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mRNA-Lipid Nanoparticles: A potent tool for manipulating neuronal genes

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Purpose: The use of RNA to manipulate gene expression in neuroscience has been limited due to the lack of an effective delivery tool. Recently, lipid nanoparticles (LNPs) have gained interest as safe and effective RNA delivery vehicles both *in vitro* and *in vivo*. However, traditional methods for the manufacturing of LNPs pose numerous challenges such as operator variability, and poor scalability. Here, we describe the robust and reproducible manufacture of mRNA-LNPs using the scalable microfluidics-based NanoAssemblr™ platform. The mRNA-LNPs exhibit exceptional transfection and exogenous gene expression efficiency in difficult-to-transfect primary neurons.

Methods: GFP mRNA was encapsulated into LNPs using the NanoAssemblr™ Benchtop instrument and the NanoAssemblr™ Spark™ (Precision NanoSystems Inc., Vancouver, Canada). The mRNA-LNPs were characterized for their size, PDI, and GFP mRNA encapsulation efficiency. Two mRNAs of ~1000 nucleotides and ~4500 nucleotides respectively, were encapsulated to observe the effect of particle size. GFP mRNA-LNP uptake and GFP expression in neural cell cultures was investigated using flow cytometry, ELISA, and confocal microscopy. Cell viability of neurons was investigated using the Presto Blue Assay.

Results: mRNA was successfully encapsulated into LNPs using the NanoAssemblr™ platform. The LNPs encapsulating the two different mRNA lengths both exhibited the same size of ~120 nm, low PDI (< 0.2), and high mRNA encapsulation efficiency (> 95%). Flow cytometry analysis after a 48 h treatment of 2.5 µg/mL GFP mRNA-LNP in rat primary neurons at DIV 7 showed > 95% uptake of nanoparticles (based on a fluorescent probe within the nanoparticle) leading to > 95% of cells expressing GFP. Similar results for GFP expression were observed using ELISA. The high expression and uptake did not significantly impact cell viability even at doses of 5 µg/mL of GFP mRNA as measured using Presto Blue Cell Viability Assay.

Conclusions: The NanoAssemblr™ microfluidics-based technology reproducibly manufactured mRNA-LNP that effectively mediated gene expression in primary neurons, demonstrating mRNA-LNPs as an effective delivery tool for neuroscience applications.

Biography

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