Optimization of lipid nanoparticle formulations of siRNA and mRNA for brain gene therapy

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BACKGROUND

Brain diseases are a significant burden to the Canadian health care system, and can be caused by both heritable and sporadic genetic mutations. Many genetic neurodevelopmental and neurodegenerative diseases are caused by either the toxic gain-of-function of a mutant protein, or a loss-of-function mutation.[1,2]

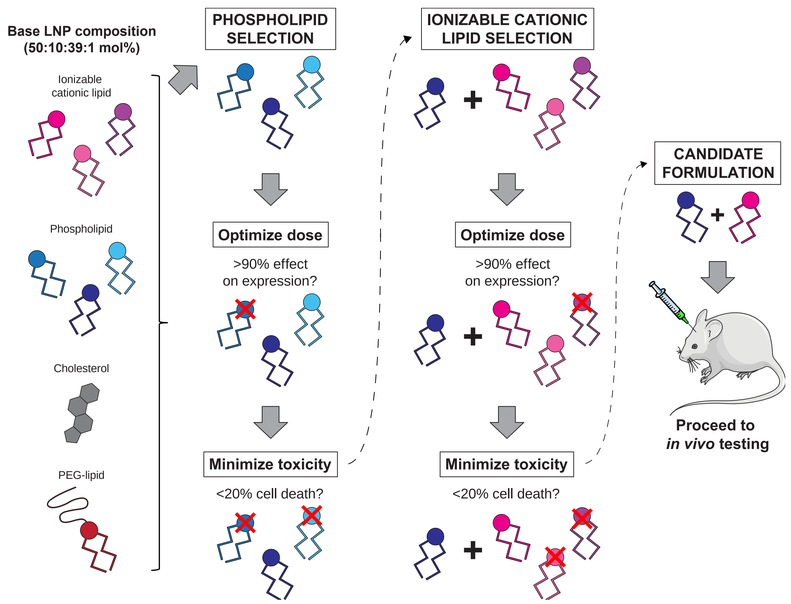
- Brain gene therapy agents must be efficiently delivered to and effective in neurons (the primary cells of interest in the brain)
- Current brain gene therapy approaches are limited by toxicity and immunogenicity
- Neurons are highly amenable to transfection by lipid nanoparticles (LNPs), and LNPs are safe and effective for the treatment of other genetic diseases[3-6]
- *In vivo* LNP administration will occur by direct injection into cerebrospinal fluid or brain tissue, so LNP formulation screening in primary neurons *in vitro* will likely translate accurately to *in vivo* work

PURPOSE

To identify and optimize lipid nanoparticle formulations and doses for the delivery of gene therapy payloads *in vitro* and *in vivo* to treat genetic brain diseases.

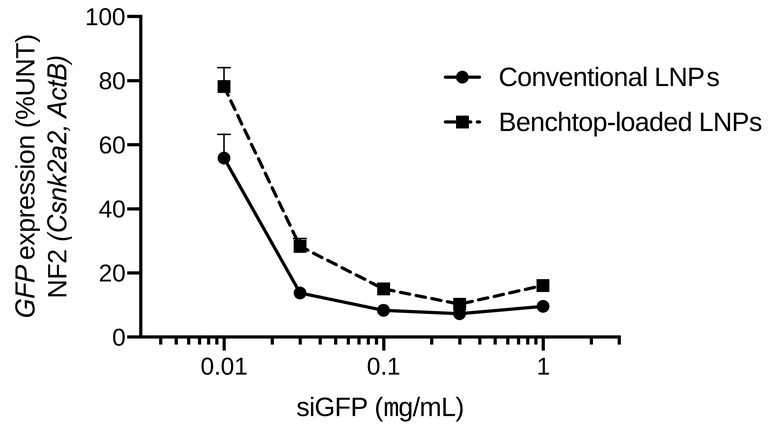
IN VITRO SCREENING STRATEGY

Workflow to optimize LNP formulation and dosage for the efficient delivery of siRNA or mRNA to primary cortical neurons *in vitro*.



