Delivery of miRNA Using a Hydrogel and Lipid Nanoparticle System for Bone Regeneration Applications

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INTRODUCTION: Research into lipid nanoparticles (LNPs) as a delivery system has accelerated rapidly in recent years. 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 delivery, especially in an orthopaedic and dental context. 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 during the extended healing process of bone formation. Furthermore, miRNAs have the ability to produce varied responses depending on the cell they are delivered to, making systemic delivery of miRNAs less desirable if the targeted response is for a site of action such as a fracture or in conjunction with an orthopedic implant. To address these problems, the goal of this project was to develop a delivery platform that will localize delivery and extend release of therapeutic miRNAs. We hypothesized that by using an injectable, biorthogonal and rapidly polymerizing click hydrogel, it could serve as a reservoir for miRNAs encapsulated in LNPs, localizing and extending the release of those LNPs to prolong a therapeutic effect.

METHODS: MiRNA mimics were acquired as mature, double stranded sequences with a Cy3 fluorescent tag (Thermo-Fisher Scientific), firefly luciferase (f-Luc) mRNA strands were obtained with an N1-methylpseudoUridine cap for enhanced stability and expression (Genscript). 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 oligonucleotide strands to be encapsulated. Outcome measures included size, polydispersity index (PDI), zeta potential, and encapsulation efficiency. Hydrogel release kinetics of LNPs was performed by staining LNPs with a fluorescent dye and incubating gels loaded with the stained LNPs in PBS over 12 days. At each timepoint the releasate was collected and fluorescence was measured using a plate reader and compared to a previously generated standard curve (n=6). Hydrogel released LNPs were also measured for their physical properties. Uptake by bone marrow stromal cells (bMSCs) was tested using PKH26, a lipophilic dye, and a fluorescently tagged miRNA strand and visualized using confocal microscopy. Representative images were quantified for total cell number and cells containing LNPs or miRNA to estimate transfection efficiency (n=8). Transfection ability of LNPs was quantified by delivering LNPs containing f-Luc mRNA to bMSCs for 24 hours. Production of luciferase was quantified by adding luciferin to the cell lysate and measuring produced luminescence on a luminometer, and adding luciferin directly to the cell monolayer and imaging on an IVIS machine (n=6). LNPs released from the hydrogel were also measured for their ability to successfully transfect cells. Hydrogels containing f-Luc LNPs were incubated for 6 days in growth media, then the releasate was used to treat bMSCs for 24 hours (n=6). Luminescence was quantified in the same manner as above.

RESULTS: LNPs were found to be around 85-100 nm in diameter with a PDI less than 0.2 indicating population size homogeneity and with a neutral surface charge (Figure 1A). Encapsulation efficiency studies showed encapsulation above 85% of the amount of loaded miRNA in the aqueous phase. LNPs released from the hydrogel were similar in size, but had a higher PDI compared to those that had not been seeded in the hydrogel. Surface charge was comparable regardless of whether they were loaded in the gel or not. Release kinetics of LNPs from the hydrogel showed a small burst release of around 25% by hour 12, and then a steady consistent release profile up to approximately 80% of loaded LNPs by day 12 (Figure 1B). Confocal microscopy showed successful cell uptake of LNPs using PKH26 as a direct indication of LNP uptake, and fluorescently tagged miRNAs as an indication of successful delivery of payload to the cytoplasm. LNPs were also shown to deliver intact mRNA strands that were successfully translated into luciferase enzyme. Significantly, LNPs released from the gel also successfully transfected bMSCs with f-luciferase mRNA. Measured luciferase activity was 30% less than the positive control group containing the same concentration of f-Luc seeded into the gels, reflecting the result that 70% of LNPs were released from the gel by day 6 (Figure 1C).

DISCUSSION: Synthesized LNPs were consistent in physical properties and capable of encapsulating miRNAs for delivery. Confocal microscopy showed stained LNPs were incorporated into the cytoplasm of bMSCs, and LNPs carrying fluorescently tagged miRNA were also uptaken by cells, proving that miRNA delivery by LNPs is possible. Furthermore, hydrogel is able to release LNPs in a steady and reproducible manner. These LNPs were also not affected by loading or release from the gel, maintaining a similar size following release. Importantly, these gel-loaded LNPs were also capable of transfecting cells with mRNA, showing that as a system, *in vitro* delivery of LNPs using the hydrogel was successful. Further study will involve examining hydrogel-LNP delivery of bone-related miRNAs to affect osteogenic differentiation and proving the system in an *in vivo* model.

SIGNIFICANCE/CLINICAL RELEVANCE: The study shows an effective local delivery system of LNP with miRNA for treating bMSCs. Our goal is to transform this technology into a clinically relevant novel RNA delivery platform for the treatment of bone in orthopaedic and dental medicine addressing the unique challenges of bone regeneration and implantology including localization and extended release.

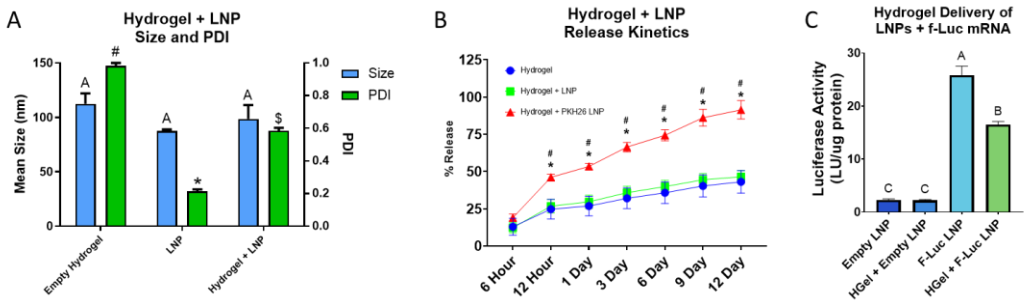

Figure 1: A) Measured physical properties of LNPs before and after loading and release from hydrogel. B.) Release kinetics of LNPs from the hydrogel. C.) Luciferase activity of cells treated with LNPs loaded with F-Luciferase mRNA and LNPs collected from hydrogel releasate media after 6 days.