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Enhanced *in-vitro* transfection and biocompatibility of L-arginine modified oligo (-alkylaminosiloxanes)-*graft*-polyethylenimine

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ABSTRACT

Branched poly ethylene imine (PEI) has been considered as the most efficient non-viral gene transfection agent. However, its clinical application is confined due to cytotoxicity. In the present study, we tried to enhance transfection efficiency and reduce toxicity of PEI by conjugating it with arginine modified oligo (-alkylaminosiloxane) [P(SiDAAr)n]. These derivatives were complexed with plasmid DNA and the resulting nanoparticles were characterised by dynamic light scattering (DLS), Atomic force microscopy (AFM), transmission electron microscopy (TEM), gel retardation and DNase I interaction to determine surface charge, particle size, morphology, complex formation and protection of DNA respectively. Among the four P(SiDAAr)n derivatives, nanoparticles of the P(SiDAAr)5/pDNA was found to exhibit 98% cell viability and around 150% more gene transfection than branched PEI in KB cell lines. Studies performed on transfection mechanism, using inhibitor study, clearly stated that the enhancement in transfection is due to the multiple pathways for cellular uptake which offered by the presence of uniformly spaced arginine moiety by oligo(-alkylaminosiloxane) arms. The nuclear localisation ability of the arginine residue was also established by using FITC stained nanoparticles on Hoechst 33342 stained nucleus.

1. Introduction

The development of safe and efficient gene delivery vectors is a prerequisite for the treatment of human genetic and acquired diseases. Non-immunogenicity, unrestricted plasmid size, and possibility of repeated administration makes non-viral gene delivery systems a safer alternative to viral vectors [1,2]. Cationic polymers are widely used as non-viral vectors. Poly ethylene imine (PEI) is regarded as the most effective one among non-viral vectors [3]. But the major drawbacks of PEI are its non-degradability, cytotoxicity, and aggregation. High transfection efficiency of PEI, along with its cytotoxicity, strongly depends on its molecular weight. The commercially available branched PEI having a molecular weight of 25 kDa, has been widely used as 'gold standard', but high toxicity of this homopolymer strictly limits its application in gene therapy [4]. Many studies have been attempted to reduce its cytotoxicity and maintain the transfection efficiency [3,5,6]. However, in most cases modified PEI is found to be less efficient than non-modified PEI. At the same time, several research groups have succeeded in enhancing the transfection efficiency of PEI by improving cell viability by its conjugation with poly ethylene glycol [7,8], by using targeted moieties [9,10], ester amine based modification [11] etc. Studies have shown better transfection than PEI at higher charge ratios, which leads to the higher usage of polymers.

Silicon derivatives, which belong to the largest class of industrial compounds, show generally low toxicity and biocompatibility [12,13]. Silica is an essential component of cells throughout the human body and amorphous silica is biodegradable and freely dispersible throughout the body which ultimately excreted by urine [14]. Kichler et al. reported that out of two main processes of siloxane synthesis such as hydrosilation and oligomerisation of alkoxysilanes, the oligoaminosiloxanes such as SiDA synthesized by alkoxysilane condensation gives useful products in the area of DNA vectorisation [15]. The oligoalkylaminosiloxane prepared by alkali hydrolysis delivers DNA into cells by adsorptive endocytosis through sulphated proteoglycans. Recently, several groups have reported the use of mesoporous silica matrix in carrying, protecting and releasing large amounts of cargo such as genes and membrane-impermeable chemicals [14,16–19]. Rosenholm et.al. reported that conjugating PEI with folic acid and mesoporous silica could improve endosomal escape, reduce toxicity and enhance targetability [14].

