DEVELOPMENT OF LIPID NANOPARTICLES CONTAINING miRNA FROM HUMAN NEURAL PRECURSOR CELLS

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Parkinson's Disease (PD) is a degenerative disorder caused by the loss of dopaminergic neurons. It is characterized by a range of motor symptoms, including akinesia and bradykinesia, tremor, and rigidity. Current treatments for PD are symptomatic and cannot stop disease progression or reverse neurodegeneration[1]. miRNA therapy has emerged as an innovative approach to treating PD. However, this approach presents challenges, such as the rapid degradation of miRNA by endonucleases and the limited ability of these molecules to cross cell membranes[2]. To overcome these drawbacks, non-viral nanocarriers, like lipid nanoparticles (LNPs), have offered a promising strategy for effectively delivering therapeutic miRNAs[3].

Given the information presented, miRNA from neural precursor cells was encapsulated in lipid nanoparticles aiming to treat PD. miRNA was isolated from neuronal progenitor cells differentiated from human umbilical cord stem cells, using the following kits: “Exosome Isolation Reagent (4478359)”, and “Total Exosome RNA & Protein Isolation Kit (4478545)” from Invitrogen™ by Thermo Fisher. miRNA-loaded lipid nanoparticles were prepared by the ethanol dilution method as previously described. Briefly, an ethanolic solution containing a cationic (DOTAP) and an ionizable lipid (DODMA) that enables nucleic acids to be complexed for release inside cells, a pegylated lipid (DSPE-PEG2000), which provides stability and allows the particles to evade the immune system, and structural lipids such as cholesterol and the DSPC, was prepared. The ethanolic solution was then mixed with an acetate buffer pH 4, containing the miRNA. The LNPs were formed spontaneously and following dialyzed against a phosphate buffer at pH 7.4. The DODMA/DOTAP:DSPC:DSPE-PEG2000:cholesterol ratio was 50:5:1.5:38.5 for all tested formulations. The total lipid concentration in the LNPs was 12.5 mM. Unloaded LNPs were also prepared using the same conditions. The LNPs were characterized according to their size and polydispersity index (PDI), and zeta potential using dynamic light scattering (DLS) and laser-doppler anemometry, respectively. Additionally, the morphology of unloaded and miRNA-loaded NPLs was assessed by cryogenic transmission electron microscope (Cryo TEM, LNNano, CNPEM, Campinas, SP).

Unloaded (LNP_{B1}) and miRNA-loaded (LNP_{miRNA}) LNP presenting nanometric size and monodisperse size distribution were obtained, but an increase in both particle size and PDI was verified upon miRNA encapsulation

(Table 1). Both unloaded and miRNA-loaded LNP displayed charge surfaces close to neutrality, as expected due to the presence of polyethyleneglycol chains at particle surface [4-6].

Table 1: Size, PDI, and zeta potential values obtained for LNPs.

Sample	Size ± SD (nm)	PDI ± SD	Zeta potencial (mV)
LNP_{B1}	110.2 ± 0.305	0.284 ± 0.028	1.13 ± 0.816
LNP_{miRNA}	154.10 ± 4.517	0.316 ± 0.018	0.77 ± 0.481

Cryo-TEM images demonstrated nanoparticles displaying a spherical and vesicular structure in which the wall is composed of a bilayer related to the lipid organization and sizes similar to those obtained by DLS. Cryo-TEM images showed that the incorporation of miRNA at the LNP allowed the formation of a concentric core, suggesting the formation of the miRNA-loaded LNP with an electron-dense core. The results evidenced the viability of obtaining LNP-containing miRNA from neuronal precursor cells. Further studies will be carried out to demonstrate the potential application of miRNA-loaded LNP to treat PD.

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