An evaluation of *in vitro* and *in vivo* toxicity of chitosan-pDNA polyplexes

**Purpose**

This study aims to 1) characterize transfection efficiency (the ability to deliver genetic materials into cells) and toxicity of chitosan-pDNA polyplexes *in vitro* and 2) determine the inflammatory response following nasal administration in mice.

**Methods**

Chitosan-pDNA polyplexes were formulated at different ratios of primary amine groups in chitosan to phosphate groups in plasmid DNA (pDNA) and using two different types of pDNA [CpG(+) and CpG(-)]. The complexes were characterized for size and zeta-potential using a Zetasizer Nano ZS. The efficiency of transfection and degree of toxicity of the polyplexes was determined in human alveolar adenocarcinoma (A549) and human embryonic kidney (HEK293) cells using luciferase expression and MTS assays, respectively. *In vivo* inflammation responses and gene expression were investigated following nasal instillation of chitosan-pDNA polyplexes to mice (n = 6/group). Two doses of 50 μl (12.5 μg pDNA/50 μl) were given one hour apart. After 24 hours of treatment, mice were sacrificed followed by isolation of lungs and the bronchoalveolar lavage (BAL) fluid was collected. The number of macrophages, neutrophils and lymphocytes in the BAL fluid were counted to quantify the inflammatory response. Total proteins were determined using a Bradford protein assay. Activity of lactate dehydrogenase (LDH) was measured using a spectrophotometric detection kit. Cytokines were measured using a multiplexed fluorescent bead-based immunoassay.

**Results**
Chitosan-pDNA polyplexes generated high luciferase expression in HEK293 and A549 cells, especially when using polyplexes prepared at N/P ratios ranging from 5 to 20. When compared to poly(ethylenimine) (PEI)-pDNA polyplexes (the gold standard in non-viral gene delivery), the chitosan-pDNA polyplexes had significantly higher cell viability. Nasal instillation of chitosan-pDNA polyplexes in mice increased the number of neutrophils as compared to control groups. LDH activity and cytokines were also increased with chitosan-pDNA polyplexes treatments.

Conclusion

Polyplexes made from chitosan and pDNA have potential as non-viral gene delivery systems. Chitosan-pDNA polyplexes have significantly lower toxicity when compared to PEI-pDNA polyplexes. However, pulmonary delivery of chitosan-pDNA polyplexes to mice induces an inflammatory response.