



NITROGEN TO PHOSPHATE RATIO IN TRANSFECTION SOLUTION (PEI2-GNP-DNA) AFFECTS TRANSGENE DELIVERY IN THE HUMAN CORNEA *IN VITRO*

Ajay Sharma^{1,2}, Ashish Tandon^{1,2}, Jonathan CK Tovey^{1,2}, Rangan Gupta^{1,2}, J David Robertson³, Jennifer A Fortune⁴, Alexander M Klibanov⁴, John Cowden¹, Rajiv R. Mohan*^{1,2,5}

¹Mason Eye Institute, University of Missouri-Columbia, MO and ²Harry S. Truman Memorial VA Hospital, Columbia, MO,

³Department of Chemistry and Research Reactor, University of Missouri-Columbia, ⁴Departments of Chemistry and Biological Engineering, MIT, Cambridge, MA, ⁵College of Veterinary Medicine, University of Missouri-Columbia



Introduction

Conventional treatments for corneal diseases provide short-term relief and often fail. No gene therapy modalities for corneal diseases are currently available. Nanotechnology has potential to provide safe and effective nonviral gene therapy treatments. Gold nanoparticles (GNP) because of their bio-inertness, non-toxicity, ease of synthesis, and efficient condensation of DNA have been tested as gene therapy vectors. Our pilot experiments showed that polyethylenimine-conjugated gold nanoparticles (PEI2-GNP) can effectively deliver foreign genes in the cornea.

Rationale and Hypothesis

For nonviral gene delivery, nature of GNP, concentration of plasmid DNA and exposure timing of vector to tissue play pivotal role. We hypothesized that plasmid concentration and PEI monomer amount in transfection solution of GNP-PEI affect gene transfer efficiency and toxicity. PEI is highly efficient in drug and gene delivery vector but is known to cause moderate cytotoxicity.

Aims

1. Whether different molar ratio of PEI2 nitrogen (N) and phosphate (P) of DNA in PEI2-GNP transfection solution regulates transgene delivery in human cornea *in vitro*
2. Examine PEI2-GNP toxicity to the rabbit cornea *in vivo*
3. Determine uptake and clearance of GNP for the rabbit cornea *in vivo*

Methods

- Donor human corneas and New Zealand White rabbits were used
- Various PEI2-GNP N/P ratios and plasmid expressing GFP were tested
- DNA complexation was tested by agarose gel-retardation assay
- *In vitro* toxicity was tested with trypan blue assay
- *In vivo* toxicity examined with slit-lamp biomicroscopy and IHC
- GNP uptake and clearance analyzed with neutron activation analysis (NAA)
- GNP entry and intracellular trafficking was studied with electron microscopy
- One way ANOVA and Tukey's test were used for statistical analysis

Conclusions and Future Studies

- PEI2-GNP are promising vector for corneal gene therapy.
- *In vivo* safety and efficacy testing of our GNP-transfection solution are underway

Results

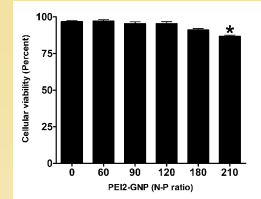


Fig 1: Effect of various N/P ratio of PEI2-GNP on cellular viability of human corneal fibroblasts evaluated by trypan blue assay. No significant loss of cellular viability detected up to N/P ratio 180.

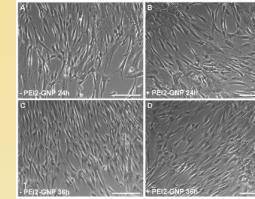


Fig 2: Effects of PEI2-GNP on human corneal fibroblasts. Detection of classical HSF morphology suggests that GNP-PEI do not alter phenotype. Scale bar = 10 μ m.

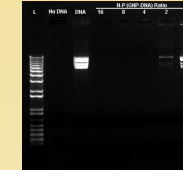


Fig 3: Gel electrophoresis showing complexation of plasmid DNA to PEI2-GNP.

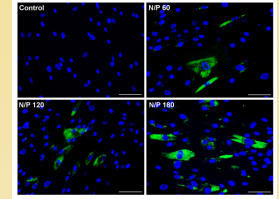


Fig 4: GFP gene delivery in human corneal fibroblasts with various N/P ratios of PEI2-GNP and plasmid solution. Nuclei are stained blue with DAPI. Scale bar = 100 μ m.

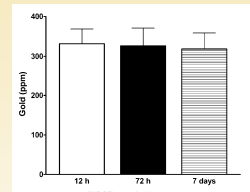


Fig 5: Quantification of gold uptake in PEI2-GNP-treated rabbit corneas, *in vivo* by neutron activation analysis.

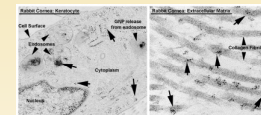


Fig 6: Electron microscopy images showing location and intracellular trafficking of GNP in keratocytes (A) and extracellular matrix (B) of the rabbit cornea.

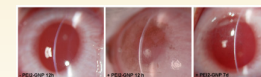


Fig 7: Clinical eye examination with slit-lamp microscope in untreated and GNP-treated rabbit corneas.

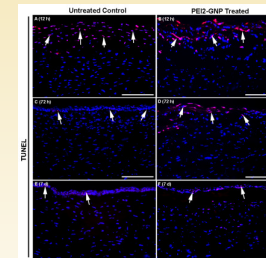


Fig 8: TUNEL-positive cells (red) detected in rabbit cornea at 12h, 72h and 7d. Nuclei are stained blue with DAPI. Scale bar = 100 μ m.

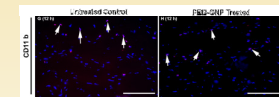


Fig 9: Immunological response detected with CD11b immunocytochemistry in rabbit cornea *in vivo*. Nuclei are stained blue with DAPI. Scale bar denotes 100 μ m.

Important Findings

- Efficient gene delivery: 53-58% ($p < 0.001$) with N/P 180 and 23-41% ($p < 0.01$) with N/P 120 or less
- Low Toxicity: N/P ratio ≤ 180 safe for human corneal fibroblasts
- Moderate apoptosis and low immune response
- Appreciable *in vivo* gold uptake (>300 ppm) in rabbit cornea with gradual clearance over time.
- Endocytosis as a possible mechanism for cellular uptake

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