BBA - General Subjects 1861 (2017) 2334–2341

Contents lists available at ScienceDirect

BBA - General Subjects

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

Graphene oxide nanosheets in complex with cell penetrating peptides for oligonucleotides delivery

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ARTICLE INFO

Keywords:
Graphene oxide
Cell penetrating peptides
Transfection
Gene delivery

ABSTRACT

A new strategy for gene transfection using the nanocarrier of cell penetrating peptides (CPPs; **P**ep**F**ect14 (PF14) or **P**ep**F**ect14 (PF14) (PF221)) in complex with graphene oxide (GO) is reported. GO complexed with CPPs and plasmid (pGL3), splice correction oligonucleotides (SCO) or small interfering RNA (siRNA) are performed. Data show adsorption of CPPs and oligonucleotides on the top of the graphenic lamellar without any observed change of the particle size of GO. GO mitigates the cytotoxicity of CPPs and improves the material biocompatibility. Complexes of GO-pGL3-CPPs (CPPs; PF14 or PF221) offer 2.1–2.5 fold increase of the cell transfection compared to pGL3-CPPs (CPPs; PF14 or PF221). GO-SCO-PF14 assemblies effectively transfect the cells with an increase of > 10–25 fold compared to the transfection using PF14. The concentration of GO plays a significant role in the material nanotoxicity and the transfection efficiency. The results open a new horizon in the gene treatment using CPPs and offer a simple strategy for further investigations.

1. Introduction

Carbon nanomaterials such as graphene (G) and graphene oxide (GO) advanced many fields such as energy, biosensing, catalytic, and biomedical applications [1–4]. GO has a high aspect ratio and surface area which is almost 10 fold of other nanomaterials [5] with high biocompatibility [6]. They provided smart system for drug delivery and site-specific controlled drug delivery systems (DDSs) [7], offered bimodal photodynamic and photothermal destruction of tumors [8]. The conjugation of graphene layers with biomolecules or other nanoparticles are simple and advance multifunctional applications [9–13]. Precoating of GO with proteins showed regulatory control of nanomaterial-triggered complement activation [14]. GO served as a modulator against protein misfolding and aggregations [15,16]. The non-covalent interactions of GO with proteins inhibited in vitro formation of the amyloid fibril in peptide solution [17]. GO based nanomaterials interact strongly with biomolecules such as ssDNA via π-π interactions [18]. Functionalized GO offered the site-specific controlled release of chemotherapy drugs such as doxorubicin (DOX) for an adenosine triphosphate (ATP) rich environment [19]. **S**mall **i**nterfering **R**NA (siRNA) encapsulated with GO and modified porous silicon nanoparticles (GO–pSiNPs) showed the controlled release of the oligonucleotide payloads in vitro by a factor of 3 [20]. Graphene-based nanomaterials advanced gene delivery and showed successful transfection to the target cells [21,22] and offered specific targeting [23]. Furthermore, it improved the dispersion of insoluble drugs [24]. A few formulations based on G nanomaterials are being evaluated for the gene delivery.

Viral and non-viral vectors have been applied for the cell transfection of gene based therapeutic such as oligonucleotides (ONs) [25]. Non-viral gene delivery vectors cause low immunogenicity compared to viral gene delivery vectors [25]. Several types of non-viral vectors, including lipids, polymers, carbon-based nanomaterials, and other inorganic nanoparticles, have been reported [26,27]. Non-viral gene delivery vectors provide higher loading capacity and are easy for fabrication [26]. Among the large number of non-viral vectors, cell penetrating peptides (CPPs) are considered as efficient nanocarriers for gene therapy. CPPs vectors provided strong interactions with cellular lipid bilayers, could penetrate the cell membrane, and showed a long blood circulation time [28]. They showed small side-effects such as inflammation, or immune response [28]. CPPs offered a wide range of peptides (1850 peptide entries were listed on CPPsite 2.0 database) [29], and multifunctionality [28]. However, most of the CPP-cargo complexes are entrapped into the endosomes post endocytosis and degraded into the lysosome, and thus decreased their efficiency [30]. One

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http://dx.doi.org/10.1016/j.bbagen.2017.07.002
Received 13 April 2017; Received in revised form 27 June 2017; Accepted 4 July 2017
Available online 06 July 2017

