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Novel glycol chitosan-based polymeric gene carrier synthesized by a Michael addition reaction with low molecular weight polyethylenimine

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ABSTRACT

A glycol chitosan-based polymer that spontaneously assembles with plasmid DNA into nanorods was evaluated as a non-viral vector for gene delivery. Glycol chitosan–methyl acrylate-polyethylenimine (GMP) was synthesized by grafting polyethylenimine onto glycol chitosan via amidation after Michael addition using methyl acrylate. Gel retardation and PicoGreen assay experiments showed complete complex formation with plasmid DNA. GMP/pDNA complexes were characterized using biophysical techniques and were found to be positively charged rod-shape structures with widths in the nanometer scale and lengths in the micrometer scale. Transfection efficiency and cytotoxicity of GMP polymer was evaluated in human epithelial ovary carcinoma (HeLa) cells, human embryonic kidney 293 (HEK293) cells, and human hepatocellular liver carcinoma (HepG2) cells, in comparison to high molecular weight polyethylenimine, a commonly used transfection reagent. Intracellular polymer uptake was compared and confirmed by confocal microscopy. The results demonstrate that GMP, a hybrid polymer of glycol chitosan grafted with branched polyethylenimine, may serve as a promising vehicle for efficient gene delivery.

1. Introduction

Genetic information is altered in many disease states and may require addition, correction or deletion as treatment (Patri, Kukowska-Latallo, & Baker, 2005; Yu et al., 2011). Thus, safe and efficient gene delivery vectors are needed (Eliyahu, Barenholz, & Domb, 2005). Indeed, gene delivery systems for cancer therapy have been the focus of active research (Conwell & Huang, 2005; Thomas, 2005).

There are two types of gene delivery vectors, viral and non-viral, and the major concern has been safety and efficiency of gene expression in target cells (Hess, 1996; Park, Jeong, & Kim, 2006; Xiang et al., 2003). Non-viral vectors are known to be less efficient than viral vectors (Hong, Gay, Karayan, Dabauvalle, & Boulanger, 1999; Vijayanathan, Thomas, & Thomas, 2002), because they have to be delivered through many membranous barriers (Barnard et al., 2011; Dailey et al., 2004). Typically, non-viral vectors are internalized by cells through endocytosis. The genetic payload must then be released from the endosome before it fuses with a lysosome. Such endosomal escape is mainly via a proton sponge effect or membrane destabilization (El-Sayed & Harashima, 2013; Michael & Curiel, 1994; Ziello, Huang, & Jovin, 2010). Although viral vectors show high gene expression efficiency, various non-viral vectors have been studied so far because they tend to be less immunogenic and cytotoxic (Borras, 2003; Dachs et al., 1997; Schatzlein, 2001; Tomanin et al., 2002).

Recently, polysaccharides have been investigated as polymeric vectors to deliver DNA, RNA and siRNA (Khor & Lim, 2003; Shigemasa & Minami, 1996). These include such polysaccharides as cellulose (Jere et al., 2009; Scherlund, Brodin, & Malmsten, 2000), chitosan and chitosan derivatives (Bernkop-Schnurch, 2000; Chen et al., 2012). Polysaccharides have the advantage of being biocompatible and biodegradable, but are limited by low solubility in water and complex chemical reactivity. Chitosan is a biocompatible and biodegradable material with low cytotoxicity (Kim, Ihm, Choi, Nah, & Cho, 2003; Lu et al., 2009b), and net positive charge. Thus, chitosan can interact with negatively charged biomolecules like DNA (Kato, Onishi, & Machida, 2003; Li et al., 2013). In addition, chitosan has been shown to protect DNA from nucleases and has been efficiently delivered into various cell types (Quong & Neufeld, 1998; Roy, Mao, Huang, & Leong, 1999; Saranya, Moorthi, Saravanan, Devi, & Selvamurugan, 2011). Glycol chitosan is a chitosan derivative with the added advantages of increased solubility in water and enhanced biocompatibility (Holmes & Tabrizian, 2013; Yoo,

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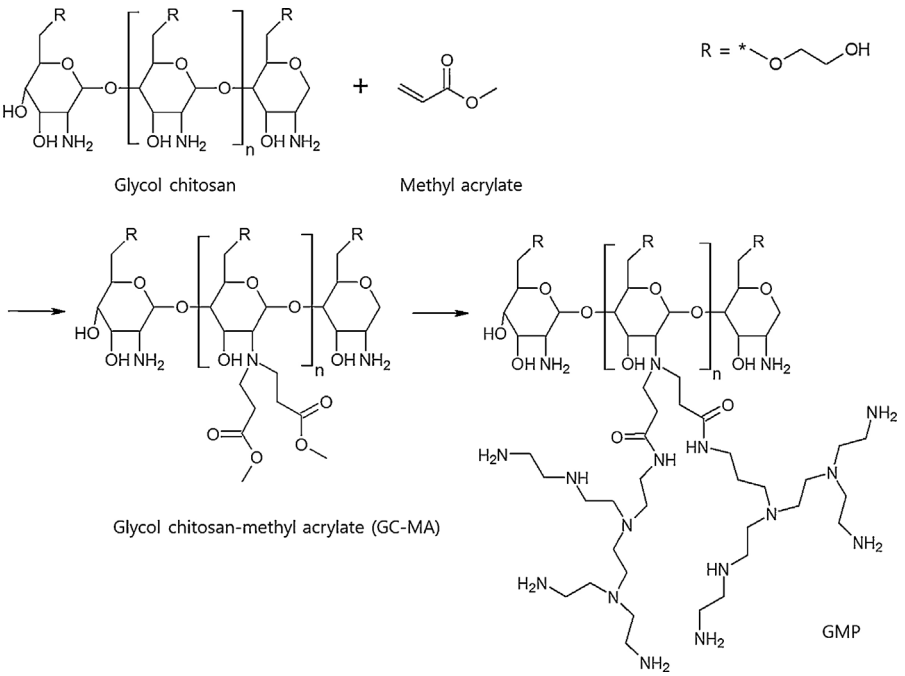

Scheme 1. Synthesis scheme of glycol chitosan (GC) conjugation with low-molecular weight polyethylenimine (PEI) via a methyl acrylate (MA).

