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Historical perspective

# Antimicrobial graphene family materials: Progress, advances, hopes and fears

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ABSTRACT

Graphene-based materials have become very popular bionanotechnological instruments in the last few years. Since 2010, the graphene family materials have been recognized as worthy of attention due to its antimicrobial properties. Functionalization of graphene (or rather graphene oxide) surface creates the possibilities to obtain efficient antimicrobial agents. In this review, progress and advances in this field in the last few years are described and discussed. Special attention is devoted to materials based on graphene oxide in which specifically selected components significantly modify biological activity of this carbon structure. Short introduction concerns the physicochemical properties of the graphene family materials. In the section on antimicrobial properties, proposed mechanisms of activity against microorganisms are given showing enhanced action of nanocomposites also under light irradiation (photoinduced activity). Another important feature, i.e. toxicity against eukaryotic cells, is presented with up-to-date data. Taking into account all the information on the properties of the described materials and usefulness of the graphene family as antimicrobial agents, hopes and fears concerning their application are discussed. Finally, some examples of promising usage in medicine and other fields, e.g. in phytobiology and water remediation, are shown.



**Contents**



*Abbreviations:* ESBL, extended spectrum β-lactam; ESKAPE, a group of the most frequent nosocomial pathogens *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp.; $G^-$, Gram-negative bacteria; $G^+$, Gram-positive bacteria; CFU, colony forming unit; CNSs, carbon nanoscrolls; EDTA, ethylenediaminetetraacetic acid; GO, graphene oxide; GrO, graphite oxide; GONMs, graphene oxide-based nanomeshes; GQDs, graphene quantum dots; MDR, multidrug resistant; MEF, mouse embryo fibroblast; MIC, minimal inhibitory concentration; MIC50, concentration that inhibits 50% of bacterial isolates; MRSA, methicillin-resistant *Staphylococcus aureus*; NIR, near infrared; NPs, nanoparticles; Pc, phthalocyanine; PEG, polyethylene glycol; rGO, reduced graphene oxide; rGrO, reduced graphite oxide; ROS, reactive oxygen species; SWCNT, single-walled carbon nanotube; MWCNT, multi-walled carbon nanotube; Vis, visible; WHO, World Health Organization.

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## 1. Introduction

Multidrug resistant (MDR) pathogens are a significant problem in infections worldwide. In 2009, the Infectious Diseases Society of America highlighted 'ESKAPE' pathogens. Six of the pathogens, the most frequently found in hospitals and the most difficult to treat, were included in this group: two of the Gram-positive bacteria ($G^+$: *Enterococcus faecium* and *Staphylococcus aureus*) and four of the Gram-negative species ($G^-$: *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp.) [1]. Multidrug resistance among pathogens is one of the major problems in therapeutics. The MDR group of pathogens (not related to 'ESKAPE' and nosocomial infections) cannot be treated with typical antimicrobials. These pathogens are resistant to most of the known and used antibiotics (e.g. *Escherichia coli* as MDR to carbapenems and cephalosporins [2]). As indicated in the report from Southern Asia [3], the most popular MDR bacteria strains from wounds of the hospitalized patients are 5 of the 'ESKAPE' pathogens: methicillin-resistant *S. aureus* (MRSA) in the $G^+$ group and extended spectrum β-lactam resistant *Enterobacteriaceae* in the $G^-$ group (ESBL: *K. pneumoniae*, *A. baumannii*, *P. aeruginosa*, *Enterobacter cloacae*, and *Proteus mirabilis*). According to WHO (data from 2015), mortality among patients infected with MDR pathogens is two-fold higher than among those infected with drug-sensitive strains of bacteria.

Antibiotics have been known for decades and their overuse and irrational application are causing increasing multidrug resistance in bacteria and fungi. Therefore, there is a strong need to find alternative ways for killing pathogens. Local and global events or programs, such as the National Program for Antibiotics Protection in Poland or the European Antibiotics Awareness Day on November 18th and World Antibiotic Awareness Week, have been established to draw attention to this problem.

Fortunately, due to the development of nanobiotechnology and novel bioactive materials, we can put into practice more and more ideas and solutions. One of the new possibilities in the research area that have appeared in the last few years is based on the graphene family materials that might be found in diagnostics, therapeutics or industry as (1) antibacterial killing factors, (2) drug and gene delivery systems, (3) bioimaging and photothermal therapy agents, (4) materials for tissue engineering, and (5) biosensors [4–7]. Due to their bactericidal and cell anti-adhesive properties, they are promising materials for other applications, such as components for food packaging foils and additives to drugs, textiles or medical and dentistry equipment.

