A MICROFLUIDIC APPROACH TO THE FORMULATION OF CATIONIC AND NEUTRAL LIPID NANOPARTICLES LOADING AND PROTECTING pDNA: TOWARDS LINKING BENCHTOP METHODS AND INDUSTRIAL SCALABILITY FOR THE PRODUCTION OF GENE THERAPEUTICS

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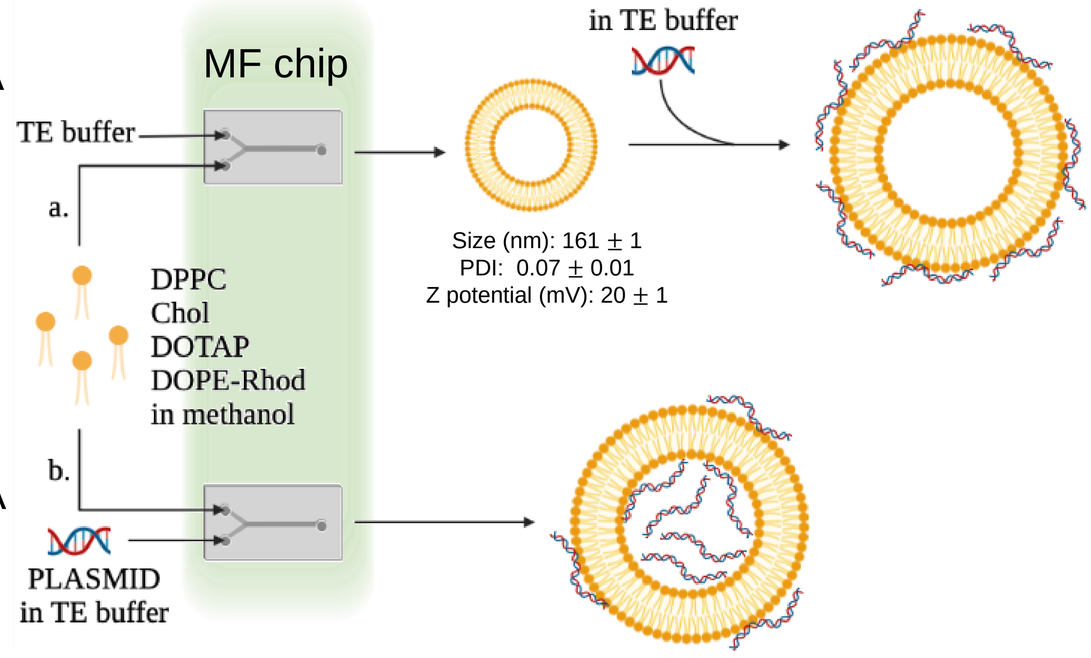
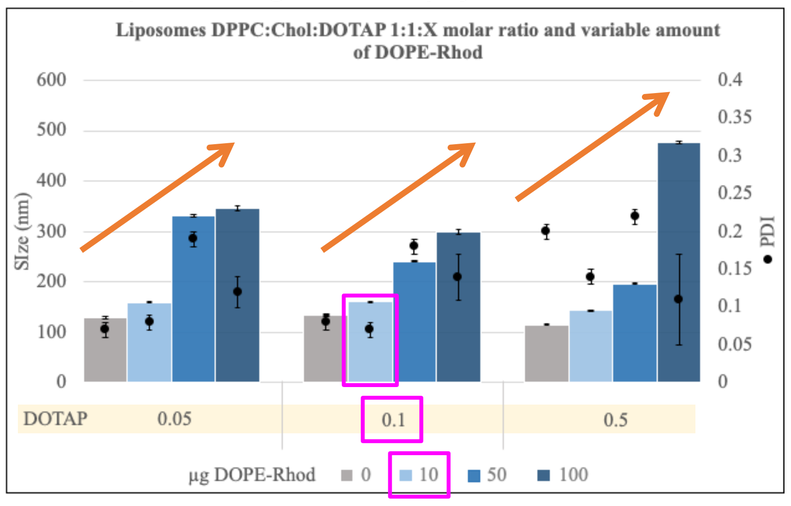
We formulated cationic liposomes with a microfluidic chip to load eGFP plasmid DNA using two methods:

