Introduction
Successful pulmonary nucleic acid therapy necessitates efficient delivery of the therapeutic gene to the lung. Delivery by inhalation is desirable for the treatment of lung diseases as the therapeutic nucleic acids can be applied locally to the affected site and minimize systemic adverse effects [1]. Previously, our group has demonstrated the use of histidine-rich amphipathic peptide, LAH4-L1, to deliver nucleic acids effectively to human lung epithelial cells, to achieve high transfection efficiency [2]. The cationic peptide can bind and condense nucleic acids through electrostatic interaction to form nano-sized complexes for efficient cellular uptake and promote endosomal escape.

Objectives
1. To prepare and characterize the spray dried and spray freeze dried powder formulations of LAH4-L1/DNA for inhalation
2. To evaluate and compare the biological activities of the spray dried and spray freeze dried powders

Results and Discussions
Shear forces and extreme temperatures typically encountered during the drying processes may cause DNA degradation. The gel retardation assay (Fig. 1) showed that the structure of plasmid DNA could be successfully preserved after SD and SFD. The extracted DNA adopted a more relaxed structure compared to the original supercoiled structure. The significant of this is uncertain because the level of supercoiling of plasmid DNA is not known to affect the transfection efficiency significantly [3].

The SD and SFD powders show a spherical morphology (Fig. 2), with SD powder surface slightly corrugated. The SD powder particle diameter was around 1-2 μm. The SFD powders were highly porous with particle diameters about 20-40 μm.

The transfection efficiency of SD powder formulation was significantly increased compared to that of the freshly prepared LAH4-L1/DNA complexes (Fig. 3A). The presence of mannitol in the formulation may enhance the cellular uptake of the DNA complexes. Although the peptide based formulations were less efficient than Lipofectamine™ 2000, they were significantly less toxic as shown in the protein assay (Fig. 3B).

Materials and Methods
Peptide: LAH4-L1 (KKALAHALHLALLALHLHALKKA) were synthesized (>70% purity) by ChinaPeptide (Shanghai, China).
Plasmid DNA: gWIZ™huc (expressing luciferase) was purchased from Aldevron (Fargo, ND,USA).
Mannitol: Pearlitol 160C was purchased from Roquette (Lestrem, France).

Preparation of Spray Dried (SD) and Spray Freeze Dried (SFD) Powders
Mannitol: LAH4-L1: DNA (1000: 10: 1, by weight) was dissolved in water with a final concentration of mannitol at 10 mg/mL. The solutions were subjected to spray drying (Büchi B-191 Mini Spray Dryer) or spray freeze drying (ultrasonic atomizer nozzle).
DNA Integrity DNA was extracted from the powders and the resuspended samples were evaluated by gel retardation assay.
Morphology The morphology of the SD and SFD powders were observed by scanning electron microscopy (Hitachi S4800 FEG SEM).
Transfection To evaluate the biological activity of the dry powders, in vitro transfection experiments were performed on A549 cells. The powders were resuspended in culture media before adding to the A549 cells. The transfection efficiency was assessed by luciferase assay at 48 h post-transfection.

Acknowledgements
The work was funded by Seed Funding for Basic Research (201111159114), The University of Hong Kong and partly by the Australian Research Council.

References