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Review

Terms of endearment: Bacteria meet graphene nanosurfaces

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ABSTRACT

Microbial multidrug resistance poses serious risks in returning the human species into the pre-antibiotic era if it remains unsolved. While conventional research approaches to combat infectious diseases have been inadequate, nanomaterials are a promising alternative for the development of sound antimicrobial countermeasures. Graphene, a two-dimensional ultra-thin nanomaterial, possesses excellent electronic and biocompatibility properties, which position it in the biotechnology forefront for diverse applications in biosensing, therapeutics, diagnostics, drug delivery and device development. Yet, several questions remain unanswered. For instance, the way these nanosurfaces interact with the microbial entities is poorly understood. The mechanistic elucidation of this interface seems critical to determine the feasibility of applications under development. Are graphene derivatives appropriate materials to design potent antimicrobial agents, vehicles or effective diagnostic microsensors? Has the partition of major microbial resistance phenotypic determinants been sufficiently investigated? Can toxicity become a limiting factor? Are we getting closer to clinical implementation?

To facilitate research conducive to answer such questions, this review describes the features of the graphene–bacterial interaction. An overview on paradigms of graphene–microbial interactions is expected to shed light on the range of materials available, and identify possible applications, serving the ultimate goal to develop deeper understanding and collective conscience for the true capabilities of this nanomaterial platform.

1. Introduction

The rise and re-emergence of infectious diseases are causing millions of deaths around the globe, every year while standard antimicrobial therapies are losing the battle against an increasing number of bacterial infections [1]. The ability of microbes to adapt and evolve in the presence of antimicrobial agents has been identified as a critical clinical threat. The rise of bacteria, resistant to multiple classes of antibiotics, the so-called "superbugs", is responsible for increasing rates of illness, mortality and high healthcare costs, as well as for the emergence of chronic diseases and repeated or prolonged hospitalizations [2]. Superbugs render standard antibiotics ineffective, and conventional diagnostic tools redundant and inefficient, therefore, biomedical research is shifting focus into new dogmas about infectious diseases [3,4].

Nanomedicine raises expectations to the development of innovative therapies in critical areas including bacterial and viral infections, cancer, cardiovascular diseases and tissue regeneration [5]. Nanomedicine is a business sector with rapid expansion estimated to reach $219 billion by 2019 [6,7].

Nanoscale size and multivalency of nanomaterials are responsible for increasing efficiency and reducing adverse effects of small molecule drugs. In general, nanosystems have been found to

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enhance drug's solubility, stability, biodistribution and pharmacokinetics [8,9]. Moreover, nanomaterials bearing multiple moieties, such as imaging agents and specific targeting groups, enable the material to bind to diseased tissues, cancer cells or bacterial cell walls, while imaging *in vitro* or *in vivo* localization through fluorescence or magnetic resonance imaging (MRI) [10,11]. A broad array of nanomaterials with promising medical applications have populated the literature, including liposomes, dendrimers, hydrogels, polymeric nanoparticles, metallic nanoparticles, mechanized nanoparticles, polyrotaxanes, tridimensional DNA-based nanostructures, fullerenes, carbon nanotubes, and graphene derivatives [12–30].

There has been an increasing progress in the application of nanomaterials in medicine; however, a number of factors have limited the development of medically useful nanotherapeutic systems. In particular, in infectious diseases where the critical problem of evolving multidrug resistance is ongoing, there is a battleground for the discovery of tailored nanomaterials with defined properties for identifying, targeting and fighting superbugs [31]. The nanomaterial clinical pipeline remains thin, and as the number of nanomaterial derivatives is increasing testing has become more rigorous to follow the pace of development. Nevertheless, there has been a steady increase in the clinical approval rate of nanomaterials by the Food and Drugs Administration (FDA) [32].

Nanomaterials based on carbon allotropes have been tested for microbial sensing applications, and provided materials with enhanced antimicrobial properties [33–35]. Carbon, due to its valency, is able to form several allotropes resulting in structures with different shapes and properties. Allotropes of carbon include *graphite*, with bonded carbon atoms in a hexagonal lattice forming layered two-dimensional (2D) sheets, *graphene* that consists of single sheets of graphite and *buckminsterfullerenes* where the carbon atoms are bonded in a hexagonal lattice forming three-dimensional (3D) hollow structures that can be spheres (C_{60}), ellipsoids (for example C_{70} and C_{84}) or tubular (carbon nanotubes) (Fig. 1).

