In vitro Expression of Myc897–Piezo1 via Lipid Nanoparticle Delivery System

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Introduction

Magnatogenetics is a novel neuromodulation method receiving attention lately, with capabilities to target deep brain regions, high special and temporal resolution, and non-invasiveness. Its implementation in further studies requires an artificial factor that responds to magnetic stimulation. Myc897-Piezo1, a modified variant of Piezo1 protein, a *bona fide* mechanosensitive cation channel, allows magnetomechanical neuromodulation.

Until now, Myc897-Piezo1 was administered through viral vectors, which have high immunogenicity and requires long periods of time for expression. LNP is faster in speed, with lower immunogenicity and higher biocompatibility compared to viral vectors. In this study, we focused on these capabilities of LNP as a novel delivery system for foreign protin expression, which can also be further applied to future gene therapies.

Scheme

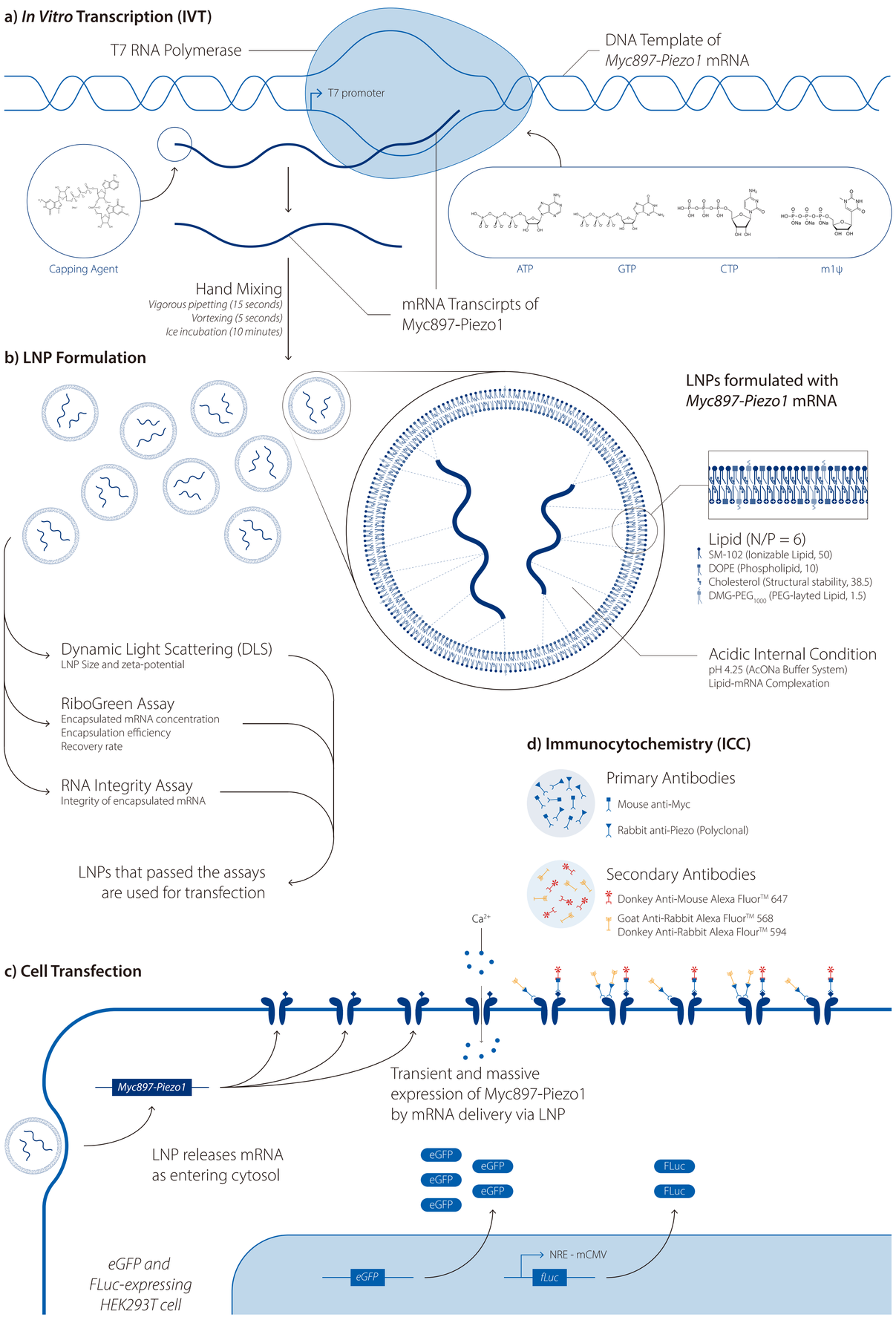

Figure 1 | **Overall Scheme of Research.**

a) In vitro transcription (IVT) produces large quantities of Myc897-Piezo1 mRNA from the DNA template. We used m1ψ to lower immunogenicity and capping agent to produce mature eukaryotic mRNA.
b) Hand mixing the mRNA-containing aqueous phase and the lipid-containing ethanol phase produces LNP. DLS, RiboGreen, and RNA integrity assays are done to ensure the quality of LNPs. The samples which passed all three tests are used for transfection.
c) LNPs containing Myc897-Piezo1 mRNAs are transfected into eGFP and FLuc expressing HEK293T cell. mRNAs are released from lipid, and then translated into Myc897-Piezo1 channel.
d) The validity of Myc897-Piezo1 expression was confirmed using ICC. The samples were visualized using an inverted confocal microscope system and co-labelled with DAPI to monitor the nucleus.

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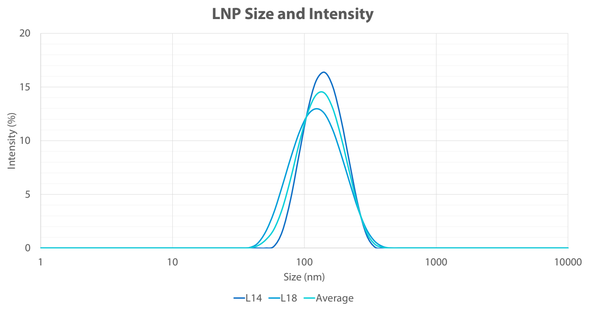
Results

Physicochemical Properties of LNPs

a) DLS Analysis Data



Sample	L14	L18	Average
Z-Average Size (nm)	133.1	113.9	138.3
PDI	0.086	0.142	0.130

