Electroporation Enables Oligonucleotide Delivery to Regulate Gene Expression in Friedrich's Ataxia Patient-Derived Cells

Background

Oligonucleotide drugs are an up-and-coming area of development that presents many possibilities for the personalized treatment of rare diseases. There are about 8,000 different rare diseases. Each individually affects fewer than one in 2,000 people—but combined, rare diseases affect over 300 million people worldwide. Many of these disorders have a genetic component, making them excellent targets for personalized, oligonucleotide therapies based on short sequences of nucleic acids designed to regulate mRNA and protein expression and address diseases at the transcriptome level.

Friedrich's Ataxia (FRDA) is a rare genetic disease caused by a mutation in the frataxin (*fxn*) gene, which codes for a mitochondrial protein. In FRDA patients, a trinucleotide repeat expansion in non-coding DNA causes the corresponding mRNA to form an R-loop, hindering transcription of *fxn*. There are no therapies yet which address FRDA at the transcriptome level by up-regulating *fxn* expression.

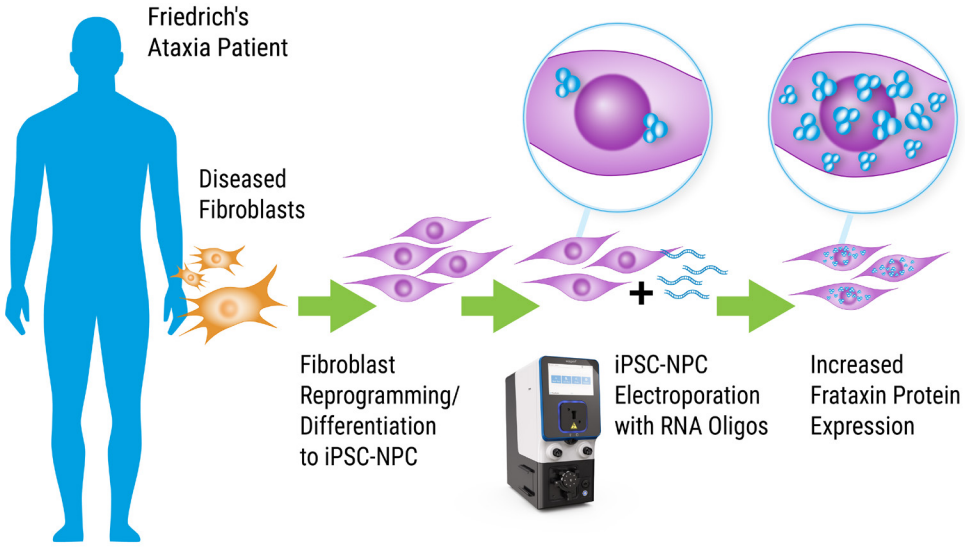
All oligonucleotide drugs operate by base-pairing with target sequences in the affected gene—for FRDA, the target is the *fxn* repeat expansion. There are two types of oligonucleotide drugs: antisense oligonucleotides (ASOs) and RNA interference (RNAi) therapies. ASOs function as a single strand; they often carry chemical modifications to enhance their stability and binding affinity with the target. RNAi drugs are based on small interfering RNA (siRNA) that work with the cells' own RNA silencing machinery to alter gene expression.

Researchers have previously demonstrated that both ASOs and siRNAs could effectively target the FXN repeat expansion and increase FXN protein expression in FRDA patient-derived fibroblasts. Now, MaxCyte® Flow Electroporation technology is helping them to investigate the efficacy of oligonucleotide therapies in induced pluripotent stem cell-derived neural progenitor cells (iPSC-NPCs) from FRDA patients.

Aim

The study presented here was designed to investigate oligonucleotide activators as candidates for FRDA treatment. The researchers' first goal was to find an efficient, reproducible way to deliver synthetic oligonucleotide drug candidates into patient-derived iPSCs; MaxCyte's Flow Electroporation® technology provided a perfect solution. Once the oligonucleotides were introduced through highly efficient and safe ExPERT™ electroporation, featured experiments examined their ability to up-regulate FXN mRNA and protein expression.

MaxCyte Flow Electroporation®: A Practical Method for Oligonucleotide Drug Discovery



Method Overview

Below is a summary of the protocol used to reprogram and differentiate patient-derived iPSCs then transfect oligonucleotides into the resulting iPSC-NPCs to assess their effect on gene expression. The procedure illustrates the perfect fit of MaxCyte Flow Electroporation in developing and delivering novel gene therapies.