Stemming from the scarcity of studies focused on biomedical applications of graphene-based nanomaterials, this review article is an effort towards: (i) better describing the antimicrobial properties of nanomaterials based on graphene as the epicenter of carbon allotropes, (ii) mapping and evaluating the interaction between the graphene family materials (GFM) and bacteria, and (iii) outlining the principles of GFM bio-functionalization for bacterial sensing and antimicrobial efficacy [33,36–38]. A discussion about the strengths and weaknesses of the graphene platform in direct comparison with the existing carbon allotropes lineage is presented. This analysis identifies knowledge gaps and opportunities for the development of efficient biocompatible, safe graphene-based diagnostic and therapeutic technologies.

2. GFM chemical properties

GFM comprise all 2D materials that contain the basic graphene sheet backbone such as pristine graphene (pG), few-layer graphene (FLG), graphene nanosheets (GNS), graphene oxide (GO) and reduced graphene oxide (RGO) (Fig. 2) [39]. These 2D materials have been thoroughly investigated due to their outstanding mechanical, electronic, thermal and optical properties [40–43]. Their detailed chemical structure and individual morphology significantly affect their properties, interaction with biomolecules, and consequently their antimicrobial activity. GFM can also vary in layer number, layer dimensions, and purity, which are the most relevant properties in terms of their behavior in biological applications [44].

2.1. Pristine graphene (pG)

G is a 2D monolayer of sp^2-hybridized carbon atoms tightly arranged in a flat hexagonal structure, similar to a honeycomb lattice. It is the thinnest material in the world - only one carbon atom thick – although the graphene sheet area may be up to 1 cm^2 [45–47]. Graphene can be obtained through micromechanical exfoliation of graphite, chemical vapor deposition (CVD), epitaxial growth or chemical intercalation [48–51].

2.2. Graphene oxide (GO)

GO bears oxygen-containing functional groups on the basal plane and edges resulting in a mixed sp^2-sp^3 hybridized carbon nanosheet, and it can be considered as an insulating and disordered analogue of the highly conducting crystalline pG [52]. GO contains an uncertain number of water molecules intercalated between the oxidized carbon layers, and variable type and coverage of oxygen containing functional groups that result from differences in synthetic procedures. Oxygen content in GO is usually high, typically characterized by C:O atomic ratios less than 3:1, and commonly closer to 2:1. According to the widely accepted Lerf-Klinowski structural model, GO contains two types of regions: (i) an aromatic region, with flat non-oxidized benzene rings, and (ii) a wrinkled region, with alicyclic six-member rings bearing double bonds, hydroxyl, and ether groups [53,54]. The relative size of each region depends on the degree of oxidation. The distribution of functional groups in every oxidized aromatic ring is not identical, and both the oxidized rings and aromatic entities are distributed randomly throughout the GO nanosheets. Therefore, each fundamental GO layer contains a dense 2D carbonaceous skeleton with more sp^3 than sp^2 carbons, and hydroxyl and carboxyl functional groups.

GO can be obtained through oxidation of graphite using four different methods, which include the usage of concentrated H_2SO_4 along with: (i) concentrated HNO_3 and $KClO_3$ oxidant (Hofmann method); (ii) fuming HNO_3 and $KClO_3$ oxidant (Staudenmaier

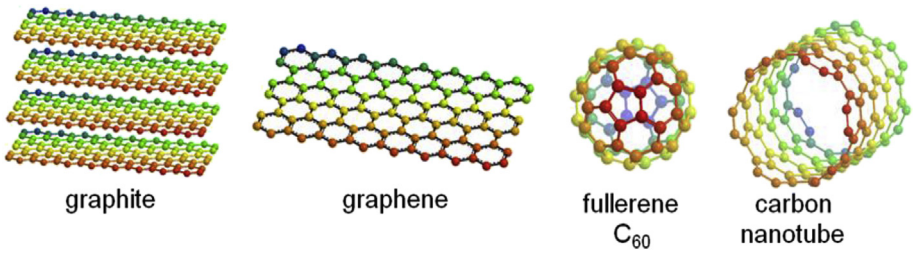

Fig. 1. 3D models of carbon allotropes graphite, graphene, fullerene C60 and carbon nanotubes (color by depth). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)