Determination of Drug Delivery Efficiency and Cytotoxicity for Combinatorial Library of pH Responsive Lipids

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General Background (Fu 2014)

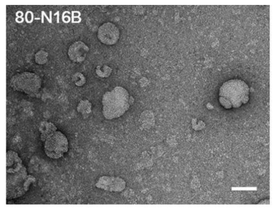
More progress is needed in finding safe/effective drug delivery methods. Current methods include mechanical strategies such as:

- Microinjection- direct injection into cells.
- Electroporation- cell permeability is increased from applying an electrical field.
- Using virus-like particles- resemble viruses but do not contain viral genetic material.

These solutions are limited by the amount of tissue that can be treated and invasive side effects from contamination.

Introduction of Lipid Nanoparticles (Shah 2015)

- Lipid Nanoparticles (LNPs) are composed of various lipids including fatty acids, glycerides, waxes, alcohols.
- Lipids form liposomes made up of amphiphilic phospholipids that can self assemble into vesicles.
- Can be formulated with surface helper lipids and steroids that could stabilize structures and increase membrane transfusion efficiency.
- Have problems in controlling timing of drug release which results in low delivery efficiency and increased toxicity to cells.



TEM images of an example of LNPs from another library. Scale bar = 100 nm. Modified from (Li et. al 2018).

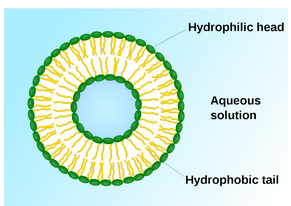
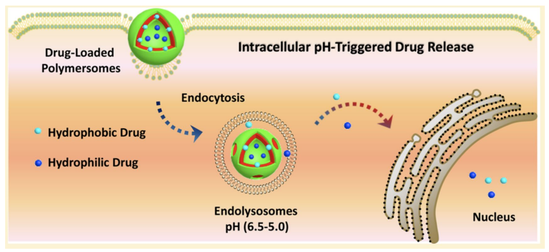


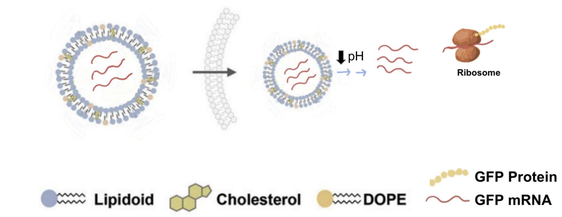
Image of LNP https://en.wikipedia.org/wiki/Liposome

Background: pH Sensitive LNPs (Zhao 2013)

- One way to control drug release timing is to create nanocarriers that can detect environmental stimuli, allowing drug release only in the right conditions.
- One useful stimuli to utilize is pH as the extracellular environment of the human body and the cytoplasm pHs is ~7.4 while organelles such as endosomes/lysosomes have pHs ~5.
- Tumor tissues have extracellular pHs ~6.8.
- pH sensitive lipids have acid degradable structures that stay intact until the environmental pH is lower.



Intracellular pH-Triggered Drug Release Modified from (Wang et. al 2015).



Intracellular Delivery of LNPS. Modified from (Li, 2019).

Methods for Delivery and Formulation of LNPs

Delivery of LNPs and GFP mRNA *in vitro*

The efficiency of GFP fluorescence induction from the uptake of green fluorescent mRNA (GFP mRNA) was measured using FACs flow cytometry.

- LNPs loaded with GFP mRNA through self assembly procedure.
- LNPs incorporated into HeLa cells through endocytosis.
- Compared to commercially available lipid Lipofectamine 2000 (positive control).
- Final LNP concentration was 7.4 µg/mL.
- Final GFP mRNA concentration was 0.74 µg/mL.
- Negative control was HeLa cells without addition of LNPs and GFP mRNA.

Unformulated LNPs

- LNPs made from pure library lipidoids.

Formulated LNPs

- Addition of 1,2-Dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) and cholesterol.

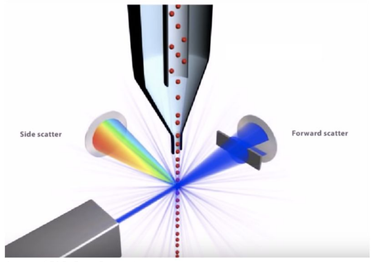
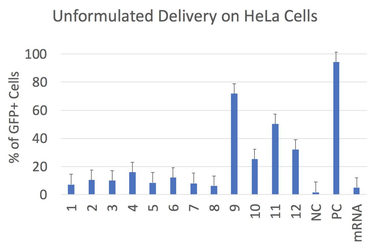


