly crucial in diagnosing other many diseases (such as cancers or inflammatory syndromes). Graphene-based biosensors have been shown to successfully detect specific proteins, for example, glial fibrillary acidic protein (a marker of central nervous system injury).[24] Graphene-based systems for detecting cancer cells have been also developed, using optical biosensors[25] or magnetic fluorescent biosensors.[26] A very good level of sensitivity has been achieved in both cases.

Another example is the use of graphene quantum dots modified with annexin V antibody to label apoptotic cells in live zebrafish. Graphene quantum dots have also been used in the imaging of human breast adenocarcinoma cell line (MCF-7 cells), human cervical cancer cell line (HeLa cells) and normal human mammary epithelial cell line (MCF-10A). The toxicity of graphene quantum dots has also been investigated; they were found to have high biocompatibility because they did not affect significantly the growth of zebrafish.[27]

In cardiovascular medicine, as in oncology, the identification of at-risk patients might bring substantial advantages, as many life-threatening conditions develop suddenly, without clear pre-existing symptoms. Such is often the case with myocardial infarction and ischemic stroke. High levels of circulating platelet-derived microparticles are an associated risk of those events. A GO-based sensor highly specific for platelet-derived microparticles has been tested by Kailashiya et al.[28] The proposed sensor is postulated to be a convenient tool for identifying individuals at a high risk of such incidents.

In another study, a platinum nanoparticle-decorated rGO field effect transistor biosensor was used for highly sensitive detection of BNP – a molecule widely accepted as a marker of heart failure – in whole blood.[29]

Detecting physical signals: Wearable sensors and ECG

It seems that the unique properties of graphene, such as its large specific surface area, outstanding mechanical flexibility, excellent thermal and electrical conductivity, high optical transmittance, and ultrahigh carrier mobility, make it a very promising material for the development of wearable sensors and implantable devices in health monitoring in all cases where detection of a physical signal is required.

Graphene on-skin wearable electrodes are characterized by high stretchability and durability.[30] The excellent air permeability of some designs is an improvement over the current electronic sensors, where constrained perspiration and inflammation risk remain issues.

The potential applications of such wearable sensors are not limited to strictly medical uses. The market demand for wearable electric devices is growing rapidly, especially with the ever-increasing popularity of exercise devices (heart rate monitors, etc.); the comfort of prolonged use and resistance to humidity, abrasion and tension are all very important features of sensors for such devices.

As opposed to wearable sensors, other designs make it possible to acquire the electrical signal directly from the heart and other tissues.

A flexible graphene-based microprobe developed using microelectromechanical technology was demonstrated to enable high-resolution detection of electrophysiological signals (including ECG) in zebrafish models. A special hydrophlization treatment was used to improve the signal-to-noise ratio of the device.[31]

The biocompatibility and suitability of graphene microelectrodes for extracellular recordings were also tested by measuring electrical activities from acute heart tissue and cardiac muscle cells. The recordings showed encouraging signal-to-noise ratios of 65:15 for heart tissue recordings and 20:10 for HL-1 cells. Due to low noise and excellent robustness, those sensor arrays might be suitable for diverse and biologically relevant applications.[32]

Targeted therapy: Delivering drugs to selected cells

The successful treatment of many malignant tumors requires high concentrations of anti-tumor agents that are also lethal for healthy tissues. Several methods have been developed to facilitate only localized release (i.e., only to the tumor cells) of those potent drugs or physical impulses. In photothermal therapy, a light-controlled release of heat is used. Graphene nanomaterials are characterized by strong near-infrared absorbance and a large surface area (which can be functionalized with active molecules). Therefore, several groups have reported graphene-based methods of photothermal therapy, in some case coupled with photodynamic therapy and targeted chemotherapy.[33,34] When treating solid tumors where penetration deep below the surface is required, an ultrasound rather than a light signal is used to provoke the desired effect (the release of the active substance or physical impulse). This approach is called sonodynamic therapy.[35] In each case, graphene-based materials were functionalized with photosensitizers, sonosensitizers, or anticancer drugs. Applications in photothermally controlled gene delivery have also been reported,[36] as has intracellular probing for specific proteins.[37]

Regenerative medicine: Potential applications in orthopedics, neurology and cardiology

In the case of tissue loss or damage, traditional treatments use the patient's own tissue (autograft) or tissues from donors (allograft). Both methods have limitations, mainly a lack of sufficient "spare" healthy tissue in the case of autografts and a limited number of donors, technical limitations of organ transplantation, and the need for immunosuppressive medication in the case of allografts. In contrast to those traditional methods, tissue engineering uses scaffold materials to control cell proliferation, differentiation, migration, and adhesion, as well as the growth of suitable extracellular matrix. Graphene and graphene-delivered materials have been proposed in tissue engineering techniques for bone, nervous and cardiac tissues.[38]

In several in vitro studies, graphene-based materials have been shown to promote growth and osteogenic differentiation of mesenchymal stem cells.[39] In some studies, a special matrix composed of poly (L-lactic-co-glycolic acid), hydroxyapatite and GO was built, where GO increased the tensile strength of the material.[40] The matrix promoted protein adsorption, induced osteogenic function, and accelerated the proliferation and differentiation of human mesenchymal stem cells.