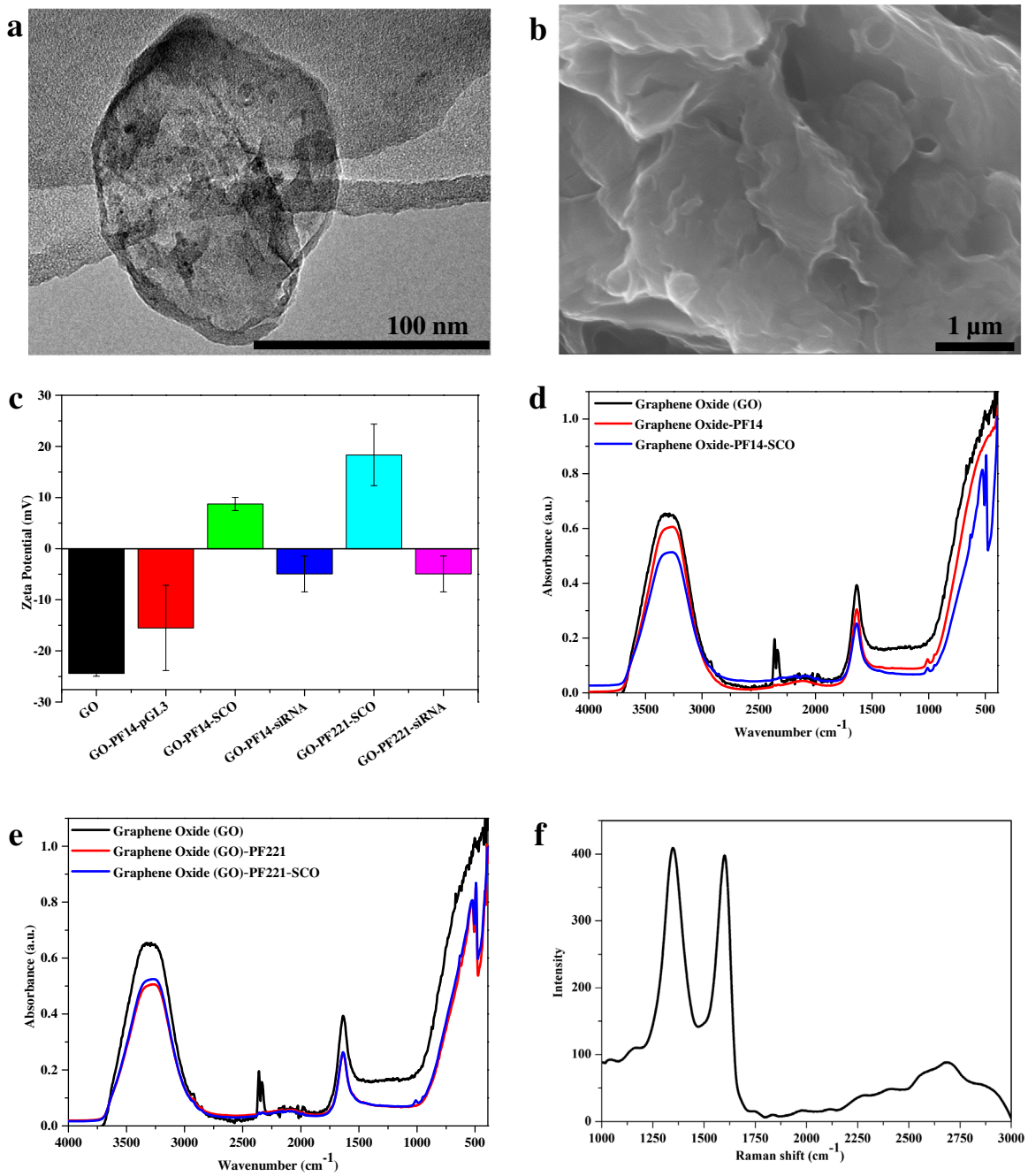

Fig. 1. Characterization of GO using a) TEM, b) SEM, c) zeta potential, d–e) FTIR for GO, and the interactions of GO and CPPs, and f) Raman spectrum of GO.

way to increase the endosomolytic activity of CPPs is to create heterogeneous multi-vectors via the combination of CPPs with other non-viral vectors such as GO.

Herein, GO combined with a cell penetrating peptide (**P**ep**F**ect14 (PF14), PepFect221 (PF221)) for the gene delivery for plasmid (pGL3), **s**plice **c**orrection **o**ligonucleotides (SCO), and siRNA are reported. The materials were characterized using a wide range of analytical tools. The complexes of GO-ONs-CPPs have been applied for gene delivery. GO mediated the efficiency of the complexes of CPPs-oligonucleotides and mitigated their cytotoxicities. GO-ONs-CPPs offered several magnitude enhancements (1.5–25 fold) of CPPs-ONs. The new combination of CPPs and GO offer multi-vector and offer high efficacy delivery system.

2. Materials and methods

Graphite was purchased from Alfa Aeser. Phosphate-buffered saline (PBS, P/S), methanol, trypsin (0.25%) and fetal bovine serum (FBS) were obtained from Sigma-Aldrich (Germany). Luciferase expressing plasmid (pGL3, 3 MDa, Promega, Sweden), splice correction oligonucleotides (SCO, 5′-CCU CUU ACC UCA GUU ACA, 6.5 kDa), or small interfering RNA (siRNA, 5′-GGA CGA GGA CGA GCA CUU CUU, 13 kDa Microsynth AG, Switzerland) were used without purification.

2.1. Synthesis of graphene oxide (GO)

GO was synthesized according to our previous publication [31] using Hummers method with modification [32]. Briefly, graphite (1 g) was dispersed in an acid mixture of nitric acid (10 ml, 69–72%) and sulfuric acid (15 ml, 96.0%). The mixture was stirred during the addition of potassium permanganate (3 g, ≥ 99%, 5 h, and temperature < 0 °C). Then, excess of hydrogen peroxide (30–32%) was added to remove unreacted $KMnO_4$. The precipitates were collected and washed