Adding DNA to the formulated liposomes



Adding DNA during the formulation

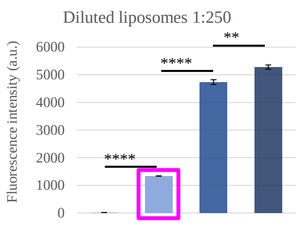
Optimizing the empty formulation



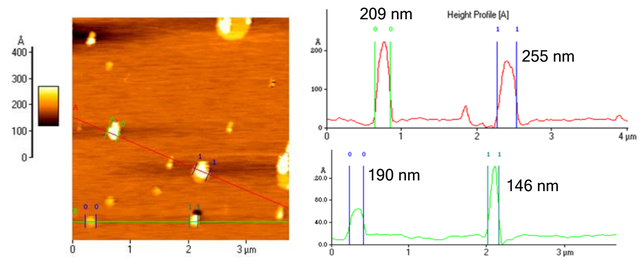
Scan me!

Stable in size and PDI when stored at 4°C for up to 14 days

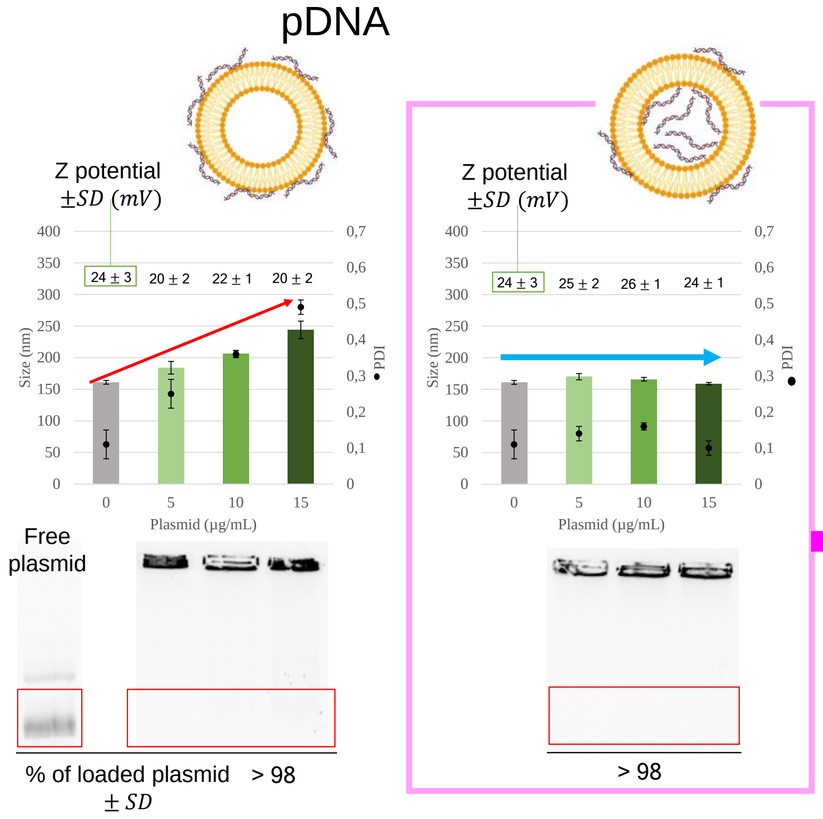
Fluorescence intensity of Liposomes with 0-10-50-100 µg DOPE-Rhod



