PL-11

# An Academic Approach to Vaccine Development in a Global Pandemic: A Personal Account

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## 1. Introduction

The severity and the toll on healthcare systems all over the world caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) led the World Health Organization to declare a pandemic on 11 March 2020 (1). The sudden spread of the disease demanded rapid development of therapeutics and prophylactic intervention in a short period of time. Since early this year, huge efforts in vaccine research were made (2).

To answer to this demand and help alleviate the still dire situation in Thailand, scientists from the University of Geneva and the Chula Vaccine Research Center (Bangkok, Thailand) entered into a collaborative effort to develop pDNA vaccine formulations against SARS-CoV-2. This is a résumé of some of the results achieved (3).

DNA vaccination is considered a potential strategy to meet the challenge. This technique, however, holds two major disadvantages: poor immunogenicity and transfection of the target cell. Both require the development of effective delivery systems and adjuvants. Liposomes are among the more widely investigated vaccine delivery systems. However, the manufacture and scale up of liposomes can be challenging due to classical method being time consuming and hard to reproduce. New generation microfluidics devices are a great tool to overcome these problems.

## 2. Materials and methods (if necessary)

A plasmid DNA (pDNA) encoding SARS-COV-2 full-length Spike (S) protein, pDNA-S was developed at Chula Vaccine Research Center, Chulalongkorn University, Bangkok, Thailand. An effective DOTAP based liposomal delivery system was prepared, which showed immunogenicity in vivo. However, thin film rehydration manufacture method is not ideal in terms of reproducibility, size tuning and scale-up. The thin film protocol was translated to a microfluidics device (NanoAssemblr Ignite, Precision Nanosystems), with the goal of obtaining the same liposomal formulation of similar properties in terms of size, zeta-potential and transfection efficiency (***Figure 1***).

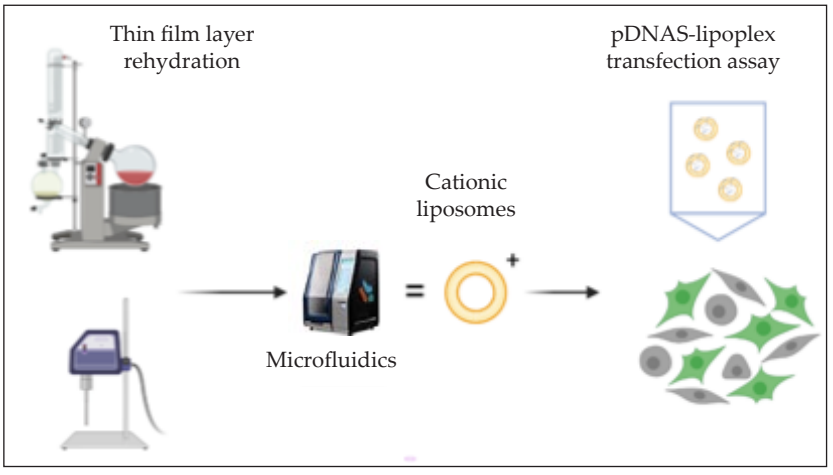


***Figure 1*** *Thin film method translation to microfluidics and in vitro characterization*

DPPC:DOPE:DOTAP (DOTAP4) liposomes where prepared by microfluidics, organic phase was prepared in ethanol, which was later removed by tangential flow filtration (TFF). Dynamic light scattering, zeta-potential measurement, and transmission electron microscopy (TEM) were used to characterize the liposomes. Liposomes and pDNA-S were

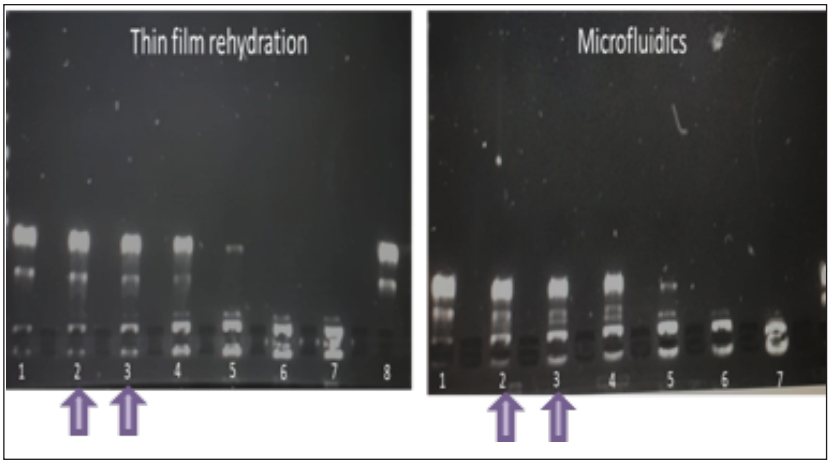


***Figure 2*** *Liposome:pDNA ratios of 1:1 and 5:1 ratio show suitable complexation efficacy for both preparation methods.*

