Plasmid 84–85 (2016) 51–60

Contents lists available at ScienceDirect

Plasmid

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

Graphene oxide–cationic polymer conjugates: Synthesis and application as gene delivery vectors

Mohsen Teimouri [a,b,1], Azadeh Hashem Nia [a,b,1], Khalil Abnous [a], Hossein Eshghi [b], Mohammad Ramezani [a,*]

[a] Pharmaceutical Research Center, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran
[b] School of Science, Department of Chemistry, Ferdowsi University of Mashhad, Mashhad, Iran

ARTICLE INFO

Article history:
Received 4 January 2016
Received in revised form 7 March 2016
Accepted 23 March 2016
Available online 9 April 2016

Keywords:
Graphene oxide
Functionalization
Polyethylenimine
Gene delivery
Polyamidoamine
Polypropylenimine

ABSTRACT

Nanomedicine as the interface between nanotechnology and medical sciences is a new area that has attracted the attention of vast groups of researchers. Carbon nanomaterials are common platform for synthesis of nanoparticles for biomedical applications due to their low cytotoxicity and feasible internalization into mammalian cell lines (Yang et al., 2007; Arora et al., 2014; Oh and Park, 2014). Synthesis of vectors based on various cationic polymers polyethylenimine (PEI), polypropylenimine (PPI) and polyamidoamine (PAMAM) and their derivatives were considered as a strategy for transferring plasmid DNA and treatment of genetic diseases. Considering the low cytotoxicity of graphene, chemical modification of its surface has led to fabrication of novel gene delivery systems based on graphene and graphene oxide. Herein we report the synthesis of three groups of vectors based on conjugation of graphene oxide (GO) with alkylated derivatives of three different cationic polymers (polyethylenimine (PEI), polypropylenimine (PPI) and polyamidoamine (PAMAM)) through different linkers including surface carboxyl group, glycine and spermidine. Two main challenges in design of gene delivery vectors is decreasing cytotoxicity while improving the transfection efficiency. All synthesized vectors showed significantly lower cellular toxicity compared to bare polymer. A plasmid encoding green fluorescent protein (GFP) was used to evaluate the transfection efficiency of nanoparticles both qualitatively using live cell fluorescent imaging and quantitatively using flow cytometry and each vector was compared to its polymer base. Most successful conjugation strategy was observed in the case of PEI conjugates among which most efficient vector was PEI–GO conjugate bearing glycine linker. This vector was 9 fold more effective in terms of the percent of EGFP transfected cells.
© 2016 Elsevier B.V. All rights reserved.

1. Introduction

Graphene, as a single-atom-thick and two-dimensional sp^2 carbon-based material, has attracted tremendous attention due to its extremely high flexibility, mechanical flexibility, optical transparency and chemical stability (Bolotin et al., 2008; Esmaeili and Entezari, 2014; Nair et al., 2008). Moreover, similar to carbon nanotubes (Yang et al., 2007; Arora et al., 2014; Oh and Park, 2014), its superior properties such as high surface area, capability of biofunctionalization and delocalized π electrons systems, can be beneficial for applications in drug delivery (Akhavan et al., 2012; Bao et al., 2011; Kuila et al., 2011; Li et al., 2008; Liu et al., 2008).

In recent years, many studies have shown that graphene can be used as a safe and effective hybrid matrix with cationic polymers to pave the way for the delivery of gene into different cells (Chang et al., 2010; Chen et al., 2011; Mao et al., 2010; Sun et al., 2008). In particular, pristine graphene material is incapable of functioning in biological trails because of low solubility and high tendency to agglomerate. Consequently, many studies have focused on functionalization of graphene oxide (GO) (Geng et al., 2009; Kuilla et al., 2010; Worsley et al., 2007). There are two main approaches used to functionalize graphene including covalent and non-covalent functionalization (Liu et al., 2012). Non-covalent modification methods are usually utilized when there is a need for preserving the electrical conductivity and large surface of graphene. On the other hand, covalent methods are normally preferred when the stability and the robust mechanical properties of modified graphene are demanded (Liu et al., 2012). GO possesses large groups of carboxylic acid at its edges as well as epoxy and hydroxyl groups on the surface (Park and Ruoff, 2009). It was shown that GO interacts through π–π interactions with plasmid DNA (Varghese et al., 2009). Many investigations have shown that both covalent and non-covalent functionalized graphene oxides are able to condense plasmid DNA for intracellular gene delivery (Chen et al., 2011; Feng et al., 2011; Kim et al., 2011; Liu et al., 2014).