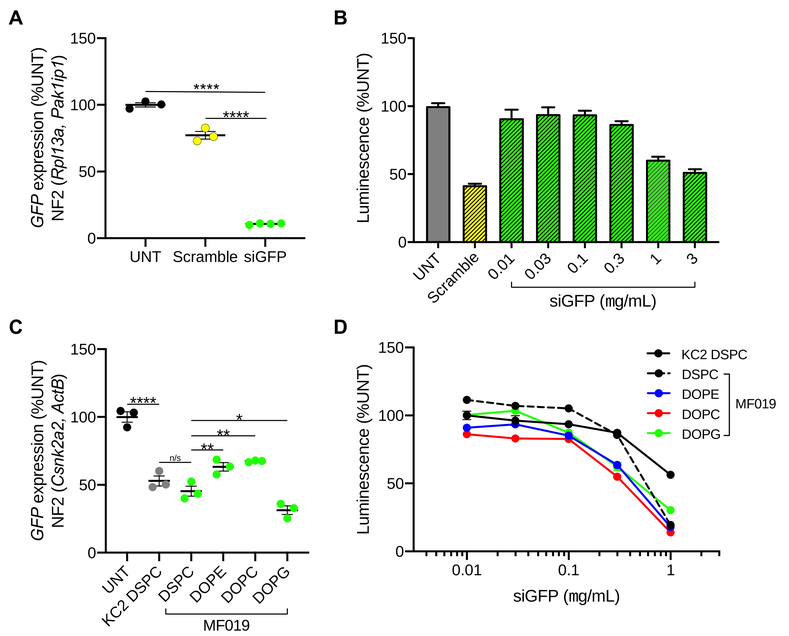
"DUMP AND MIX" LNP PREPARATION

Combining preformed vesicles at pH 4 with siRNA by pipette mixing on the benchtop produces LNPs that perform similarly to particles produced using rapid mixing (for details, scan QR code, top left).



RESULTS: siRNA

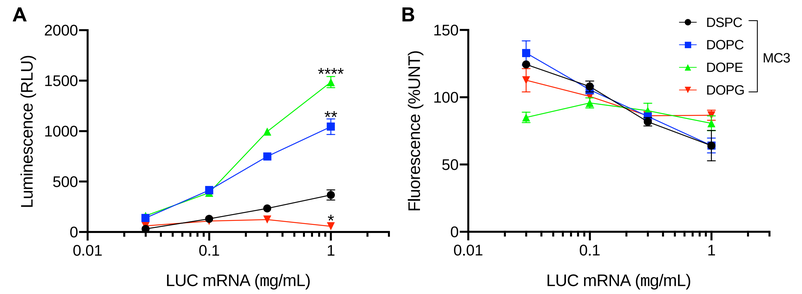
siRNA dose and lipid type affect GFP reporter knockdown efficiency and primary neuronal cell viability *in vitro*.



(A) LNP-mediated GFP knockdown (measured by RT-qPCR) is efficient at 3 µg/mL siGFP. (B) siRNA dose impacts primary neuronal viability *in vitro*. (C) In LNPs prepared using the ionizable lipids DLin-KC2-DMA (KC2) or MF019 (proprietary lipid), phospholipid species impacts GFP knockdown efficiency at 0.01 µg/mL siGFP (measured by RT-qPCR). (D) Higher doses result in decreased primary neuronal viability *in vitro,* regardless of phospholipid species.

RESULTS: mRNA

mRNA dose and phospholipid type affect luciferase reporter expression and primary neuronal cell viability *in vitro*.



(A) In LNPs prepared using the ionizable lipid DLin-MC3-DMA (MC3), phospholipid species impacts the magnitude of luciferase expression at 1 µg/mL luciferase mRNA (measured by microplate luminometer). (B) Higher doses result in decreased primary neuronal viability *in vitro,* regardless of phospholipid species.

FUTURE DIRECTIONS

- Assess the impact of ionizable lipid type on GFP reporter knockdown efficiency and luciferase reporter expression in primary neuronal viability *in vitro.*
- Evaluate the performance of optimized neuronal LNP formulations in other primary brain cell types *in vitro*.
- Evaluate the performance of *in vitro*-optimized LNPs *in vivo*.

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