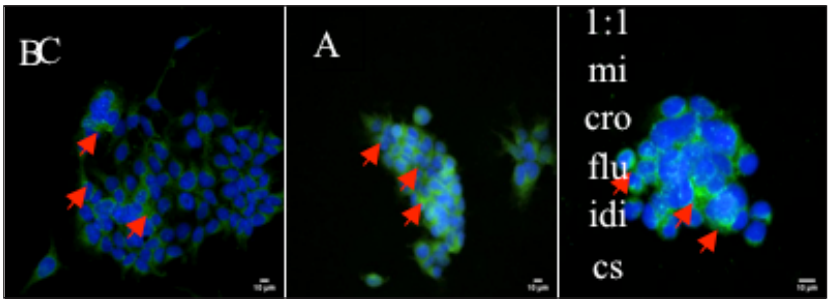


***Figure 3*** *HEK 293 cells were transfected with pDNA S lipoplexes 1:1 (A) and 5:1 (B) ratio, and (C) with lipofectamine (ctlr+).*

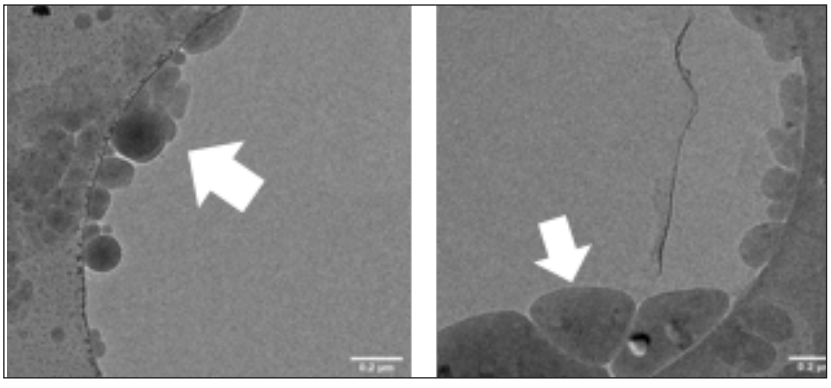

***Figure 4*** *Cryo-TEM images of liposomes, (A) microfluidics, (B) film layer rehydration.*

mixed at different N/P ratios and incubated 30 minutes at RT to allow complexation. Size, zeta-potential and electrophoretic mobility were measured. HEK293 adherent cells were transfected 24h before the experiment with pDNA-S/liposome complexes and used to detect protein expression. Complexes of Lipofectamine 2000-pDNA S were used as positive control, and pDNA S alone and non-transfected cells were used as negative control. After fixation and antibody staining, cells were mounted on slides. Images were taken using Nikon A1r Spectral with a 40x oil immersion objective.

## 3. Results

DOTAP4 blank liposomes prepared by thin film rehydration had a size of 130.9±5.8 nm and a PDI of 0.210±0.028, zeta-potential was +48±12 mV. Liposomal positive charge allowed for the complexation with negatively charged pDNA-S. N/P ratios from 0.25:1 to 100:1 were tested. A size increase with increasing positive charge was observed, which corresponded to a switch from negative charged samples (nucleic acid excess) to positive charged samples (liposome excess), confirming the fact that aggregation is increased when charge is neutral. Gel permeation assay as well as zeta-potential results (not shown) were equivalent for the two methods (***Figure 2***).

However, some differences were observed for ratios 10:1, 25:1 and 100:1 regarding the size. Transfection was confirmed in cells treated with liposome-pDNA-S complexes, which was observed for both microfluidics manufactured sample and thin film layer rehydration sample at a 1:1 N/P ratio and in pDNA-S/lipofectamine samples (***Figure 3***).

Cryo-TEM images show a multilamellar structure in particles manufactured by microfluidics and a unilamellar structure for particles manufactured by thin film method (***Figure 4***).

## 4. Conclusions

Liposomes manufactured by microfluidics method seems comparable in characteristics with thin film rehydration method manufactured liposomes.

Main structural difference is observed by Cryo-TEM images where lamellarity of the liposomes appears to be different.

Early results from ongoing in vivo immunogenicity studies in ICR mice appear to confirm comparable antibody production and T cell activation by pDNA-liposome formulaitons manufactured by both methods.

## 5. Acknowledgements

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