Gene therapy is one of the most serious challenges in nanotechnology and biomedicine. In recent years, non-viral vectors have become an alternative therapeutic strategy which has taken the place of the usual approaches of viruses as gene carriers.

Despite the high efficiency of viral vectors in gene transfer to eukaryotic cells, they have several drawbacks including inflammatory

* Corresponding author.
E-mail address: ramezanim@mums.ac.ir (M. Ramezani).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.plasmid.2016.03.002
0147-619X/© 2016 Elsevier B.V. All rights reserved.

reactions and carcinogenicity, which must be overcame before their widespread use (Behnam et al., 2013; Glover et al., 2005; Verma and Somia, 1997). Although a non-viral gene transfer has low yields, their efficacy can be tuned by structural modifications. Cationic polymers and dendrimers such as PEI, PAMAM and PPI have the ability to form polyplexes with DNA in nanoscale sizes which are appropriate for in vitro and in vivo gene transfer applications (Verma and Somia, 1997; Boussif et al., 1995; Godbey et al., 1999). Among these, high molecular weight PEI 25 kDa has high transfection efficiency. Dendrimers are spherical branched molecules with repetitive units that are mostly symmetrical around the center. In principle, dendrimers bearing amine groups have the highest density of positive potential charges (Šebestík et al., 2012). It is believed that cationic polymers transfer gene through "proton sponge" mechanism (Behr, 1997). A main factor in enhancing the gene transfection efficiency is the stability of GO-based nano-construct carriers. It has been reported that the appropriate size of PAMAM dendron attached to the oleic acid (graphene–oleate–polyamidoamine) had a remarkable effect on biocompatibility and transfection efficiency (Liu et al., 2014). Besides attachment of linkers to GO through acylation and amidation reactions increases the stability, it also preserves buffering capacity of carriers by adding predominantly more amine groups to them.

To the best of our knowledge, there has been no prior investigation on the comparison of three types of low molecular weight dendrimers with different linkers. Application of linkers with different chain length will provide a distance between GO surface and polymer and may lower the shielding effect of GO sheet for surface amine groups.

To achieve this objective, spermidine and glycine were grafted onto the surface and edge of GO through the formation of amide linkages between the terminal carboxylate using 1-ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride (EDC) and N-hydroxy succinimide (NHS) chemistry (Fig. 1). In first approach, dendrimers were directly attached onto graphene oxide through amide bond, while in second and third approaches the conjugation was carried out via glycine and spermidine linkers, respectively.

The characterization of functionalized GO was established by Fourier transform infrared (FT-IR) spectroscopy, transmission electron microscopy (TEM), zeta potential measurement, and ethidium bromide exclusion assay. It was assumed that conjugation of polycations with oligoamine- and glycine-modified GO can improve transfection efficiency while lowering cellular toxicity.

2. Materials and methods

2.1. Material

A natural graphite powder (particle diameter of ≤20 μm) was purchased from Loba-Chem (India). G3 PPI dendrimer was purchased from SyMO-Chem BV (Eindhoven, Netherlands). PAMAM dendrimers (ethylene diamine core, G3), branched PEI with an average molecular weights of 25 and 1.8 kDa, glycine, 2-bromoacetic acid, 1-ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride (EDC), N-hydroxy succinimide (NHS), spermidine, methylthiazoletetrazolium [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] (MTT; tissue culture grade) and all other solvents at the highest available purity were purchased from Sigma-Aldrich (Munich, Germany). Dulbecco's modified Eagle's medium and fetal bovine serum were purchased from GIBCO (Gaithersburg, USA). Plasmid pRL-CMV (Renilla luciferase under the control of the cytomegalovirus enhancer/promoter), and luciferase assay kit were obtained from Promega (Madison, WI, USA). Ethidium bromide (EtBr) was supplied by Cinnagen (Tehran, Iran). Dialysis membranes with various MWCO were purchased from Spectrum Laboratories (Houston, USA).