AFM morphology of empty liposomes

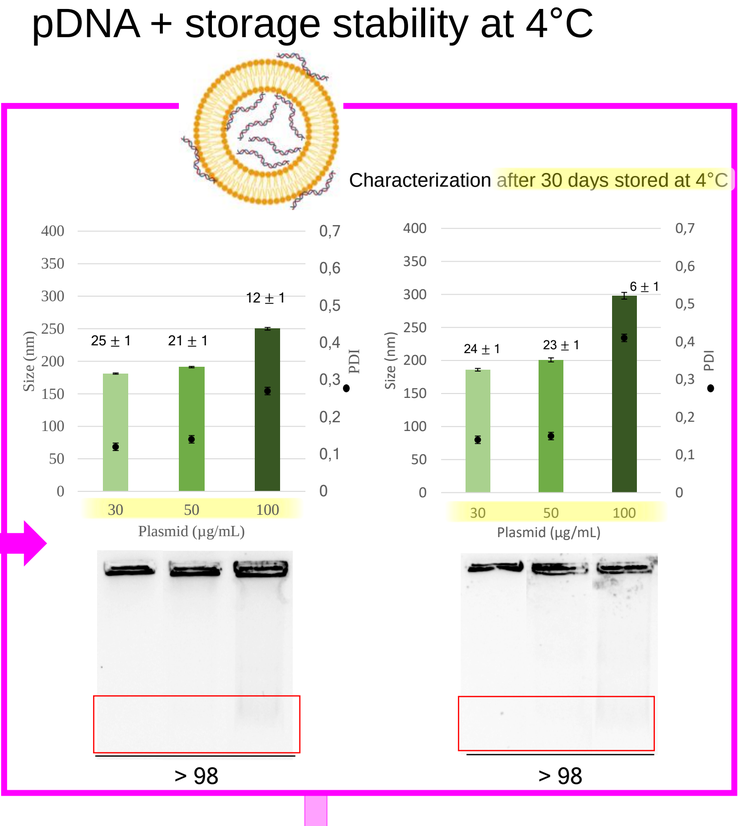


Optimized **cationic** liposomes loaded with **5, 10, or 15 µg/mL** pDNA



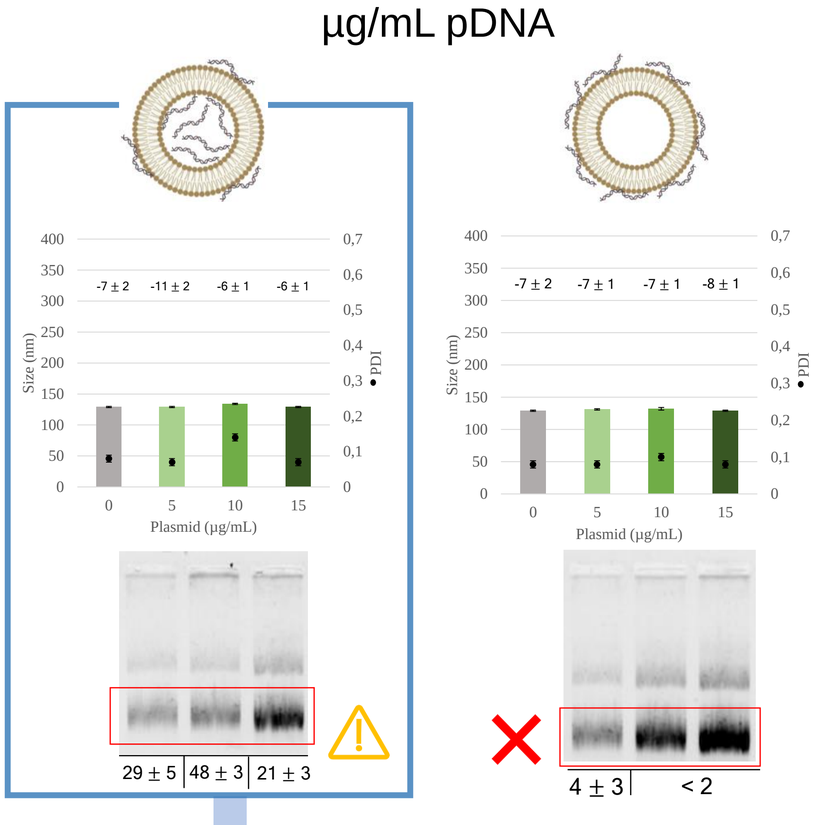
- ✓ Both methods allowed for a full **complexation of > 98% pDNA**
- ✓ Addition of pDNA to formed liposomes caused an **increase in size** and PDI dependant on the amount of DNA added

Optimized **cationic** liposomes loaded with **30, 50, or 100 µg/mL** pDNA + storage stability at 4°C



- ✓ Almost **100% of pDNA** at the highest concentration of **100 µg/mL** was loaded into cationic liposomes
- ✓ Size, PDI, and content are **stable for 1 month** at 4°C

