NanoImpact 5 (2017) 109–118

Contents lists available at ScienceDirect

NanoImpact

journal homepage: www.journals.elsevier.com/nanoimpact

Review article

Immunological effects of graphene family nanomaterials

Jabran Saleem [a], Liming Wang [b,*], Chunying Chen [a,*]

[a] CAS Key Laboratory for Biomedical Effects of Nanomaterials and Nanosafety & CAS Center for Excellence in Nanoscience, National Center for Nanoscience and Technology of China, Chinese Academy of Sciences (CAS), Beijing 100190, PR China
[b] CAS Key Laboratory for Biomedical Effects of Nanomaterials and Nanosafety, Institute of High Energy Physics, Chinese Academy of Sciences (CAS), Beijing 100049, PR China

ARTICLE INFO

Article history:
Received 22 September 2016
Received in revised form 26 December 2016
Accepted 25 January 2017
Available online 26 January 2017

Keywords:
Graphene family nanomaterials
Immune system components
Immunomodulation

ABSTRACT

Graphene and its derivatives are called graphene family nanomaterials (GFNs). Over the past few years, they have been heavily investigated in biomedical arena due to their extraordinary physiochemical properties and potential biomedical applications. However, the biocompatibility of GFNs is becoming important for biomedical applications such as drug and gene delivery, tissue engineering, biosensing and imaging. In this regard, it is crucial to understand the process of interaction of GFNs with immune system, which is also meaningful to manipulate their interaction for safe and efficient applications. Herein, different modalities of GFNs interaction with various components of immune system and the outcome of these interactions are described and evaluated. This review also summarizes different mechanisms involved in immunological effects of GFNs and techniques that are employed for GFNs, to escape the clutches of immune system. We elucidate the intricate balance between immune-stimulation and immune-suppression and expect that understanding of immunological effects of graphene derivatives would help evaluate and estimate the possible biomedical applications as far as immune system is concerned.

Contents

1. Introduction

Nowadays, engineering nanomaterials have been reported to have unique and novel characteristics and hold promises in broad applications in many fields. From the esteemed family of nanomaterials, one shining star is graphene, often called “miracle material”-nicknamed so, for its extraordinary properties. It is a single atom thick sheet of sp^2-hybridized carbon atoms arranged in a continuous series of hexagons, resembling a honey comb structure. It has become one of the world's most attractive materials (Lee et al., 2008) with great electrical and thermal conductivity (Schweirz, 2010; Baladin et al., 2008) and is impermeable even to an atom as small as helium (Bunch et al., 2008). All of these properties make it very attractive for potentially various applications. Graphene and its derivatives, collectively can be termed as graphene family nanomaterials (GFNs), which include single or few layered graphene (FLG), graphene nanoribbons (GNRs), graphene nanoplatelets (GNPs), graphene oxide (GO), reduced graphene oxide (rGO), graphene quantum dots (GQDs) and a few more of their sort. GFNs have gained promising applications in many

* Corresponding authors.
E-mail addresses: wangliming@ihep.ac.cn (L. Wang), chenchy@nanoctr.cn (C. Chen).

http://dx.doi.org/10.1016/j.impact.2017.01.005
2452-0748/

fields, such as photonics/plasmonics (Bonaccorso et al., 2010; Grigorenko et al., 2012), electronics (Jang et al., 2016), sensors Shao et al. (2010), catalysis (Machado and Serp, 2012), drug delivery (Liu et al., 2013) and DNA sequencing (Heerema and Dekker, 2016). GFNs can be prepared chemically and their properties can be changed by number of synthesis techniques (Sun et al., 2011).

Study of the interaction between GFNs and biological systems has rapidly increased recently in scientific community. As carbon nanomaterials vary in their lateral size, layer number, defect density, surface chemistry, purity and composition, their interaction with biological systems becomes complicated, validating a detailed investigation for their health effects or safety (Zhao et al., 2015). Toxicity profiles of GFNs have been well assessed by researchers over the years and means of biocompatibility have also been elucidated (Guo and Mei, 2014; Seabra et al., 2014). The most important factor, however, when it comes to using a GFN in human body, is the nature of its interaction with immune system.

Animals, higher in the evolutionary tree, have developed a very sophisticated defense mechanism to ward off any foreign entity. It distinguishes foreign bodies like bacteria, viruses and parasites from body's tissue and is a layered system with cascade of barriers making it hard for these invaders to affect body's physiological functions. As a component of immune system, phagocytic cells like macrophages and dendritic cells help clear the foreign bodies with the help of complement system, which is a set of proteins dedicated for better recognition of these foreign bodies by these cells. Moreover, once phagocytic cells are properly stimulated, they can recruit another type of cells in this fight, called lymphocytes – a layered defense system as already mentioned. Nanomaterials are known to be immunostimulant as well as immunosuppressant (Jiao et al., 2014; Hussain et al., 2012) and so are GFNs. That fact makes it complicated to understand their biological outcome after interaction with immune system (Fig. 1).