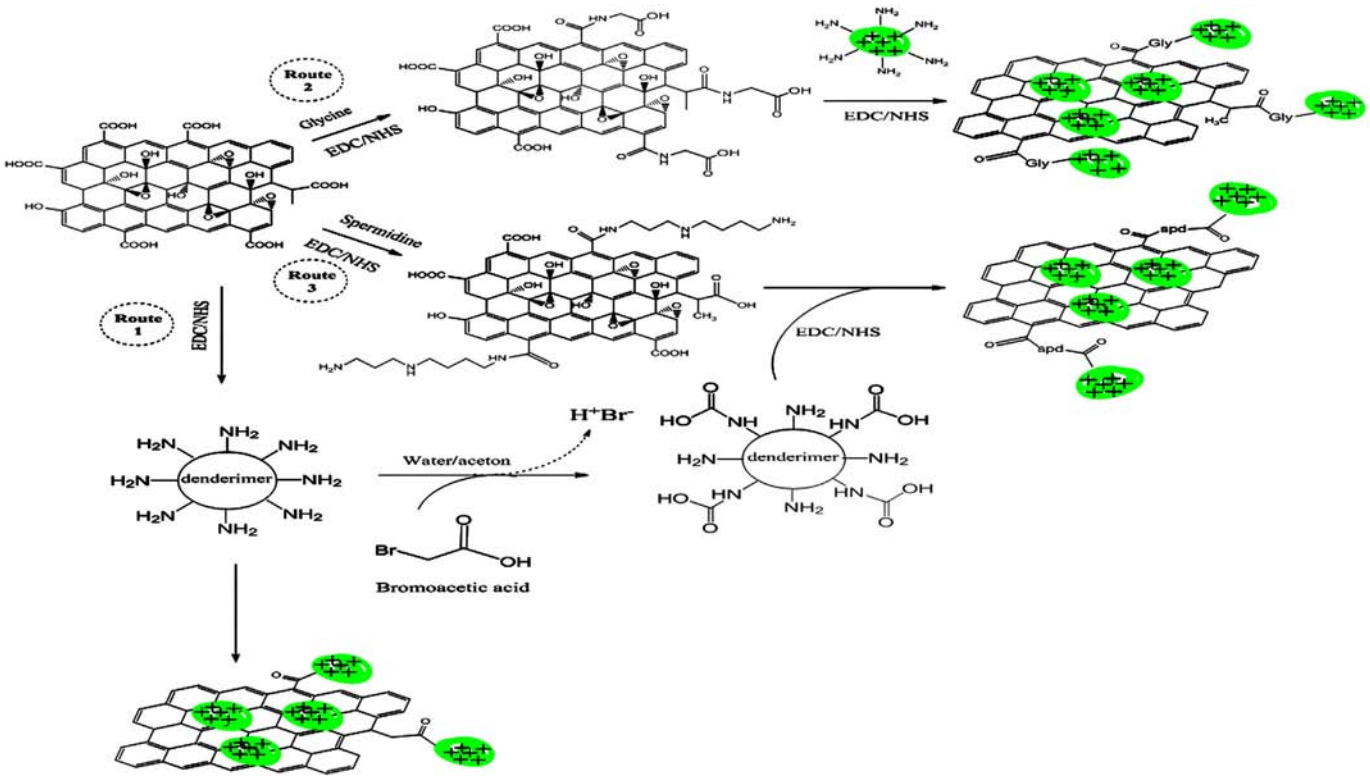

Fig. 1. Schematic illustration of the three approaches in the synthesis of GO–polycation conjugates (Route 1) and GO–Gly–polycation conjugates (Route 2) and GO–Spd–polycation conjugates (Route 3) via covalent attachment.

Download English Version:

https://daneshyari.com/en/article/2824047

Download Persian Version:

https://daneshyari.com/article/2824047

Daneshyari.com