It is well known that arginine residues of tat peptides have the capability of intracellular translocation. [20–22]. Several studies conducted by conjugating arginine residues on dendrimers such as

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poly (amido amine) (PAMAM) dendrimer [23], poly (propylene imine) (PPI) dendrimer [24], and poly (L-lysine) (PLL) dendrimer [25], showed enhanced transfection efficiency than unmodified dendrimers. Similarly, linear polymers like, chitosan [26] and poly (ester-amide)s [27] also displayed high transfection efficiency when conjugated to arginine. The enhanced transfection may be due to the membrane permeability and nuclear localisation ability of arginine residues [28] despite their existence as oligopeptide form.

In this study, a non-viral system have been designed comprising reduced toxicity and adsorptive endocytosis of siloxane derivative, endosomal disrupting capacity of branched PEI, along with membrane permeability and nuclear localisation ability of arginine residues. Our objective has been attained by the synthesis of arginine conjugated oligo- (alkylaminosiloxane) and its conjugation with PEI at different compositions. (Scheme 1) Physicochemical properties of the polymer and its nanoparticles with DNA were thoroughly characterised. The biocompatibility, transfection efficiency, cellular uptake mechanism and nuclear localisation ability were also examined on comparison with PEI as control.

2. Materials and methods

2.1. Materials

3-(2-aminoethylamino)propyl-methyl-dimethoxysilane was from Fluka and branched PEI (Mw 25 kDa), arginine from Aldrich. Luciferase expression plasmid pGL3 control vector, Luciferase 1000 Assay System and Reporter Lysis Buffer were purchased from Promega, GFP expression plasmid, pEGFP-N3, (Clontech) were kindly provided by Dr. E. Sreekumar at RGCB (Thiruvananthapuram, India), 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), dicyclohexylcarbodiimide (DCC), N-hydroxysuccinimide (NHS),L-Arginine, and 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT), were purchased from Sigma (USA). Fetal bovine serum (FBS), Minimal Essential Medium (MEM), and Trypsin/EDTA were obtained from Gibco (USA). RPMI 1640 (RPMI), Hoechst 33342 were from Invitrogen.

