Developing a Targeted, Localized Delivery Platform for miRNA using Lipid Nanoparticles

Thomas Jacobs[1], Anushka Phadke[1], D. Joshua Cohen[1], Barbara D. Boyan[1], Zvi Schwartz[1]
[1]College of Engineering, Virginia Commonwealth University, Richmond, VA

Statement of purpose: Research into lipid nanoparticles (LNPs) as a delivery system has accelerated rapidly since the FDA's approval of LNP-based vaccines in response to the COVID-19 pandemic, unlocking the ability to deliver mRNA sequences across the cell membrane. A potential new application of this LNP delivery system is for therapeutic microRNAs (miRNAs). MiRNAs are short, typically between 18 and 22 base pairs, noncoding RNAs that serve as posttranscriptional regulators of gene expression and have been implicated as powerful modulators of a variety of cell processes including proliferation, differentiation, and disease. While miRNAs represent an exciting new form of treatment to explore, there are also significant challenges related to bioactivity and targeted response to address before they can be considered a therapeutic and translated to clinical use. Therapeutic response is limited by how fast the miRNA is cleared from the cell, producing short cellular responses that can be beneficial in some situations, but lack relevance for chronic diseases or extended healing processes. Furthermore, miRNAs have the ability to produce varied responses dependent on the cell they are delivered to, making systemic delivery of miRNAs less desirable if the targeted response is for one site of action or isolated to a single cell type. In order to address these problems, the goal of this project was to develop a miRNA delivery platform that will localize targeted delivery and extend release of therapeutic miRNAs through the use of LNPs. In the present study we used miRNA-145, which is known to regulate differentiation of human bone marrow stromal cells (bMSCs) during bone regeneration.

Methods: MiRNA mimics and inhibitors were acquired as mature, double stranded sequences. Lipid nanoparticles (LNPs) were synthesized using a flow controller set at a constant pressure and microfluidic mixing chip and composed of a channel for the lipid formulation phase and an aqueous phase containing the miRNA. Different aqueous phases were tested for their effect on LNP physical properties. These included water as a neutral pH aqueous phase, sodium acetate at a pH of 5.01 and sodium citrate at a pH of 4.01. Outcome measures included size, polydispersity index (PDI), zeta potential, and encapsulation efficiency. Cell uptake for the chosen formulation was tested using a similarly sized fluorescently tagged miRNA strand and imaged using confocal microscopy. Transfection ability was quantified by delivering firefly luciferase (f-Luc) mRNA. Production of luciferase was quantified by adding luciferin to the cell lysate and measuring produced luminescence on a luminometer. For cell experiments, bMSCs were cultured on 24 well culture plates and grown to confluence in MSC growth media (GM) or differentiation media (DM: GM + 0.1 mM dexamethasone; 10 mM beta-glycerolphosphate; 50µg/mL ascorbic acid). Cells were treated with miR-145 mimic or inhibitor at 20 nM and 40 nM concentrations for 48 hours. Afterwards, the cell layers were lysed (DNA; alkaline phosphatase specific activity); and conditioned media were assessed for production of osteocalcin (OCN), osteopontin (OPN), osteoprotegerin (OPG), and vascular endothelial growth factor (VEGF), which were normalized to DNA. This was conducted on bMSCs sourced from two different donors, one male, and one female.

Results: LNPs were found to be around 115nm in diameter with a PDI less than 0.2 indicating population size homogeneity and with a neutral surface charge regardless of whether they encapsulated miRNA-145 or not and independent of aqueous phase. Encapsulation efficiency studies showed that water and sodium acetate buffer provided encapsulation at or above 90% of the amount of miRNA suspended in the aqueous phase, while sodium citrate had less than 20% encapsulation. Confocal microscopy showed successful cell uptake of miRNA. LNPs were also shown to deliver intact mRNA strands that were successfully transcribed into luciferase. Treatment with miRNA-145 mimic decreased production of osteogenic differentiation markers OPN and OPG. Treatment with miRNA-145 inhibitor increased production of osteogenic markers OCN and OPN. Effects of both the mimic and inhibitor only impacted bMSCs actively differentiating into osteoblasts, as there were minimal observed differences between cells cultured in growth media and treated with the miRNA mimic or inhibitor compared to the growth media control.

Conclusions: The data demonstrate selecting an appropriate aqueous phase for synthesizing miRNA loaded LNPs is critical to successful encapsulation. Our chosen aqueous phase produced LNPs capable of being up taken by cells and producing a biological response. Furthermore, miRNA-145 plays a role in bMSC fate determination. We postulate the reason why miR-145 only appears to exert an effect in differentiating bMSCs may reflect its role as a modulator of the rate and extent of osteoblastic phenotypic expression in cells committed to the osteoblast lineage. Further studies will explore using the LNPs to modulate bMSCs differentiation by delivering miRNA-145 as a clinically relevant delivery method.