A few reviews on biologically active carbon-based materials have already been published [4,5,8–14]. Using this information, as well as recently published data and our experience in graphene oxide and antibacterial nanomaterials, we would like to briefly present antimicrobial properties of the graphene family materials and to draw attention to the promising applications of these structures and their functionalized derivatives, and to the worries related to their usage.

## 2. Graphene oxide and other graphene family nanomaterials

### 2.1. Short characterization of graphene materials

Graphene, a layer of single-atom-thick carbon atoms closely packed into a honeycomb two-dimensional lattice, is the main representative of the carbon-based family nanomaterials. Other 2D structures which belong to this group are few-layer-graphene, ultrathin graphite, graphene oxide, graphite oxide, and reduced graphene oxide, as well as carbon nanotubes. They have been hailed as materials of the future due to their unique thermal, mechanical, electrical or optical properties. Biological studies have recently shown that they are also promising candidates for different medical applications, e.g. as antimicrobial agents.

The properties of graphene are a result of the $sp^2$ hybrid carbon framework, whereas in graphene oxide (GO) and graphite oxide (GrO), a large fraction (0.5–0.6) of carbon is $sp^3$ hybridized and covalently bonded with oxygen in the form of epoxy (C—O—C) and hydroxyl (C—OH) groups. The remaining carbon is $sp^2$ hybridized and bonded either with neighboring carbon atoms or with oxygen in the form of carboxyl (—COOH) and carbonyl (C═O) groups which predominantly decorate the edges of graphene sheets. GO is therefore a 2D network of small $sp^2$ carbon domains in a $sp^3$-bonded matrix.

GrO can be easily obtained from graphite flakes at high yield under oxidizing conditions and three main synthesis routes can be pointed out. The modified Hummers method [15,16] is the most conventional way, where a strong oxidant $KMnO_4$ is used for graphite oxidation in the presence of $H_2SO_4$, $NaNO_3$, and $H_2O_2$. The second method is based on the work performed by Brodie [17] who investigated the reactivity of flake graphite by adding potassium chlorate to slurry of graphite in fuming nitric acid. A similar procedure, but with adding chlorate in multiple aliquots over the course of the reaction (in the presence of concentrated $H_2SO_4$ to increase the acidity of the mixture), was used by Staudenmaier [18]. Importantly, it has been demonstrated that the products of these reactions show strong variance, depending not only on the particular oxidants used, but also on the graphite source (natural or synthetic graphite flakes) and reaction conditions. Different lateral sizes (from several nanometers up to several micrometers) or the composition of GO structures affects physical, chemical, optical, and electrical properties of the nanosheets.

Oxidized graphite usually retains its stacked structure (Fig. 1a). Chemically, the two oxides – graphite oxide and graphene oxide – are similar, but GO flakes are usually one or a few layers thick. Graphite oxide can be exfoliated to graphene oxide (Fig. 1b) by thermal, mechanical or sonochemical methods [19,20]. In this review, we have tried to use the correct term – GrO or GO – to name the described materials. However, in the literature authors sometimes do not specify which oxide forms they have in mind and in this case, we decided to use the GO definition.

The surface oxygen functionality of GO and GrO ensures its hydrophilicity, so the flakes can be easily dissolved (at low concentration) or dispersed in water or other polar solvents. Thus, the oxides can be successfully used to be further functionalized, to form nanocomposites or can be uniformly deposited from water-based solutions as thin films on different substrates. The epoxy, hydroxyl, and carbonyl groups of platelets as well as double bonds on the GO and GrO sheets are chemically active. An ideal approach to the chemical modification of graphene (graphite) oxide would utilize reactions of these groups [19,21]. GO (and other graphene-based materials) can also exhibit non-covalent binding via π–π or σ–π stacking and van der Waals interaction [22–24]. The non-covalent approach offers a non-destructive way to modify the surfaces of carbon-based materials. A number of surface modifiers including aromatic compounds, small-molecular surfactants, amphiphilic polymers, and biomacromolecules have already been described [25,26]. These features of GO and GrO – different than in the case of graphene – make this material particularly interesting. Therefore, we would like to focus mainly on this carbon material.

### 2.2. Antimicrobial properties

The mechanism of antibacterial activity of graphene materials is still not well explained. In the literature, few possibilities have been

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