Neutral liposomes prepared with both methods, adding 5, 10, 15 µg/mL pDNA

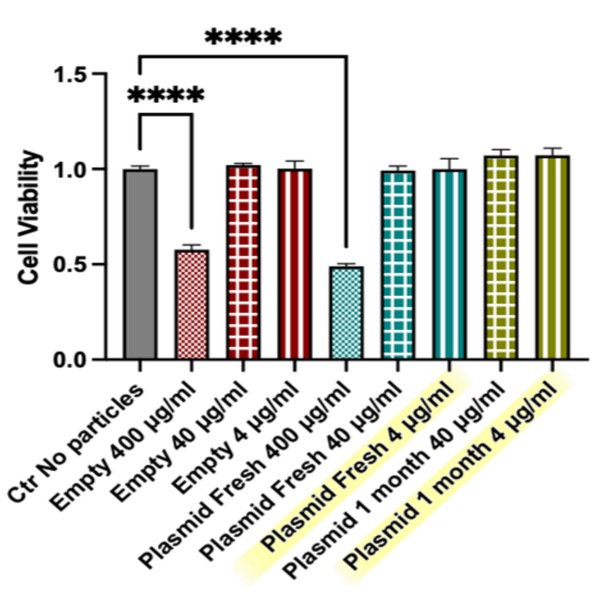


- ✓ Adding pDNA to formulated **neutral liposomes** resulted in < 2% loading due to unspecific bindings
- ✓ Up to **50% of pDNA** was loaded when added **during the formulation of neutral liposomes**
- ✓ Neutral liposomes might be able to load large amounts of pDNA without the cost and the toxicity linked to cationic particles

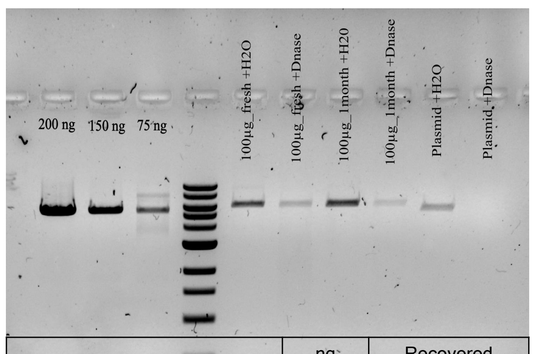
Further experiments are now ongoing to :

- Improve the **transfection efficiency** of the cationic particles
  - ➢ Modifications in the lipidic composition of liposomes
- Investigate the efficacy of **neutral** particles
  - ➢ Stability, DNA release, protection ability

Cell viability on A11 cells



Protection from DNAse

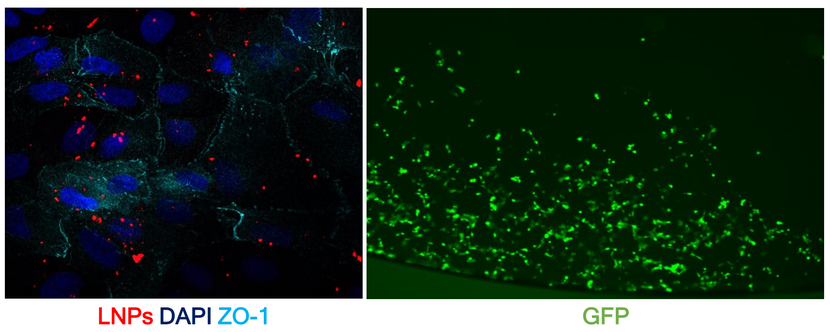


	ng	Recovered
100 µg/mL 1month	75	100%
100 µg/mL 1month + DNAse	53	71%
50 µg/mL 1month	84	100%
50 µg/mL 1month + DNAse	87	105%
30 µg/mL 1month	46	100%
30 µg/mL 1month + DNAse	26	57%
Plasmid	124	100%
Plasmid + DNAse	/	0

Transfection ability *in vitro of LNPs loaded with 100 µg/mL pDNA*

- In COS7 cells there was **no transfection** from DNA-LNPs (left)
- DNA was extracted and used with metafectene (right) showing that the DNA is still functional

➢ **The DNA is not released from the LNPs**



LNPs DAPI ZO-1 GFP

- Concentrations < 40 µg/mL of fresh or 1 month stored Liposomes showed no toxicity
- Exposure to DNAse showed 30% loss of DNA

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