Colloids and Surfaces B: Biointerfaces 171 (2018) 260–275

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

Functionalization of graphene family nanomaterials for application in cancer therapy

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ARTICLE INFO

Keywords:
Cancer therapy
Graphene oxide
Nanostructures
Photothermal therapy
Tumors

ABSTRACT

Graphene family nanomaterials' (GFN) ability to interact with near-infrared light has propelled their application in cancer photothermal therapy. Furthermore, the graphitic lattice of GFN can adsorb different types of molecules, which has motivated their use in cancer drug delivery. However, the direct application of GFN in cancer therapy is severely hindered by their poor colloidal stability, sub-optimal safety, inefficient tumor uptake and non-selectivity towards cancer cells. To overcome these limitations, GFN have been functionalized with different types of materials. This review is focused on the different functionalizations used in the design of GFN aimed for application in cancer therapy, disclosing their role on surpassing the critical issues related to GFN-based therapies.

1. Introduction

Graphene family nanomaterials (GFN) have been receiving a growing attention in various fields, including cancer therapy [1–6]. In this context, GFN have been explored as photothermal agents due to their near infrared (NIR; 750–1000 nm) absorption [7]. The responsiveness of GFN to NIR light is crucial since this type of radiation has weak or insignificant interactions with biological components (e.g. water, proteins, melanin), thus ensuring minimal off-target heating and deep tissue penetration during the photothermal therapy [7]. Moreover, GFN have also been employed as delivery vehicles due to their ability to adsorb drugs (and other molecules) on their surface [8]. Compared to other drug delivery systems, GFN display a high loading capacity, which has been attributed to their large surface area [9,10].

Despite the enormous potential of GFN, the poor water solubility and weak stability of these materials limit their use in cancer therapy [11,12]. Moreover, as-synthesized GFN are not suited to become passively accumulated in the tumor site nor tailored to be selectively internalized by cancer cells [13–16]. Furthermore, depending on the surface chemistry, size or impurities content, some GFN have shown to be cytotoxic [17–20]. To overcome these limitations, researchers have been modifying the surface of GFN with a variety of materials.

In this review, the different functionalizations performed to allow an efficient application of GFN in cancer therapy and enhance their therapeutic performance and safety are discussed. Firstly, the physicochemical properties of GFN as well as the most convenient routes for functionalizing these materials are analyzed (Section 2). Afterward, the functionalization of GFN for application in cancer therapy and its implications are discussed (Section 3). The functionalizations performed to improve GFN hydrophilicity and colloidal stability are discussed in Section 3.1, and those that aim to improve their biocompatibility and hemocompatibility are analyzed in Section 3.2. The approaches employed to enhance the blood circulation time and tumor uptake of GFN are reviewed in Section 3.3. The functionalizations that improve GFN cellular uptake and selectivity towards cancer cells are discussed in Section 3.4. Finally, an overview about the future directions are presented (Section 4). For the sake of brevity, this review does not cover modifications that aim to improve GFN tumor accumulation by means of external forces (e.g. by using magnetic fields), nor those that enhance GFN therapeutic performance through the incorporation of other nanostructures (e.g. gold nanostructures). Functionalization routes that are not used when designing GFN aimed for cancer therapy are also not analyzed.

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https://doi.org/10.1016/j.colsurfb.2018.07.030
Received 15 May 2018; Received in revised form 13 July 2018; Accepted 14 July 2018
Available online 17 July 2018