1. **Reprogramming** – Fibroblast cell lines were reprogrammed to iPSCs using integration-free Sendai virus transgene delivery.
2. **Differentiation** – The iPSCs were differentiated into NPCs by inhibition of TGF-β/SMAD signaling.
3. **Transfection** – iPSC-NPCs were processed using the MaxCyte STx™ instrument with preset Optimization protocols 4 and 6 and OC-100 processing assembly to deliver synthetic ASOs and siRNA drug candidates. After transfection, cells recovered briefly at 37°C and were then transferred to 12- or 24-well plates.
4. **Analysis** – *fxn* mRNA and protein expression were monitored by qRT-PCR and western blot, respectively, 24-96 hours post-transfection.

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Results

Efficient Electroporation and Transcript Knockdown

Human motor neuron cells (HMN, iXCells Biotechnologies) and a previously identified target gene and benchmark oligonucleotide compound (*MALAT1* and anti-*MALAT1*) were used for initial evaluation of transfection methods. Following either MaxCyte electroporation, Lipofectamine® stem transfer reagent, or gymnotic (unassisted) uptake, post transfection cell viability and knockdown of *MALAT1* 24 h were compared for each method. A 1 µM dose of the anti-*MALAT1* ASO delivered by MaxCyte electroporation gave over 95% RNA knockdown in HMNs and NPCs (Figure 1A, 1C), significantly better than lipid transfection or gymnotic delivery. Similarly, cell viability post electroporation proved equal to or better than lipid transfection or cells that received oligo alone via gymnotic free uptake (Figure 1B, 1D).

Figure 1

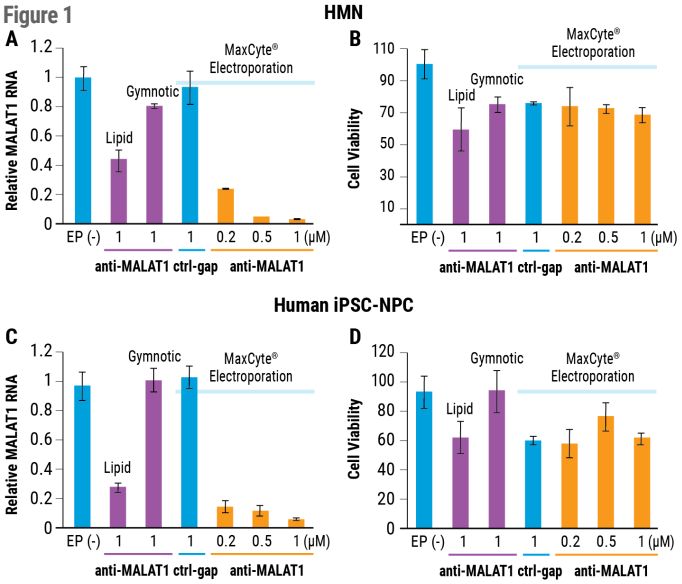

Figure 1. Electroporation for optimized cell viability and RNAi efficiency. A) Relative *MALAT1* RNA levels (measured by RT-qPCR) and B) relative HMN cell viability (measured by Trypan blue staining) after transfection by either lipid transfection, gymnotic delivery or MaxCyte electroporation. C) Relative *MALAT1* RNA levels in FRDA patient-derived iPSC-NPCs after transfection by MaxCyte electroporation. D) Relative cell viability was measured by Trypan blue staining. All assays performed 24h post transfection with anti-*MALAT1*. EP(-) is no treatment/no electroporation control. Ctrl-gap is a control oligonucleotide. Error bars represent ± SD.

Up-Regulated Frataxin Expression

fxn mRNA expression in FRDA patient-derived iPSC-NPCs was compared with and without three different oligonucleotide activators: ss-siRNA-1, a single-stranded siRNA which uses the RNAi mechanism; BNA-2, an ASO which contains a constrained (bridged) ethyl modification on the ribose for enhanced binding affinity to the repeat expansion; and a control, ctrl-ASO.

Figure 2

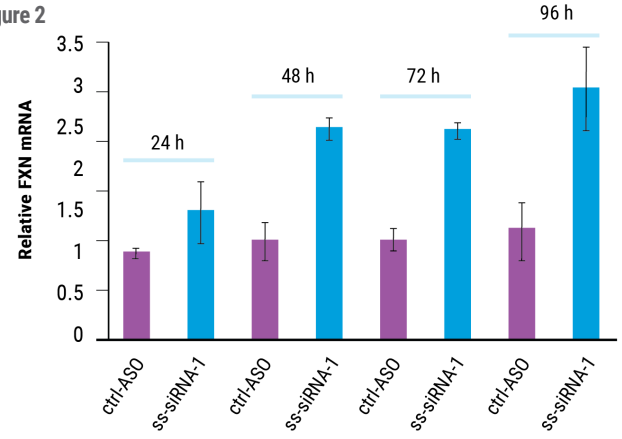

Figure 2. Time course of FRDA activation. *fxn* mRNA expression in FRDA patient-derived iPSC-NPCs with ss-siRNA-1 (5 µM, n = 2, optimization 4) and control oligonucleotide ctrl-ASO. Error bars represent ± SD.

iPSC-NPCs from a healthy person expressed 2.5 times more *fxn* mRNA than FRDA patients. MaxCyte electroporation of 5 µM ss-siRNA (Figure 2) or BNA-2 (data not shown) increased *fxn* expression in FRDA patient-derived iPSC-NPCs to normal levels within 48h. *fxn* mRNA remained elevated until at least 96h post transfection.

