Chemico-Biological Interactions 244 (2016) 27–36

Contents lists available at ScienceDirect

Chemico-Biological Interactions

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

Polyurethane dispersion containing quaternized ammonium groups: An efficient nanosize gene delivery carrier for A549 cancer cell line transfection

Mahsa Yousefpour Marzbali [a, b, c], Ahmad Yari Khosroushahi [d, e, *], AliAkbar Movassaghpour [f], Hamid Yeganeh [g, **]

[a] Biotechnology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
[b] Student Research Committee, Tabriz University of Medical Sciences, Tabriz, Iran
[c] Department of Medical Nanotechnology, Faculty of Advanced Medical Science, Tabriz University of Medical Sciences, Tabriz, Iran
[d] Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
[e] Department of Pharmacognosy, Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran
[f] Hematology and Oncology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
[g] Iran Polymer and Petrochemical Institute (IPPI), P.O. Box: 14965/115, Tehran, Iran

ARTICLE INFO

Article history:
Received 12 August 2015
Received in revised form
29 October 2015
Accepted 25 November 2015
Available online 30 November 2015

Keywords:
Cationic polymers
Cytotoxicity
Gene delivery
Gene therapy
Transfection

ABSTRACT

A novel polyurethane containing cationic ammonium groups (QPU) was synthesized and used as vector for gene therapy and cancer gene targeting. The synthesized QPU was characterized by Fourier transform infrared and nuclear magnetic resonance spectroscopy methods. An agarose gel retardation electrophoresis assay was conducted to verify the complete complex formation between QPU and pDNA. The particles size and zeta potential of neat polymers, plasmid DNA, polymers/DNA polyplexes were determined by the dynamic light scattering technique. The polyplexes cytotoxicity was determined using [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay and its transfection efficiency was examined qualitatively by fluorescent microscopy and quantitatively by flow cytometery methods. The gel retardation assay, particle size and zeta potential measurements were confirmed that the synthesized cationic polymer could condense DNA efficiently in the physiologic condition. QPU polyplexes showed a significantly lower cytotoxicity compared to Polyfect® polyplexes in the examined human cancerous (A549) or normal cells (KDR). Based on our findings, the transfection efficiency by QPU was 2.2 fold higher than Polyfect® in the A549 cells whereas in the KDR cells, the cell transfection by Polyfect® was 18.1 fold higher than QPU. Due to low cytotoxicity for normal cells and high transfection efficiency in cancer cells, the potential applicability of designed QPU as a non-viral gene carrier for targeting of cancer gene therapy was confirmed.

1. Introduction

Gene therapy is an approach for treating diseases by correcting or modifying the defective genes through expressing the inserted genes into the cells' genome. Silencing and suppressing of not malfunctioning genes are other aims of gene therapy [1].

Discovering and introducing biosafe and efficient gene carriers are continuing challenges in gene therapy, for instance, the lack of proper biosafety of viral carriers limits their application for gene delivery into the human cells [2]. The non-viral carriers, based on cationic polymers [3] and lipids [4], with unique physical and biological properties are good alternatives for overcoming the main limitations of viral gene carriers. Formerly synthesized nano-size polycations like polyethylene glycol-polyethyleneimine (PEG-PEI) [5–7], several of Chitosans [8], and poly(2-(dimethylamino)ethyl methacrylate) (PDMAEMA) [9] could protect DNA against degrading enzymes. Conjugated polymer nanoparticles can provide the opportunity to inject genes into the cell through endocytosis [10]. Meanwhile, Biodegradable cationic polymers can assist the DNA

* Corresponding author. Faculty of Pharmacy, Tabriz University of Medical Sciences, Daneshgah Street, P.O. Box 51664-14766, Tabriz, Iran.
** Corresponding author. Polyurethanes Department Faculty of Polymer Science Iran Polymer & Petrochemical Institute (IPPI), P.O. Box: 14965/115, Tehran, Iran.
E-mail addresses: Yarikhosroushahia@tbzmed.ac.ir (A. Yari Khosroushahi), h.yeganeh@ippi.ac.ir (H. Yeganeh).

http://dx.doi.org/10.1016/j.cbi.2015.11.028
0009-2797/

release via pH buffering or "proton sponge" effects [11]. Low immunogenicity, stability in physiological solutions, capability for carrying large nucleic acid loads, and possibility of adapting their structural characteristics are other fascinating features of cationic polymeric gene carries [12,13].

Reducing toxicity and improving efficiency of such cationic polymeric carriers through controlling their biocompatibility and biodegradability are still challenging topics in this area [14–16]. Polyethylenimine (PEI) [17], poly(L-lysine) (PLL) [18], poly(-dimethylaminoethyl methacrylate) (pDMAEMA) [19], and their derivatives are examples of-cationic polymers widely used as gene carries. However, these carriers have their own disadvantages and limitations regarding their cytotoxicity [20] and biocompatibility balance as well as maintaining the efficient gene delivery property [21].