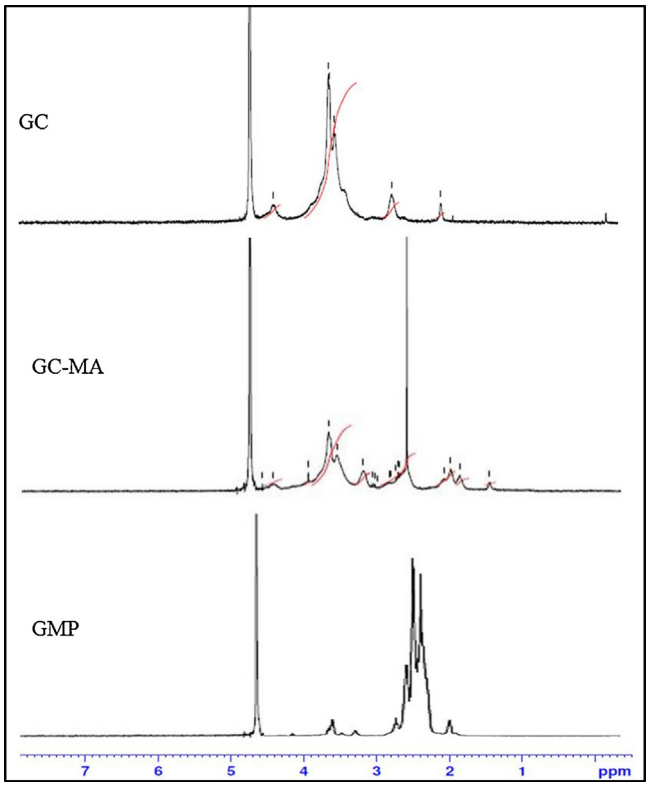

Fig. 1. ^{1}H NMR spectra of GC, GC-MA, and GMP.

Lee, Chung, Kwon, & Jeong, 2005; Zhang, Pan, Zhang, Luo, & Wang, 2008).

Polyethylenimine (PEI), a cationic polymer, is also an effective non-viral vector (Fischer, Bieber, Li, Elsasser, & Kissel, 1999; Lungwitz, Breunig, Blunk, & Gopferich, 2005) that binds negatively charged molecules by electrostatic interactions (Chen et al., 2012; Malek, Czubayko, & Aigner, 2008). The polymer has high transfection efficiency because of a proton sponge effect and high buffer capacity (Akinc, Thomas, Klibanov, & Langer, 2005). However, polyethylenimine is highly toxic and poorly biodegradable in vitro and in vivo (Neu, Fischer, & Kissel, 2005; Tripathi, Goyal, Kumar, & Gupta, 2012b), and is known that low molecular weight PEI has low cytotoxicity and it is not good for DNA condensing in transfection (Lu, Xu, Zhang, Cheng, & Zhuo, 2008).

In this study, we describe a new DNA delivery system using glycol chitosan and polyethylenimine that have been conjugated via methyl acrylate through a Michael addition reaction of the primary amine. Interestingly, the particles assumed a nanorod shape when the polymer self-assembled with plasmid DNA. The complexes are efficiently delivered via cellular uptake, as confirmed by confocal laser scanning microscopy. In addition, the conjugate polysaccharide polymeric vector displays robust gene transfection efficiency and negligible cytotoxicity in HeLa, HEK293, and HepG2 cells.

2. Materials and methods

2.1. Reagents

Glycol chitosan (GC, degree of deacetylation = 91.6%) (Lavertu et al., 2003), methyl acrylate (99%), methanol (anhydrous, 99.8%), 800 Da PEI (water-free, low-molecular weight polyethylenimine), and 25 kDa PEI (water-free, high-molecular weight polyethylenimine) were purchased from Sigma-Aldrich (Seoul, South Korea). The molecular weight of glycol chitosan (M_n = 198 kDa, M_w = 490 kDa, polydispersity index = 2.47) was determined by Waters GPC (Gel permeation chromatography) system using TSKgel G5000PWxl-CP and TSKgel G3000PWxl-CP columns (Korea Polymer Testing and Research Institute). The EZ-Cytox Enhanced Cell Viability Assay kit was sourced from Daeil Lab Service Co., Ltd (Seoul, South Korea). Lipofectamine™ 2000 reagent was purchased from Invitrogen (Carlsbad, CA). Fetal bovine serum (FBS), Dulbecco's modified Eagle's medium (DMEM), and 100× antibiotic–antimycotic agent were obtained from Gibco (Gaithersburg, MD, USA). The luciferase assay system kit was procured from Promega (Madison, WI, USA), while the Micro BCA Protein Assay Kit was obtained from Pierce (Rockford, IL, USA).