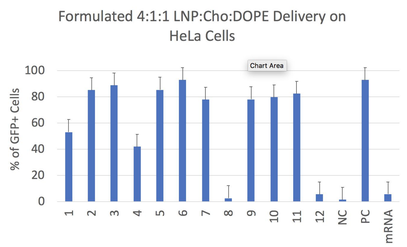
Image of FACs Flow Cytometry https://www.youtube.com/watch?v=T0RhCWaYRuc

Results for Delivery

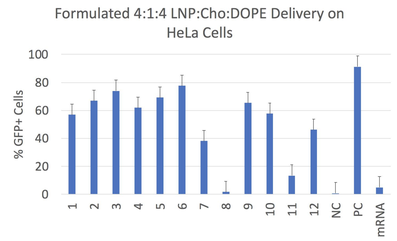
Note that numbers on the x axis indicate lipid types or negative control (NC), positive control (PC), and mRNA



- Without LNPs, there is low GFP + transfection (mRNA < LNPs).
- However, still low with LNPs and most are <20% and < PC (lfp2k).
- 9, 10, 11 and 12 slightly higher.
- 9 is at 71.9%.
- Error bars are SE.

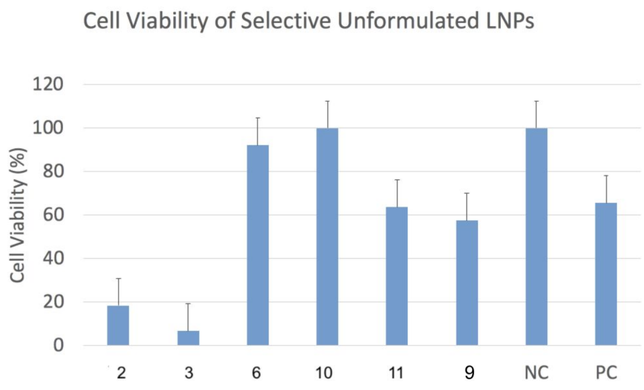


- Overall higher efficiency than unformulated.
- 2, 3, 5, 6, 9, 10, 11 induced +GFP fluorescence ~80% of the time.
- Most efficient was 6 at 92.6%.
- Least efficient was 8 and 12 < mRNA alone.
- Most efficient LNPs similar to positive control Lpf2k (92.65%).



- Increasing DOPE does not really increase transfection rates.
- In fact, efficiencies are slightly lower than 4:1:1 formulation.
- 2, 3, 5, 6, 9 could induce GFP fluorescence ~70% of the time.
- 6 highest at 77.6%.
- Lpf2k was at 91.2%.
- 8 still relatively inefficient, However, 12 increased and 11 decreased in efficiency.

Methods and Results for Cytotoxicity



MTT Assay Methods

- Cytotoxicities of unformulated LNPs were measured using MTT assay.
- Formulated LNPs would have similar toxicities as unformulated.
- Compared to commercially available lipid Lipofectamine 2000.
- Final LNP concentration was 7.4 µg/mL.
- Final GFP mRNA concentration was 0.74 µg/mL.

MTT Assay Results

- 6 and 10 have low cytotoxicity with cell viabilities over 90%.
- 9 and 11 have moderately high cytotoxicity and cell viabilities ~60%.
- 2 and 3 have high cytotoxicity levels with cell viabilities below 20%.
- Lpf2K moderately high in cytotoxicity.

Interpretations of Results

Helper lipids stabilize LNPs, increasing their formation.

Before coming into contact with cells...

- Cholesterol and DOPE can fill in gaps between lipidoids in the liposome structures.

After coming into contact with cell membrane…

- Helper lipids destabilize LNP structure due to being natural lipids and having higher affinities for cell membranes compared to artificial lipids.
- This effect arises because helper lipids' structures are more similar to the structures of cell membranes compared to artificially synthesized lipidoids.
- As a result, stronger hydrophobic interactions arise, accelerating the breakdown of LNP structures and release of cargo.

Conclusions

- Low proportion (~5%) of GFP positive cells in solutions with only mRNA shows how GFP mRNA does not effectively get transferred into cells without LNPs.
- Formulated LNPs with helper lipids DOPE and cholesterol tend to do better than unformulated ones in inducing GFP fluorescence, suggesting that future LNPs should be formulated in similar manner.

LNPs with high efficiencies and low toxicities may be favorable for use in drug delivery.

- Formulations with DOPE and cholesterol increase efficiency without drastically affecting toxicity
- This includes formulated 4:1:1 LNP:Cho:DOPE 6 and 10 which have comparable efficiency as lipofectamine 2000 but higher cell viabilities (> 90%).

Future Directions

- Address problem of cytotoxicity.
- Investigate efficient low toxicity formulated lipidoids in further in vitro/in vivo studies.

Acknowledgements

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