Polyurethanes (PUs) were conventionally used as scaffolds in tissue engineering [22–24], hydrogels [25], and drug delivery systems [26]. Desirable physico-chemical properties as well as excellent biocompatibility were the main driving forces for the wide application of polyurethanes in the aforementioned domains [27]. Interestingly, several kinds of cationic PUs have been synthesized and applied as gene delivery carriers [28]. For instance, the result of PU film-based gene delivery has shown that the film is a suitable platform for the localization of green fluorescent protein (GFP) vector delivery system which also prevents the systemic spread of vector in pig implants [29]. Other PU-based positively charged complexes, polyurethane 2-diethylaminoethylamine-polyurethane/DNA complexes with lower cytotoxicity than PEI/DNA complexes, have shown the capability of transfecting COS-7 cells *in vitro* [30]. In other studies, the researchers have demonstrated that the embedment of miR122 complexed with polyurethane-graft-short-branch polyethylenimine copolymer (PU-PEI) in nano-structured amphiphatic carboxymethyl-hexanoyl chitosan (CHC) led to dramatically enhanced microRNA (miR122) delivery into human dental pulp-derived Induced pluripotent stem cells (DP-iPSCs) and assisted these DP-iPSCs to differentiate into hepatocyte-like cells with mature hepatocyte functions [31]. In fact, cationic PUs have potential bio-features and positive charges which can be developed for non-viral vectors. The bio-safety, performance and transfection efficiency of these cationic PUs were dependent on crucial factors like types and amounts of embedded positive charge carriers, particle size, cytotoxicity, and biodegradability of these carriers [32]. The cationic PU possessing tertiary amines in the side chains have shown no significant cytotoxicity [33]. Meanwhile, they showed good hydrophilicity, and a moderate biodegradability to enhance the gene release efficiency in the target cells [34]. Cationic Pus, comprised of secondary amines in the backbone, with different molecular weight of tertiary amines in the side chain have also been prepared for establishing stronger electrostatic interaction between DNA and positively charged moieties of polymer [30,35]. The molecular weight of cationic PU carriers has played a significant role in the optimal DNA condensing ability and the protection of DNA from nuclease attack in a harsh cellular environment [30]. In addition, the cationic PU has also been combined with other cationic polymers such as PEI [36–38] for decreasing the cytotoxicity of PEI vector.

By considering the mentioned issues, this study aimed to synthesize and introduce quaternized polyurethane (QPU) thanks to possessing favorable biological properties like low cytotoxicity, high biocompatibility, and superior cell internalization ability for the effective delivery of genes into the mammalian cells. To evaluate the QPU gene transfecting efficacy, the green fluorescent protein (GFP) as a reporter gene was used in this study for facilitating the desirable assessments.

2. Materials and methods

2.1. Materials

Poly(tetramethylene ether) glycol (PTMEG, OH functionality = 2.0, M_n = 2000 g mol^{-1}) was purchased from Aldrich (British Drug House, Poole, UK) and dried by heating at 90 °C under vacuum overnight. Toluene diisocyanate (TDI mixture of 2,4 and 2,6-isomers) was purchased from Merck and purified by distillation under reduced pressure. N-Methyldiethanolamine (NMDA) and 1,4-buthane diol (BD) were purchased from Merck and freed from moisture by heating at 60 °C under vacuum. Glacial acetic acid and dibutyltin dilaureate (DBTL) were purchased from Merck and used as received. Tetrahydrofuran (THF) was purchased from Aldrich and dried via vacuum distillation over sodium wire.

Glycine, NaCl, chloroform, and diethylpyrocarbonate (DEPC) were obtained from Merck (Merck, Darmstadt, Germany). 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyltetra-zoplium bromide (MTT) was purchased from Sigma–Aldrich (St. Louis, MO, USA). RPMI-1640, penicillin and streptomycin and trypsin-EDTA solutions, L-glutamine, phosphate buffered saline (PBS), and fetal bovine serum (FBS) were purchased from Gibco Life Technologies Ltd. (Tulsa, OK, USA). All materials were analytical/cell culture tested grade and were used without further purification.

2.2. Characterization

Fourier transform infrared (FTIR) spectra were obtained using a Bruker IFS 48 instrument. All spectra were taken under air as a function of time with 16 scans at a resolution of 4 cm^{-1} and a spectral range of 4000–500 cm^{-1}. Nuclear magnetic resonance (^{1}HNMR) spectra were recorded on a Bruker model AVANCE DPX 400 MHz instrument with $CDCl_3$ as solvent. Spectra were averaged from 8 transients and calibrated by proton lock method.

2.3. Cell lines and culture conditions

A549 cells, human lung cancer cell line, and KDR cells, human normal epithelial cell line, were obtained from cell resource center of Pasteur institute of Iran (Tehran, Iran). The cells were cultured in RPMI 1640 medium supplemented with 10% (v/v) fetal bovine serum (FBS), and 1% penicillin–streptomycin (100 U/ml penicillin and 100 μg/ml streptomycin) and were incubated at 37 °C under a humidified atmosphere containing 5% CO_2. All treatments were performed on cells at logarithmic phase of the growth.

2.4. Plasmid

The pmaxGFP (Amaxa Biosciences, Cologne, Germany), as an expression vector for green fluorescent protein (GFP) with a molecular weight of 2300 kDa (3486 bp) was cloned in *Escherichia coli* (DH5α strain), then high-quality plasmid DNA for transfection was prepared using a QIAGEN Plasmid Mini Kit (Qiagen, Valencia, CA, USA) according to the kit user's guide. The concentration and purity of dissolved plasmid DNA (in Tris–EDTA buffer; 10 mM–1 mM, pH 8.0) were determined by measuring the photometric absorbance at 260 nm and calculating the ratio of the absorbance 260 and 280 nm, respectively [39]. The agarose gel electrophoresis analysis, having used the restriction enzymes, confirmed the size of 3.4 kb for linear plasmid DNA.

2.5. Synthesis of cationic polyurethane carrier (QPU)

Into a 250 ml four-necked polymerization kettle equipped with mechanical stirrer, reflux condenser, dropping funnel and N_2 inlet