The incapacitating nature of many degenerative neurological diseases (such as Alzheimer's, Parkinson's, Huntington's, or amyotrophic lateral sclerosis) makes the potential regeneration of central nervous system cells and tissues a very interesting area of study. The effective differentiation of neural stem cells into uniaxially arranged neurons remains a substantial challenge. Nevertheless, some studies have reported promising results. As an example, a novel composite scaffold structure including aligned electrospun silk nanofibers and conductive reduced graphene paper enhanced the directional growth and differentiation of neurons.[41] The presence of neuron marking proteins was confirmed in this study as well as the alignment and conductivity of the axons. In another study, a graphene-based hybrid nanofibrous scaffold promoted differentiation into oligodendrocytes.[42]

Muscle tissue engineering has great potential value for treating damage and degeneration of skeletal muscles and many internal organs in which smooth muscle cells constitute an important part. Differentiation of murine myoblasts into myotubes on a graphene-containing platform was reported in 2 studies.[43,44]

Given the prevalence of cardiovascular disease and its significant impact as one of the leading causes of mortality and morbidity in modern industrialized societies, any new treatment modality could have a huge impact on the healthcare system. Advanced heart failure is often associated with poor prognosis. This is mainly due to the fact that damage to cardiac tissue is irreversible in adults. Therefore, any progress in cardiac tissue engineering would be eagerly welcomed by cardiologists. Some interesting results in this area have been achieved with the use of graphene-derived materials. Biocompatibility and conductivity remain an important challenge for any cardiomyocyte scaffold. Such scaffolds using reduced GO were built by 2 groups and proved to be a satisfactory microenvironment for cardiomyocyte culturing.[45,46] It was also found that GO improves the engraftment

of mesenchymal stem cells used to repair ischemia–reperfusion injury of the heart.[47]

However, in another experiment on mouse embryos, graphene decreased the stem cell proliferation, probably by accelerating cell differentiation. The graphene also enhanced the mechanical properties and electrical conductivity of the tissue. Interestingly, the cardiac differentiation of the embryonic bodies with graphene was significantly greater than for those without graphene applied. The result was confirmed by high-throughput gene analysis.[48]

In a study by Norahan et al., a collagen–GO composite cardiac patch showed angiogenic activity. Such a property might be useful when the patch is applied in the area of post-infarction injury.[49]

In a rat model (both in vivo and in an isolated heart), a polymer chitosan scaffold containing GO was implanted in an infarcted heart. It was characterized by better (twofold) conductivity than conventional chitosan scaffolds. It supported cell attachment and growth and showed no signs of toxicity; improved conductivity and contractility were also demonstrated.[50]

An interesting effect was found by Ray et al.: GO interacted favorably with the His118 residue of NDPK to potentially prevent it from binding with adenosine triphosphate (ATP). As ATP would otherwise trigger the phosphorylation of the mutated G protein, the observed effect might eventually lead to increased cAMP levels during heart failure.[51]

Applications in cardiology: Electrotherapy

Modern electrotherapy is based on the presence of leads in the vascular system. Lead failure, infections and tricuspid valve insufficiency are the most common complications that arise. In patients with heart failure, the outcome of such infections may even be fatal, hence the different concepts of leadless pacing methods and the need to research new materials. Graphene and its derivatives, with known antimicrobial properties, might be considered interesting candidate materials for electrotherapy devices (both electrodes and device cans).

Graphene has been successfully used for sensors, including devices that provide a real-time electrocardiogram (ECG) signal.[45] On a zebrafish model, Chen et al. proved the concept of using a graphene microprobe to obtain high-resolution electrophysiological signals, including ECG, in vivo.[31] This result provides a strong incentive for further research.

Park et al.[47] presented an extremely interesting concept that could become a form of electrotherapy in the treatment of heart failure. An epicardial mesh made of mechanically elastic and electrically conductive material was integrated with the heart and acted as a structural element with elastic properties similar to those of epicardial tissue. The mesh detected the electrical signals of the moving rat heart and synchronized electrical stimulation over the ventricles. This design shortened total ventricular activation time, reduced wall stress and improved several indices of systolic functioning. The mesh was also successfully used to deliver a shock in order to terminate ventricular tachycardia. The features of this design constitute a comprehensive form of electrotherapy, currently a standard in heart failure treatment. The concept was very a sophisticated form of cardiomyoplasty – an idea of externally stabilizing cardiac muscle to prevent post-infarction remodeling.

Another potentially very promising field of research is the attempt to fabricate implantable protein-based bioelectrochemical capacitors (bECs) employing new nanocomposite heterostructures. In this model, 2D reduced GO sheets were interlayered with chemically modified mammalian proteins.[52] The GO nanocomposite material showed no toxicity to mouse embryo fibroblasts. These unique capacitors, being protein-based devices, use serum as an electrolyte. They may be the first step in developing bio-friendly, protein-based batteries or supercapacitors using human bodily fluids as electrolytes. In the future, such solutions might eliminate the need to replace the electrotherapy devices when the battery is drained.

