Using MDIs to deliver pDNA

Investigating whether plasmid DNA can remain viable after aerosolization and delivery by a standard MDI

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For some time, researchers have been investigating the possibility that certain lung diseases, including inheritable conditions like cystic fibrosis (CF) and malignancies such as carcinomas, could potentially respond to gene therapy delivered directly to the lung. Studies have already shown some success in correcting genetic defects [1]. Pulmonary delivery of DNA could also provide an effective immunization against diseases such as influenza and measles, as that route mimics the natural route of infection.

To date, laboratories and clinics studying respiratory gene therapy have generally used jet nebulizers to deliver the DNA formulations [2]. Nebulizers have certain advantages in that they deliver large doses of medication while patients inhale medication easily through a mask or mouthpiece without the need to breathe deeply; however, the high shearing forces they generate can damage the genetic material, rendering much of it non-viable. In addition, genetic material may adhere to the relatively large plastic surfaces on the interior of the nebulization chamber. One study has shown that up to 90% of the genetic material in a nebulized formulation may remain in the chamber while the patient inspires mostly solute [3].

Although researchers are working on advanced nebulizer technology that might improve gene delivery, other delivery methods might have advantages. Dry powder inhalers (DPIs) offer portability and rapid delivery; however, formulation of biologicals for delivery by DPI presents a number of challenges, especially with agglomeration, hygroscopicity, and particle size issues. Metered dose inhalers (MDIs) also offer portability and quick administration. If an MDI formulation can deliver DNA that remains viable after aerosolization, without any toxicity to cells from the formulation, MDIs could potentially become the delivery method of choice for gene therapy.

Preparing the pDNA formulation

Gene therapy may use a virus to carry DNA into cells or, as in this model, may introduce DNA into cells through non-viral vectors, where the genetic material enters cells without a viral carrier (transsection). Plasmid DNA (pDNA), which generally exists as ring structures outside of the chromosomal genetic material in bacterial cells, and which contains the gene that codes for the protein missing in lung diseases such as CF, can transfect epithelial cells when complexed with a lipid as a non-viral vector. First, the pDNA must be available and viable in a stabilized form.

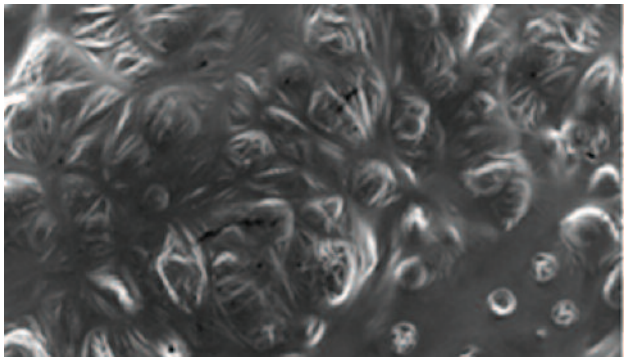
The plasmid used for this study, pEGFP, contains a gene that codes for enhanced green fluorescent protein (EGFP), which fluoresces under blue light. The EGFP plasmid provides a useful method of measuring the success of transfection because cells successfully transfected with pEGFP will fluoresce green if the plasmid has entered the nucleus and is able to use the cell's transcription apparatus to code for the EGFP protein. Measuring the fluorescence emitted therefore provides a good measure of the integrity of the pDNA after formulation processing and aerosolization from an MDI since only undamaged pDNA has the ability to use the cell's transcription apparatus.

Coating pEGFP with surfactant aids redispersibility in the HFA propellant used in an MDI formulation. The coating technique used for this formulation involves creating a water-in-oil microemulsion of sucrose solution, iso-octane, and lecithin:propan-2-ol as a surfactant. Surfactants have amphiphilic properties and reside at the interface of oil and water, and under these conditions, the surfactant molecules form a spherical structure called a reverse micelle, with the aqueous phase containing the pEGFP in the center.

