A Human iPSC-Based Platform for Functional Toxicity Screening of CNS-Targeted RNA Therapeutics

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Ncardia

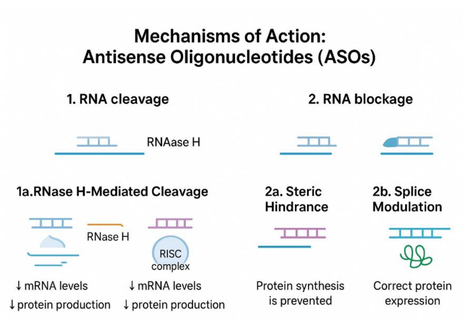
#3798

Background and Purpose

Human induced pluripotent stem cell (iPSC)-derived neural co-cultures represent an advanced in vitro model that recapitulates key structural and functional features of the human central nervous system (CNS). Such models enable the study of neuron-glia interactions and provide a more physiologically relevant context compared to traditional cell lines or animal-derived systems. Given the growing therapeutic interest in antisense oligonucleotides (ASOs) and the ongoing concerns regarding their potential CNS toxicity, iPSC-derived neural co-cultures offer a valuable platform to evaluate ASO safety, elucidate mechanisms of neurotoxicity, and support the development of safer oligonucleotide-based therapeutics

Methods

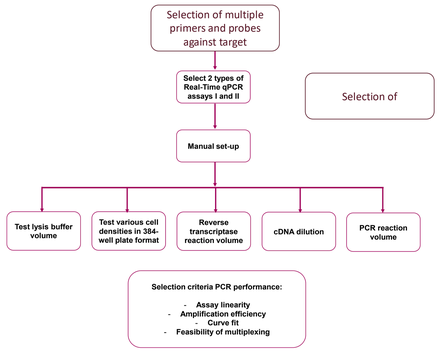
Building on evidence that ASO-induced neurotoxicity correlates with suppressed calcium transient activity and altered electrophysiology in vivo, we sought to develop complementary in vitro functional assays to better characterize ASO effects on neuronal activity. To this end, we established high-throughput calcium imaging and multi-electrode array (MEA) platforms containing human iPSC-derived neurons and astrocytes. The calcium imaging assay enables quantitative evaluation of spontaneous calcium transients, providing insights into network excitability and functional integrity, while the MEA assay allows for real-time monitoring of electrical activity across neuronal networks. Together, these physiologically relevant and scalable systems facilitate comprehensive assessment of ASO-induced functional perturbations and support early identification of potential neurotoxic liabilities



Designed to bind complementary RNA targets, ASOs can modulate RNA processing to reduce, restore, or modify protein expression through several distinct mechanisms. These include promoting RNA degradation via RNase H, altering splicing patterns, or blocking translation, thereby enabling precise and targeted regulation of gene expression at the RNA level. *Adapted from J. Clin. Med.* 2020, 9(6), 2020 10.3390/jcm9062004

Automated platform for ASO screening

A



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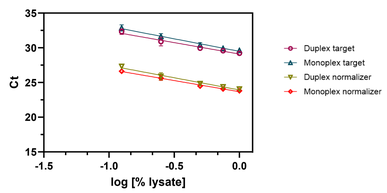
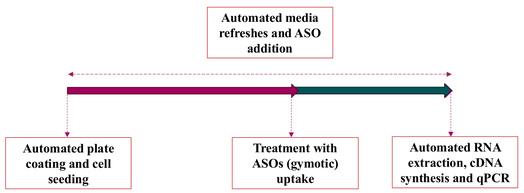


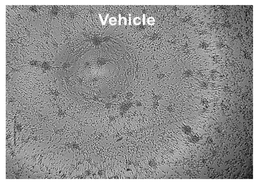
Before automation, two Real-Time qPCR assays were compared and 384-well conditions optimized based on performance metrics in untreated lysates (A).
(B) shows linear regression of the selected monoplex and duplex conditions across concentrations (mean ± SD).

A



B

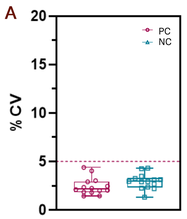
C



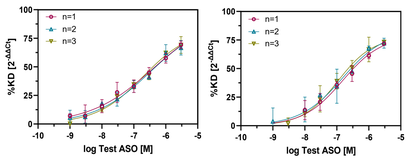
(A) illustrates the assay setup from cell seeding until downstream assay. All steps were performed using a cell culture-grade fully automated liquid handling system, as shown in (B). No significant morphological changes were visualized in vehicle controls as illustrated by phase contrast imaging (C). Neurons can be maintained in culture for extended periods.