Immunomodulation induced by GFNs (immunostimulation or immunosuppression) can, however, be enhanced by biofunctionalization or changing their physiochemical parameters. Specific modulation of immune system by GFNs can also be beneficial as it may lead to development of immunotherapeutic agents and vaccines. Here, in this review, we describe the immunological outcomes of the interaction of GFNs with various immune system components as well as mechanism of immunomodulation.

2. Interaction of GFNs with immune system components

Immune system comprises both innate and adaptive defense part. In innate immunity, macrophages and monocytes are recruited to infection site through production of special class of chemical mediators called cytokines. Then, complement system is activated that ultimately results in phagocytosis of the antigen by phagocytic cells. Adaptive immunity includes recruitment of antigen specific T and B lymphocytes which help in release of cytokines and antibodies. This kind of immunity may exist over a larger time span by remembering the antigen in terms of specific memory of antibodies. In this respect, a detailed insight into the relationship between GFNs and immune system components is importance. It has been known that surface chemistry and dose of carbon nanomaterials, govern its bioaccumulation and toxicity in vivo (Zhao et al., 2015) As most immune system components work together to determine the fate of GFNs, step by step account of the interaction of GFNs with these components is supplied here.

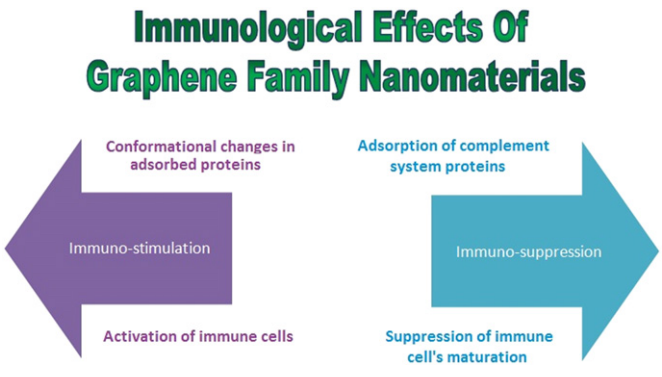

Fig. 1. A schematic diagram showing immunological routes followed by GFNs, when they interact with immune system.

2.1. Complement system

Complement system comprises over 40 proteins, which are produced by liver and found in blood circulatory system. It works as a cascade system in which, involved proteins trigger one after another, helping phagocytosis of foreign body by phagocytic cells. This system seals the fate of antigen in three different ways; opsonization, inflammation or/and cytolysis. Three major pathways are followed in this regard, namely; classical pathway, alterative pathway and lectin pathway. In this way complement system is crucial to surveillance and homeostasis of the body (Ricklin et al., 2010). The dynamics of interaction of nanomaterials with complement system is necessary to understand, if nanomaterials are to be employed in nanomedicine (Moghimi and Hunter, 2011). Nanomaterials have been known to interact with complement system in a variety of ways (Thomas et al., 2014), however, only few studies have shed light on the interaction of graphene with complement system. Recent studies of interaction of complement system with CNTs have been widely reported. It has been shown that single walled CNTs can trigger classical pathway whereas double walled ones can trigger both classical and alternative pathway (Salvador-Morales et al., 2006). Functionalization grants CNTs some protection from complement system (Andersen et al., 2013).

Like CNTs, PEGylation affords graphene some relief from the complement system activation. Tan et al. (2013) showed that that un-functionalized GO, when incubated with human sera, was able to adsorb a large number of serum proteins and induced C3 cleavage. Comparably, PEGylated GO showed much less protein adsorption and greatly reduced the C3 cleavage. About six serum proteins, however, got adsorbed onto PEGylated GO with great specificity, four of which were complement proteins. These complement proteins like C3a/C3a(des-Arg), when adsorbed to PEGylated GO, became unavailable for their receptors, which reduced the complement activation by PEGylated GO. This study showed that functionalization of GO can tune complement activation. Another study showing effect of functionalization on complement system activation was performed by Chowdhury et al. (2013), where they used dextran functionalized graphene nanoplatelets (GNP-Dex). Three concentrations of GNR-Dex (1, 7 and 10 mg/mL), were treated with blood samples from two healthy individuals and levels of SC5b-9 were measured as a reflection of complement activation (SC5b-9 is a complement protein produced in classical, alternate and lectin pathways). An increase of 20% in complement activity, as compared to untreated blood, for sample 1 and 12% for sample 2 was recorded at the concentration of 1 mg/mL of GNR-Dex. However, this 20% (474 ng/mL) and 12% (504 ng/mL) increase of SC5b-9 levels still are in normal range (176 to 624 ng/mL). To test whether dextran is responsible for complement activation, the effect of various concentrations of dextran on two blood samples was also evaluated. As dextran comprises about 40% weight of GNR-Dex, three concentrations of dextran (0.4, 2.8 and 4.0 mg/mL) were used for the experiment. An increase in alternate pathway activation (evaluated by measuring levels of Bb, which is a complementary protein special to alternate pathway only) of about 11% and 25% for sample 1 and that of 23% and 37% for sample 2 was observed at two higher concentrations.

In another study by Chowdhury et al. (2015), graphene nanoplatelets (GNPs) were non-covalently functionalized with PEG-DSPE (1, 2-distearoyl-*sn*-glycero-3-phosphoethanolamine-*N* [amino