2.2. Synthesis of SiDA

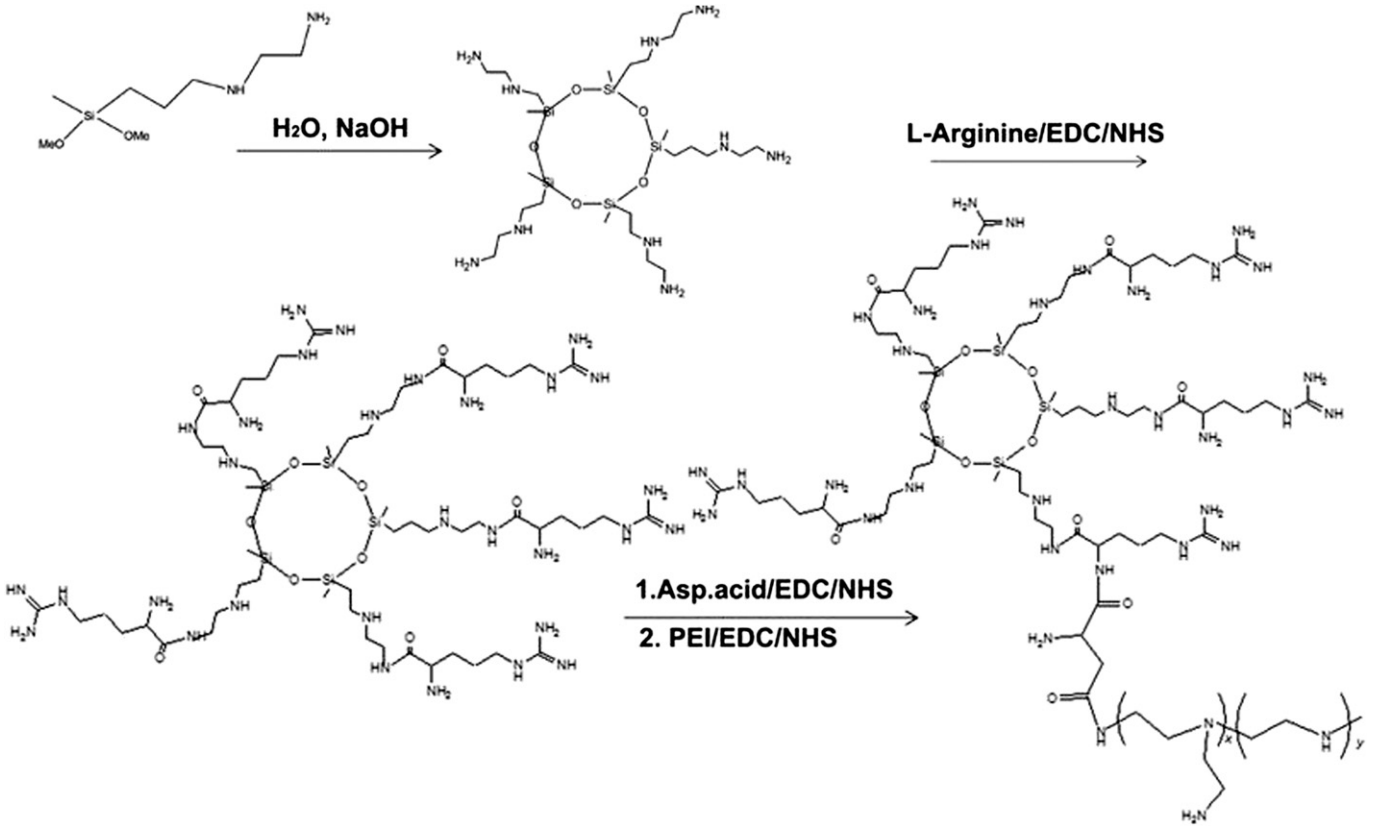
Oligo-(alkylaminosiloxane) was prepared according to the procedure given elsewhere [15]. Briefly, to 1.8 mmol, (1eq) of 3-(2-aminoethylamino) propyl-methyl-dimethoxysilane, 1 equivalent of 1 N NaOH solution was added and mixed. The sample was stirred for 20 h at room temperature. Later, the oligomers were exposed to reduced pressure to remove the volatiles. The crude sample was then diluted to 5 ml with water and neutralized to pH 7 by using 1 N HCl. The product was analyzed by ^{1}H NMR and mass spectrometry. Mass spectra were determined by micromass quattro micro API mass spectrometer. Mass: $m/z = 641,143,302,321,481,802$.

2.3. Synthesis of SiDAAr

Arginine conjugated oligo amino alkyldialkoxymethylsilane was prepared by EDC/NHS chemistry. Briefly, the acid group of arginine (5 eq) was activated using EDC/NHS for 4 h at 4 °C in PBS at pH 8. SiDA (1eq) was added to the activated arginine and the reaction kept for 18 h at room temperature. The product was then dialyzed (MWCO 1000) against deionised water to remove un-reacted substrates and lyophilized.

2.4. Synthesis of P(SiDAAr)n

SiDAAr coupling to PEI was performed by EDC/NHS chemistry using N-BOC protected aspartic acid as the linker group. Briefly, one of the acid group of aspartic acid was activated using half the equivalents of EDC and NHS for 4 h at 4 °C in PBS of pH 8. After the acid activation, SiDAAr (*n* eq) was dissolved in PBS and added into the reaction mixture. The reaction took place for 18 h at room temperature. The reaction mixture was kept for dialysis (MWCO 1000) to remove the un-reacted substrates. The second acid group of the reaction mixture was again activated using EDC and NHS at 4 °C for 6 h. After acid activation, one equivalent of PEI was added into the reaction mixture and the reaction was kept for 18 h at room temperature. The resultant mixture was dialyzed (MWCO 12000) and then lyophilized. ^{1}H NMR (D_2O): δ(ppm): 0.1-(bs, Si−C**H3**), 1.66-arginine(−HCCH2C**H2**CH2NH−); 1.86-arginine



Scheme 1. The reaction scheme of P(SiDAAr)1 derivative.