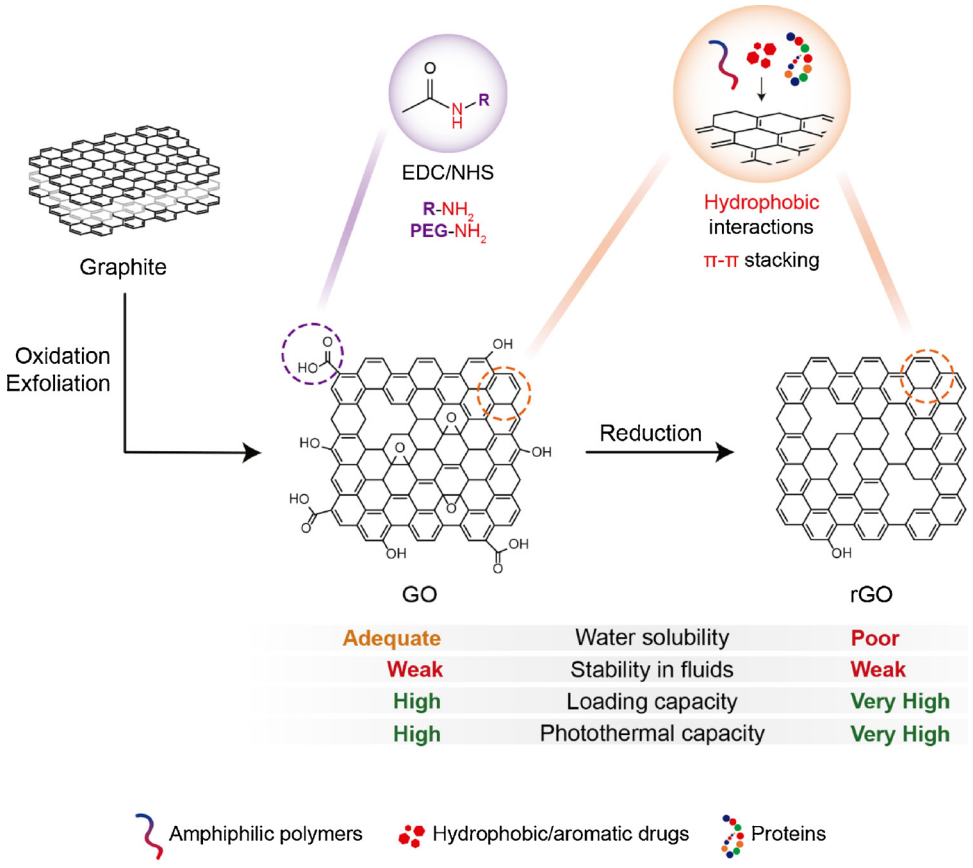

Fig. 1. Schematic representation of the synthesis of GO and rGO. The reduction of GO provokes alterations on physicochemical properties of the materials, which impact on their application in cancer therapy. The most commonly used routes for functionalizing GO and rGO are also represented. 1-ethyl-3-(-3-dimethylaminopropyl) carbodiimide (EDC), Graphene Oxide (GO), *N*-Hydroxysuccinimide (NHS), reduced GO (rGO).

2. GFN: physicochemical properties and functionalization routes

Graphene oxide (GO) is probably the most applied GFN for cancer therapy and it is also used as the precursor for the synthesis of other graphene-based nanomaterials. GO is composed by a monolayer of graphite containing several types of oxygen functional groups such as carboxyl, hydroxyl or epoxy groups (Fig. 1). This material is generally produced through the chemical oxidation of graphite and exfoliation of the resulting material (graphite oxide), into a single layer material (GO). Over the years, several methods have been developed to synthesize GO, being the Hummer's and the improved Hummer's (also known as Tour's method) methods the most commonly employed [21,22].

GO aromatic lattice can adsorb several types of molecules through hydrophobic interactions or π-π stacking. A variety of cancer-relevant molecules such as chemotherapeutic drugs or proteins can be loaded on GO by exploring these non-covalent interactions [8]. Therapeutic agents may also be covalently conjugated to the carboxyl-groups of GO. Nevertheless, such approach is not usually pursued since these groups may be required for the covalent functionalization of GO with other materials (e.g. hydrophilic polymers). Furthermore, the NIR absorption of GO also allows its application as a photothermal agent or as a light-responsive delivery vehicle in cancer therapy [23,24].

However, the direct applicability of GO in cancer therapy is limited by its poor colloidal stability since it promptly precipitates in biological fluids [11]. Moreover, GO has a sub-optimal biocompatibility and hemocompatibility, as well as inefficient tumor uptake and non-selectivity towards cancer cells (discussed in detail in Section 3). These limitations can be surpassed by functionalizing GO with different types of materials (discussed in Section 3). Based on its structure, GO is generally functionalized through the formation of amide bonds established between the carboxyl groups of GO and primary amines of other materials using 1-ethyl-3-(-3-dimethylaminopropyl) carbodiimide (EDC; a water soluble carbodiimide crosslinker) chemistry [25]. These oxygen functional groups may also be conjugated with polymerization initiators, allowing the direct growth of polymers on GO surface [26,27]. Alternatively, GO can be non-covalently functionalized with amphiphilic materials [28].

GO derivatives such as base treated GO and carboxylated GO (GO-COOH) have also been explored in cancer therapy due to their improved properties [29–31]. Base treated GO is obtained by washing GO with sodium hydroxide, a process that removes the oxidation debris from GO lattice, improving its loading capacity [32,33]. GO—COOH is produced by reacting GO with chloroacetic acid or sodium chloroacetate [34–36]. This modification introduces additional carboxyl groups for chemical conjugation and improves GO NIR absorption [34–36]. However, these GO derivatives suffer from the same stability, biocompatibility, tumor uptake and selectivity problems that affect GO, which impose their functionalization.

Reduced GO (rGO) is another GFN applied in cancer therapy. rGO is generally obtained by treating GO with reducing agents such as hydrazine hydrate (for the sake of clarity, *rGO* will refer to rGO attained using hydrazine hydrate) [12,37]. Other reducing agents such as ascorbic acid or dopamine have also been successfully applied in the reduction of GO [14,38–40]. The reduction of GO aims to restore its graphitic lattice by removing the oxygen functional groups, and such procedure drastically changes the properties of the materials (Fig. 1). In fact, when compared to GO and its derivatives, rGO displays a higher NIR absorption and loading capacity, conferring an improved photothermal and drug delivery potential to this material [12,41]. However, rGO has a weak water solubility (and stability) and its application in cancer therapy is also hindered by the same factors that affect GO, thus demanding its functionalization (discussed in detail in Section 3). As rGO has limited (or close to none) oxygen functional groups available for conjugation, this material is generally functionalized with amphiphilic polymers through non-covalent interactions (hydrophobic