Applications in cardiology: Endovascular implants

As many disorders of the cardiovascular system are primarily caused by atherosclerosis, endovascular therapy remains crucially important in cardiology. The state-of-the-art coronary stents are characterized by very satisfactory mechanical properties and their biocompatibility is much better than that of the first bare-metal stents and the first drug-eluting stents designed almost 2 decades ago. Nevertheless, even after what seems to be a successful angioplasty, late complications such as restenosis and thrombosis may eventually lead to life-threatening events. The current understanding is that interactions between the stent surface, the cells of the vascular wall, and circulating blood are the underlying causes of these events. The quest for a perfect stent material and coating remains a leading research topic in interventional cardiology. Given the very structure of graphene itself (very thin sheets) and its chemical dissimilarity from both metal and the polymers so far investigated as stent materials, it seems logical to study the performance of graphene-related materials as coatings for cardiovascular stents.

A method of coating a stainless steel stent with graphene and graphene with TiO_2 was developed by ElSawy et al. The researchers examined the hemocompatibility of such coated stents and found neither platelet adhesion nor an adverse effect on erythrocytes or leukocytes.[53]

In a study by Yang et al. a composite stent coating including GO performed better than other composite coatings – it had lower platelet adsorption and induced longer activated partial thromboplastin times (APTT) than other designs.[54]

In another experiment, a novel composite drug-eluting coating was composed from magnetic mesoporous silica nanoparticles and carbon nanotubes. The nanostructured coating proved to be mechanically flexible and biocompatible with blood as well as offering very good drug release and loading properties. In vivo studies showed rapid endothelization.[55]

Pro-healing properties of GO coatings have also been investigated on a titanium stent. The coating enhanced endothelial cell adhesion and proliferation as compared with a polydopamine coating and the titanium blank. Loading heparin onto the GO coating significantly reduced platelet adhesion and prolonged the APTT, but it did not influence endothelial cell adhesion and proliferation. It was concluded by the authors that the heparin-loaded GO coating can simultaneously enhance the cytocompatibility to endothelial cells and blood compatibility of the biomaterials.[56]

Another interesting design was evaluated by Ge et al. They covered a stainless steel stent with graphene and an antiproliferative drug. The stents were successfully implanted into rabbit carotid arteries. Several weeks after implantation, there were significantly fewer smooth muscle cells and less fibrin on the study stents than on the uncovered control stents. Most likely, the observed effect was due to the action of the antiproliferative drug, but the experiment demonstrates the feasibility of a graphene-coated drug-eluting stent. It is also worth noting that no toxic effects of the GO were observed on histological examination of the rabbit organs.[57]

In a study performed by authors of this review, it was shown that graphene coating on 316L stainless steel supported the adhesion and proliferation of human primary coronary artery endothelial cells to a greater extent than the uncoated substrate. It was also proven that the coated surface has a unique potential to affect the endothelial cell phenotype by diminishing the endothelial-to-mesenchymal transition, thus possibly reducing the risk of in-stent restenosis.[58]

As an example of a different approach, Misra et al. attempted to use graphene and similar materials, not as a coating but as a scaffold. A personalized 3D-printed cardiovascular stent was built from a biodegradable polymer carbon composite doped with graphene nanoplatelets (which ensured the controlled release of 2 types of drugs – antiproliferative and antirestenotic).[59]

Artificial heart valves represent another type of intravascular implants where limited biocompatibility may lead to adverse events. Graphene oxide was tested as a potential coating for heart valves in a study by Wilczek at al. Platelet adhesion and activation was found to be similar to that of the control materials.[60]

All those examples show that endovascular implant coating with graphene-derived materials is feasible and can offer satisfactory biocompatibility. Some results suggest that graphene promotes vascular healing and prevents both restenosis and thrombosis.

Summary

As this concise review indicates, graphene is a novel material with unique physical and chemical properties. Its biocompatibility has not yet been thoroughly investigated, but it seems that at least low concentrations are well-tolerated by cells and multicellular organisms. There are many biomedical applications proposed for graphene and its derivatives. They include areas as diverse as antimicrobial materials, physical and chemical sensors, targeted therapy and drug delivery, regeneration-promoting tissue scaffolds, and endovascular implant coatings. Many studies have demonstrated that at least basic conceptions of potential use of graphene-derived materials seem to be correct and reproducible on cellular and tissue scales. Nevertheless, only experiments involving whole multicellular organisms (of which very few have been conducted so far) will yield valid conclusions on the potential use of graphene in future diagnostics and therapy.

ORCID iDs

Jacek Arkowski https://orcid.org/0000-0003-2761-7580
Marta Obremska https://orcid.org/0000-0003-1937-1891
Kamil Kędzierski https://orcid.org/0000-0003-1340-7021
Agnieszka Sławuta https://orcid.org/0000-0001-5671-9864
Magdalena Wawrzyńska https://orcid.org/0000-0001-6855-5510

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