Formulating the pEGFP with sucrose in the aqueous phase protects the genetic material during snap freezing with liquid nitrogen followed by lyophilization to create a stable dry powder. After lyophilization, particles remain coated with excess amounts of surfactant (Fig. 1A). Washing those particles with organic solvents and centrifugation removes excess surfactant coating, leaving pEGFP particles coated with smaller amounts of surfactant (Fig. 1B).

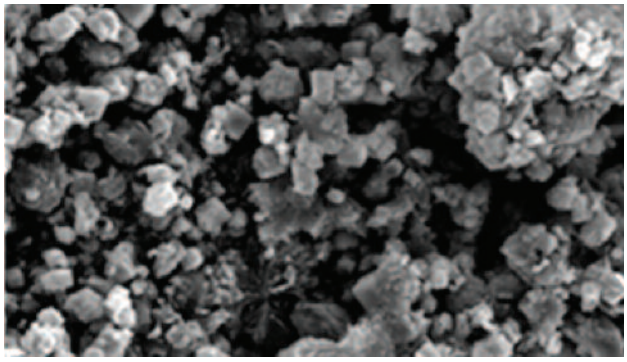
Figure 1

Lyophilized pEGFP particles before and after washing to remove excess surfactant



(a) Unwashed

— 500 µm



(b) Washed

— 20 µm

Assessing the integrity of lyophilized pEGFP

The pEGFP DNA comes in several forms: supercoiled, linear, and open circular. Of these, only the supercoiled pDNA has the most consistent ability to transfect cells while maintaining its ability to code for the EGF protein, but is also the most vulnerable to damage during the lyophilization process.

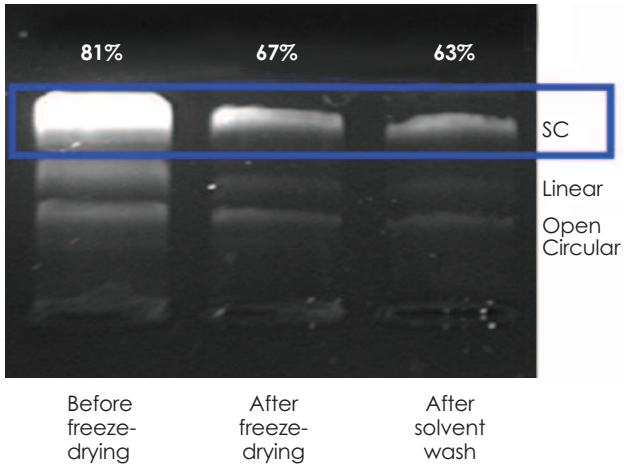
Agarose gel electrophoresis, in which an electric current is applied to an agarose gel that contains the pDNA molecules, separates the different forms of pDNA into bands. A semi-quantitative analysis that compares the fluorescence of the supercoiled band with that of the other bands shows that 81% of the pre-lyophilization pEGFP exists in the supercoiled form, with 67% surviving in that form after lyophilization and 63% after both lyophilization and solvent washing (Fig. 2), a reasonable amount of supercoiled pEGFP for transfection.

Assessing transfection ability

Measuring the transfection competence of the pEGFP after aerosolization requires formulating the

Figure 2

Qualitative gel electrophoresis of pEGFP before and after freeze drying and solvent washes



lyophilized pDNA for MDI delivery, depositing the aerosol on a culture of an appropriate cell type, and measuring the amount of fluorescence generated by the cells after they have had a chance to take up the pEGFP.

Entry into a cell during transfection can damage naked pDNA; however, complexing the pDNA with a cationic lipid usually facilitates entry and reduces damage since liposomes encapsulate the pDNA, providing protection. For that reason, these studies compared formulations of washed and unwashed pEGFP actuated onto cells grown in media with and without a cationic lipid, in this case DOTAP (dioleoyl trimethylammonium propane). All of the formulations included HFA134a as a propellant and ethanol as a co-solvent.

The cells used in this model are human Caucasian lung carcinoma cells grown in a single layer in a cell culture flask. After experiments with Brilliant Blue dye, the best location for actuating the MDI for maximum deposition on the cells proved to be from the top of the flask (Figs. 3, 4). As a result, all actuations of the pEGFP-loaded MDIs took place from the top orientation.