Automated platform for ASO screening (ctd)

A



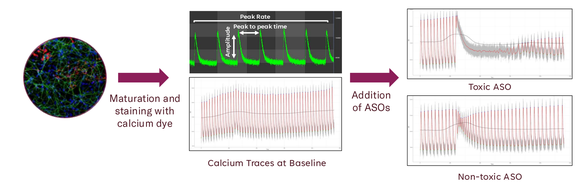
B Dose response curves in biological duplicate plates of a test ASO



(A) shows the mean of negative control conditions across 14 cell plates. Each cell plate was run as technical PCR triplicate. In both cases, the inter-plate variability in positive or inter-plate variability between technical replicates was below 5%, and the inter-plate variation among biological replicates was 2.8% for vehicle and 3.9% for the control, concluding a robust assay performance.

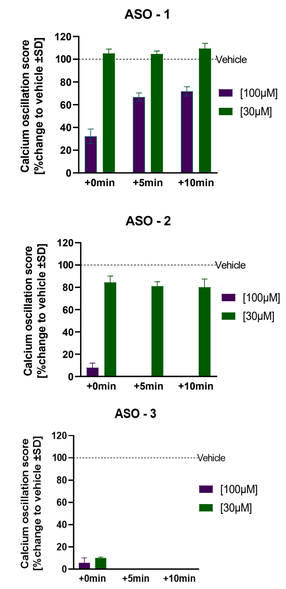
(B) shows an exemplary dose response of a test ASO in biological replicate. Data were plotted as %KD and fitted as non-linear, 4-parameter sigmoidal curve ± SD. Each line represents the fit of a technical PCR triplicate.

In vitro safety assessment of ASOs: Ca^{2+} traces



Ncyte CNS composed of glutaminergic, cholinergic and GABAergic neurons and astrocytes → Recording of spontaneous calcium peaks → Quantification of calcium peak amplitude, peak to peak time and peak rate

Exemplar calcium traces from Ncyte CNS cultures treated with toxic (upper trace) and non-toxic ASOs (lower trace). Toxic ASOs cause the disruption of spontaneous calcium activity.



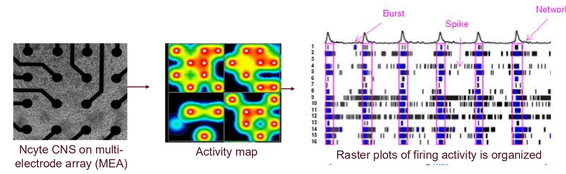
ASO-1 shows an example of a putative "safe" ASO, indicated by the preserved neuronal function.

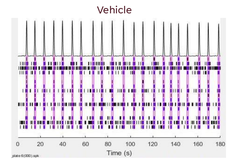
ASO-2 depicts an ASO that appears acutely safe at lower concentrations but begins to show toxicity as the concentration increases.

ASO-3 demonstrates a "toxic" ASO, which causes marked disruptions in calcium transient patterns complete loss of activity consistent with impaired neuronal viability or excitability.

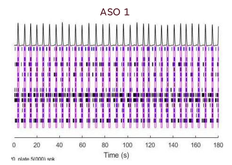
These examples show how the Calcium assay can be used to distinguish between safe and toxic ASOs

In vitro safety assessment of ASOs: MEA traces

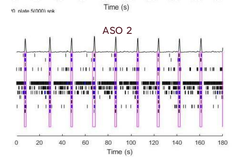




Raster MEA plots used to evaluate the effects of ASOs. In vehicle treated wells, spikes and network bursts are strong and at regular intervals



In neuronal co-cultures treated with ASO-1, a significant decrease in amplitude with an increase in event frequency was observed, suggesting a hyperactive neuronal profile.



ASO 2 demonstrates a "toxic" ASO, which causes marked disruptions in electrical activity such as reduced amplitude and network burst frequency, consistent with impaired neuronal excitability.

Collectively, these examples highlight how MEA can be applied as a sensitive, functional screen to discriminate between safe and neurotoxic ASOs.

Conclusions

This iPSC-based platform enables scalable, human-relevant, functional screening of ASO neurotoxicity, supporting the development of safer CNS-targeted RNA therapeutics:

- Fully automated RT-qPCR assay established to assess ASO efficacy in human iPSC-derived neurons, delivering highly robust data (<5% inter-plate variability) and applicable across multiple iPSC-derived cell types.
- High-throughput calcium transient assay using Ca^{2+} imaging in hiPSC-derived CNS cultures to evaluate dose-dependent acute neurotoxicity of ASOs.
- MEA (microelectrode array) assay developed to assess ASO-induced effects on neuronal network activity, capturing changes in firing rate, burst frequency, and synchrony.

Ncardia's hiPSC-derived neuronal cell models enable early screening for ASO efficacy and neurotoxicity, providing a powerful platform for improving drug development efficiency.

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