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| **Developing Dry Powder Gene Therapies for Pulmonary Delivery** |
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| **Background:** Pulmonary gene delivery is desirable due to its non-invasive nature and the possibility of achieving an increased local drug concentration. Inhaled gene vaccines (e.g., for Covid-19) are particularly attractive as they may provide improved protection by inducing an IgA-mediated mucosal immune response. While formulating genes as dry powders can provide additional benefits such as superior stability and longer shelf-life, it is highly challenging as nucleic acids are prone to degradation. The objective of this work is to develop a biocompatible gene delivery construct that is inhalable for deposition in the deep lung, mucopenetrating, and that enables transcytosis of nucleic acids (e.g., *via* the 100 – 200 nm nanoparticles arising from the micrometre construct). |
| **Methods:** Glycol chitosan (GC)-based polymers of varying properties (surface chemistry modification and molecular weight) were synthesized based on a method described previously, characterized post-complexation with plasmid DNA (pDNA) and screened *in vitro* to locate the most transfection-competent gene construct. The most promising formulation was then converted into an inhalable dry gene powder *via* spray drying and further characterized as microparticles (microparticle size, shape, and physical structure), as nanoparticles after powder reconstitution (nanoparticle size and pDNA integrity assays) as well as evaluated *in vitro* (imaging and transfection competence by flow cytometry) in short-term stability studies. |
| **Results:** During polymer screening, we identified the optimal polymer/polyplex properties resulting at a minimum toxicity (<5% cell death) and maximum transfection efficiency of 68 ± 5% of cells compared to the positive control, Lipofectamine, with a transfection efficiency of 77 ± 3% of cells. The dry gene powder formulation exhibited a spherical shape, low water content, a microparticle size of 1 – 8 μm (suitable for inhalation) and gave rise to the original nanoparticles after aqueous reconstitution while all other properties remained unchanged. Most importantly, the biological activity of pDNA was preserved post-drying; the reconstituted formulation exhibited a transfection efficiency of 60 ± 2% of cells (~12% loss of activity) and only a minor transfection decrease (8%) when stored at refrigeration and room temperatures over 30 days. |
| **Conclusions:** We have developed a pDNA-containing dry powder with promising transfection data and a microparticle size suitable for inhalation making this system a promising candidate for pulmonary gene delivery. This system is currently being tested *in vivo* in a healthy mouse model. |