Figure 3

Brilliant Blue pMDI actuated from the top and side orientation of a T25 flask

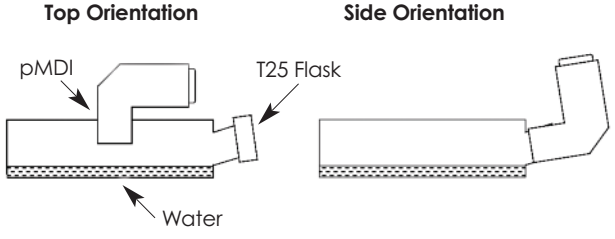


Figure 4

Deposition efficiency of Brilliant Blue when actuated from a pMDI in the top and side orientation relative to control

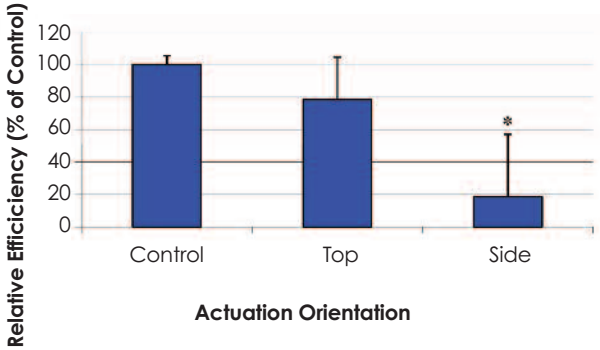
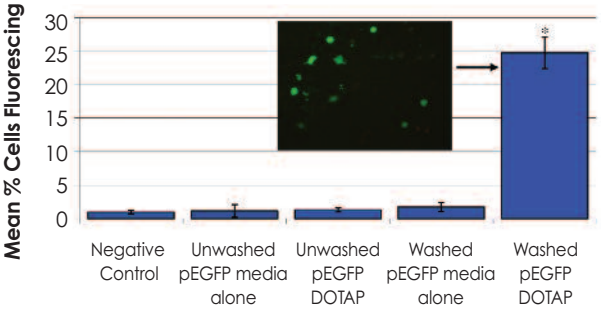


Figure 5

Mean percentage of A549 cells emitting fluorescence post pEGFP transfection

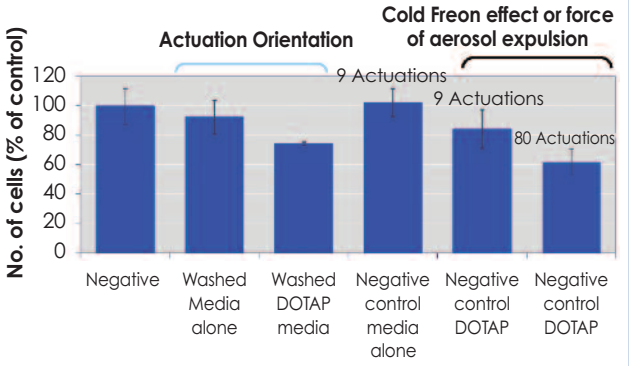


Measurement of the fluorescence of the cells treated with the 4 different formulations showed that approximately 25% of the cells treated with the formulation of washed pEGFP actuated onto media with DOTAP subsequently expressed GFP (Fig. 5), indicating that the plasmids have successfully transfected those cells. Because toxicity assays showed that DOTAP caused a certain amount of cell death (Fig. 6), future work with this cell line would require finding an alternative transfection reagent.

This study demonstrates that pDNA can remain viable in an HFA 134a formulation and during aerosolization with a standard MDI, and further studies might be able to refine the technique. Compared to nebulization where only 10% of the genetic material might be delivered through the mouthpiece, MDI delivery that achieves a 25% transfection rate holds the promise of more convenient and effective delivery of pDNA in the future.

Figure 6

Percentage cell viability relative to the control



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2. Griesenbach, U. and Alton, E. Gene transfer to the lung: Lessons learned from more than 2 decades of CF gene therapy. Advanced Drug Delivery Reviews. In press, corrected proof.

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