As predicted, activation of *fxn* mRNA expression led to increased protein expression. Delivered at 5 µM by MaxCyte Flow Electroporation, ss-siRNA-1 and BNA-2 increased frataxin protein threefold, near the level expressed in healthy cells (Figure 3).

Figure 3

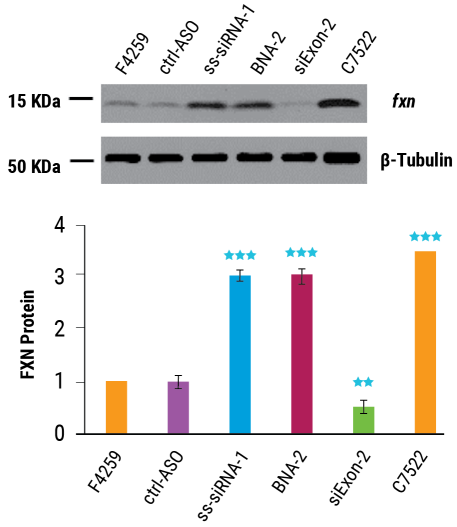

Figure 3. Activation of Frataxin protein expression. FRDA patient-derived NPCs (F4259) and wild-type NPCs (C7522) were electroporated using the MaxCyte STx™ with oligonucleotides (5 µM, n = 3) and FXN protein measured 96 hr post transfection by western blot. Error bars represent ±SD. ** indicates P < 0.01 and *** indicates P < 0.001 relative to F4259-90% by student t-test.

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Enhanced Potency through Backbone Modification

MaxCyte Flow Electroporation enabled evaluation of a matrix of siRNAs and ASOs with different backbone modifications (bridged ethyl and 2'-methoxyethyl) to determine each potential drug's efficacy for up-regulating *fxn* mRNA. *fxn* expression was measured at several doses and half maximal effective concentration (EC_{50}) calculated for each oligonucleotide (Table 1). The most potent (lower EC_{50}) drugs were constrained ethyl ASOs and siRNAs.

Table 1: Synthetic oligonucleotide effect on *fxn* mRNA expression in FRDA patient-derived iPSC-NPCs. cET = constrained ethyl, 2'-O-MOE = 2'-methoxyethyl. The most potent drug from each type of backbone chemistry is highlighted.

Oligonucleotide	Type	Backbone Chemistry	Length (bases)	EC50 (μM)
ss-siRNA-1	siRNA	ssRNA	21	0.62 ± 0.19
siGAA	siRNA	dsRNA	21	0.80 ± 0.44
BNA-2	ASO	cET	16	0.50 ± 0.09
BNA-9	ASO	cET	16	0.71 ± 0.19
BNA-17	ASO	cET	18	–
M-1	ASO	2'-O-MOE	16	2.00 ± 0.84
M-2	ASO	2'-O-MOE	16	2.18 ± 0.99
M-3	ASO	2'-O-MOE	16	2.06 ± 0.68
M-4	ASO	2'-O-MOE	18	1.07 ± 0.38
M-5	ASO	2'-O-MOE	18	0.75 ± 0.37
M-6	ASO	2'-O-MOE	18	1.74 ± 0.63

Advantages of MaxCyte Electroporation

This study demonstrates a unique solution for introducing synthetic oligonucleotide therapies into iPSC-derived cells. MaxCyte Flow Electroporation was more efficient, less toxic, and easier for novice users to implement to achieve successful outcomes compared to lipid transfection. The authors anticipate that electroporation will be an invaluable tool for testing new therapies and enabling rapid drug development process.

Conclusion and Future Applications

In summary, MaxCyte technology is helping to advance research towards innovative gene therapies to address rare genetic diseases. The study presented here demonstrates that MaxCyte Flow Electroporation is a reproducible, effective method for testing panels of oligonucleotide drugs in patient-derived neural progenitor cells with promising ability to up-regulate gene and protein expression to therapeutic levels. The results featured provide a framework for use of Flow Electroporation as an effective tool for development of oligonucleotide therapies as well as candidate screening and lead optimization strategies for genetic disorders at the transcriptome level.

References

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2. Simic. *Croat Med J*. 2019 Dec; 60(6): 485–487. doi: 10.3325/cmj.2019.60.485