An arginine-based poly(aminoglycerol ester) facilitates the transport of genetic information across the cell membrane of lung epithelial cells

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Statement of Purpose: A safe and efficient delivery of genetic information is still an un met demand. Presently, viral vectors are the most efficient gene delivery vehicles, but this efficiency is burdened with safety concerns including: immunogenicity, toxicity, mutagenicity, and potential danger of oncogenicity. Consequently, there is an increased interest in non-viral vectors as gene delivery agents, specifically cationic polymers and lipids. The polymer reported here uses a positively charged amino acid, arginine, to efficiently compact genetic information and transport it across the cell membrane.

Methods: The arginine-based polymer was synthesized via polycondensation reaction of diglycidol succinate and arginine ethyl ester, with 0.1% molar amount of $Mg(ClO_4)_2$ in anhydrous N,N-dimethylforamide (DMF) under N_2. The reaction mixture was stirred and kept at 60°C for 7 days. The resultant polymer (PSR, **Figure 1**) was precipitated with diethyl ether, and lyophilized overnight. The polymer was characterized by FTNMR, FTIR, differential scanning calorimetry, and gel permeation chromatography. The polymer's potential to compact genetic material was investigated by gel retardation assay, particle size analysis, and zeta potential analysis. To ensure PSR was not cytotoxic to cells, MTT assay was performed using rat lung epithelial (L2) cells. PSR's ability to transport nucleic acids across the cell membrane was evaluated with L2 cell cultured in 24-well plates. YOYO-1 was intercalated into the nucleic acid prior to complexation with PSR, which enabled the tracking of the DNA/polymer nanoparticles (polyplexes) with fluorescent microscopy.

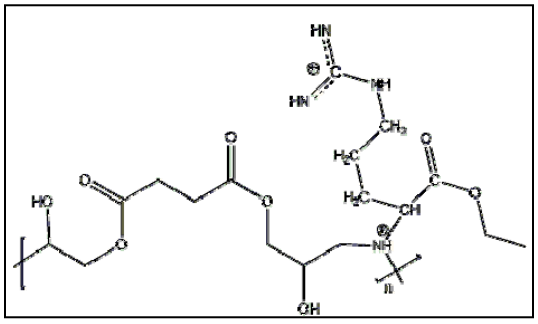

Figure 1: The chemical structure of PSR.

Results/Discussion: PSR was a yellow powder soluble in water, alcohols, and DMF. The spectroscopic characterization of PSR indicated that both arginine and the diglycidol ester were incorporated into the polymer in a 1:1 molar ratio. Gel retardation assay revealed that PSR completely retarded plasmid DNA at a nitrogen/phosphate (N/P) ratio between 4/1 – 8/1. This experiment led to the use of an N/P ratio of 10/1 for further analysis because the plasmid DNA was entirely neutralized by PSR at this ratio. Particle sizing and zeta potential measurements were conducted using dynamic light scattering. The diameter of the polyplex was 58 nm and carried a charge of +41 mV at an N/P ratio of 10/1. The polyplex size indicated that it could enter the cell through endocytosis and its positive charge improves the interaction with negatively charged proteoglycans on the cell surface. Polyplexes were then formed with plasmid DNA tagged with the fluorescent dye YOYO-1 to examine PSR's capacity to deliver nucleic acids across the cell membrane (**Figure 2**). The polyplexes were observed inside of the cells 4-5 hours after transfection, similar to the positive control, linear polyethyleneimine (PEI 25kD) at a N/P ratio of 7/1. The polyplexes appeared to be contained within endosomes, as indicated by the punctated fluorescent signals. This demonstrated that PSR can transport genetic information into cells.

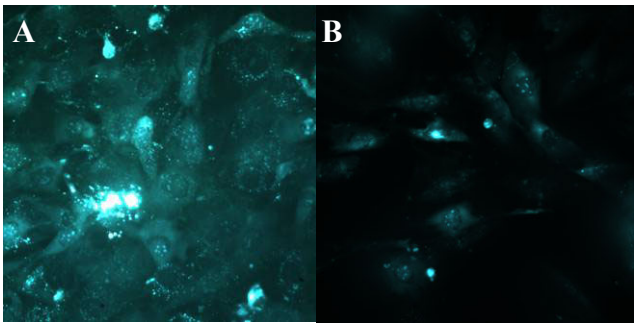

Figure 2: (A) Plasmid DNA labeled with YOYO-1 was complexed with PSR and was used to transfect lung epithelial cells. Polyplexes appeared to be present in vesicles instead of evenly distributed in cytosol. (B) Transfection using linear PEI under same conditions.

Conclusions: We have designed and synthesized a biocompatible polymer with integrated arginine functional groups. This polymer has proven to be positively charged thus enabling PSR the aptitude to compact genetic materials to particles less than 100 nm, which will allow for endocytosis. Furthermore, PSR has demonstrated it can transport this genetic information across the cell membrane of lung epithelial cells. The versatile design of PSR allows for a tunable polymer that can be modified for optimal transfection efficiency. Future investigation includes incorporation of endosomolytic agents and increasing the molecular weight of the polymer.