POSTER 113

N/P RATIO IN THE PEI2-GNP-DNA COMPLEX AFFECTS TRANSGENE DELIVERY IN THE HUMAN CORNEA *IN VITRO*

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Purpose: Recently, we discovered that polyethylenimine-conjugated gold nanoparticles (PEI2-GNP) could be used as gene therapy vector for the cornea. It was hypothesized that DNA concentration, incubation timing and PEI monomer amount in transfection solution affect gene transfer efficiency and toxicity. The aims of this study were to test whether molar ratio of PEI2 nitrogen (N) and phosphate (P) of DNA in PEI2-GNP transfection solution regulates transgene delivery in human corneal fibroblasts *in vitro*, and examine PEI2-GNP toxicity, uptake and clearance for the cornea *in vivo*.

Methods: Donor human corneas for *in vitro* and New Zealand White rabbits for *in vivo* studies were used. Various N/P ratios of PEI2-GNP-plasmid expressing GFP transfection solution were tested. DNA complexation was tested by agarose gel-retardation assay. The toxicity was tested with trypan blue assay, slit-lamp biomicroscopy and immunostaining. PEI2-GNP *in vivo* uptake and cellular entry into corneal cells were analyzed with neutron activation analysis (NAA), silver staining and electron microscopy.

Results: The N/P ratios 60 and 120 showed moderate (23-41%, $p < 0.01$) and 180 high (53-58%, $p < 0.001$) transgene delivery into human cornea *in vitro*, without altering cellular viability and phenotype significantly. Appreciable gold uptake (>300 ppm) in treated rabbit cornea with gradual clearance over time was detected with NAA. Electron microscopy studies suggest GNP uptake through endocytosis. Slit-lamp biomicroscopy in live rabbits detected no inflammation, redness or edema whereas moderate cell death and immune reactions were noted with immunocytochemistry.

Conclusion: Selected PEI2-GNPs can offer effective non-viral gene therapy modalities for treating corneal diseases.