Figure 3 | **DLS Results of Selected LNP Samples.**

b) RiboGreen Assay Data

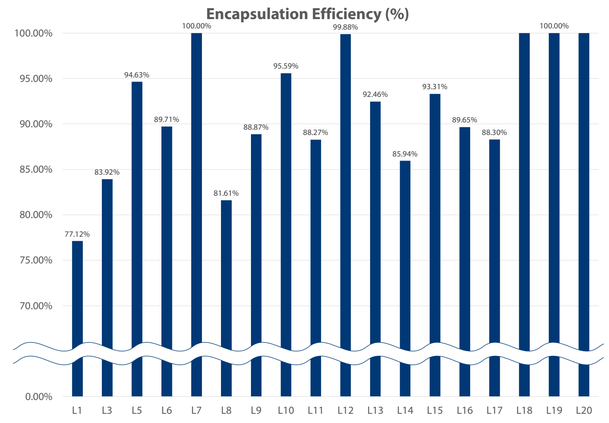


Figure 4 | **Encapsulation Efficiency of LNP Products.**

Expression of Myc897-Piezo1 in HEK293T by mRNA delivery via LNP

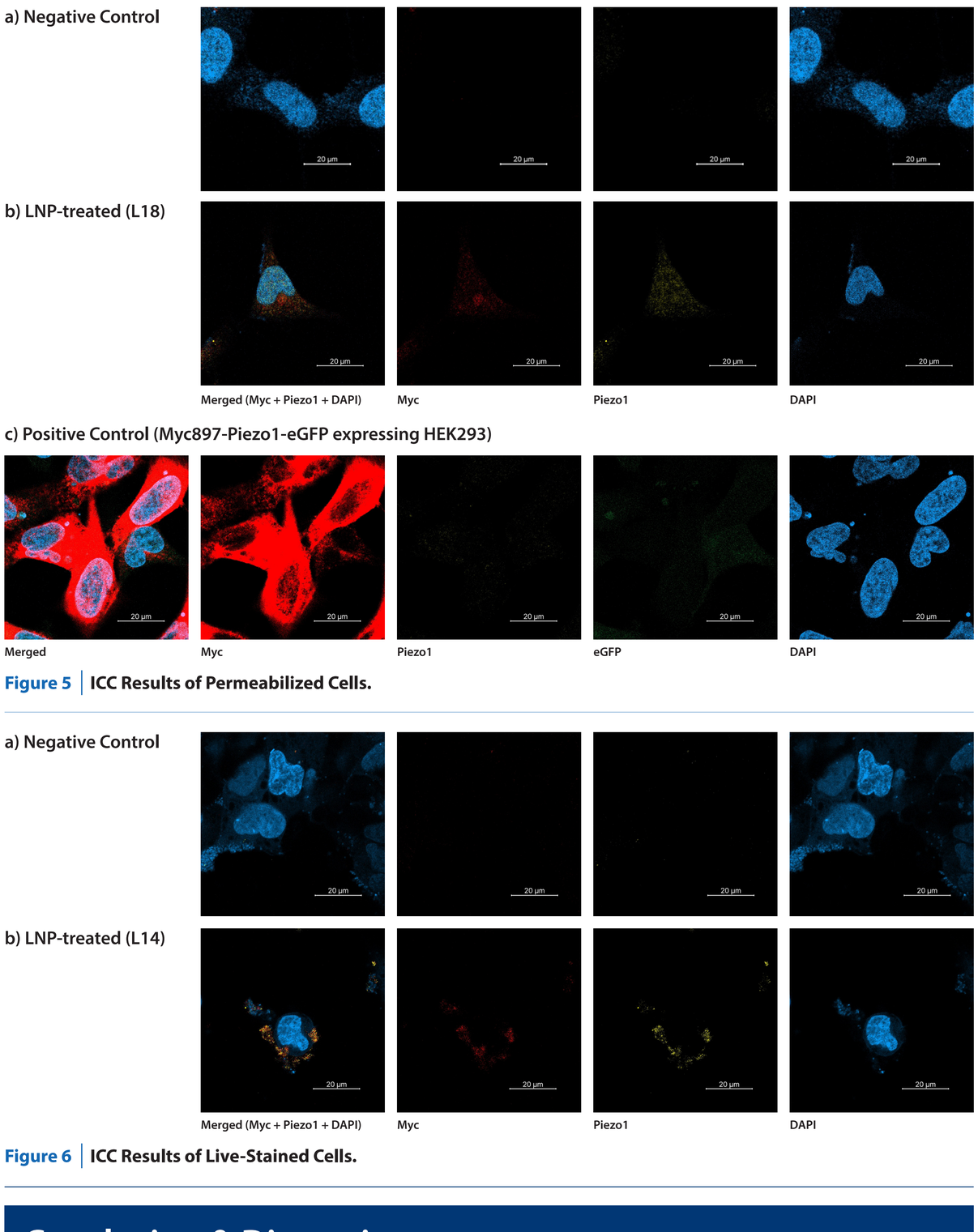


Figure 5 | **ICC Results of Permeabilized Cells.**

Figure 6 | **ICC Results of Live-Stained Cells.**

Conclusion & Discussion

In this study, we attempted to deliver Myc897-Piezo1 mRNA through LNP. From the Ribogreen and RNA integrity assay, we could confirm that target mRNA was successfully encapsulated into LNP, without damage. The target mRNA in this study, Myc897-Piezo1 (8.1 kb) is significantly bigger in size than current LNP-delivered mRNAs in market, such as Pfizer BNT16262(4.3 kb) or Moderna mRNA1273 (5.5 kb). This success poses a significant milestone of possibility to the gene therapy using LNP, where studies regarding the size capacity of mRNA for LNP delivery is scarce.

Furthermore, we confirmed the increased signal of Myc and Piezo1 in cells transfected with produced LNPs through ICC results of permeabilized cells, and observed the localization of proteins in membrane after 48 hours from transfection in live cell staining ICC results. The Myc and Piezo1 signals colocalized in ICC results confirms that the Myc897-Piezo1 is expressed in HEK293T cells, which do not express Piezo1 channel endogenously.

Additional investigations are necessary to determine the extent of protein expression, the functionality of expressed proteins, and the responsiveness of expressed proteins to magnetic stimulation. These investigations can be carried out using a luciferase assay or other calcium-sensing methods. Despite the need for further research, the present study has met the requirements for the use of LNP in future magnetogenetics research, as we have validated the expression of the Myc897-Piezo1 channel through a successful LNP delivery system.

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