Targeted Delivery of Gene Therapies to Chondrocytes via Peptide-Modified Lipid Nanoparticles for the Treatment of Osteoarthritis

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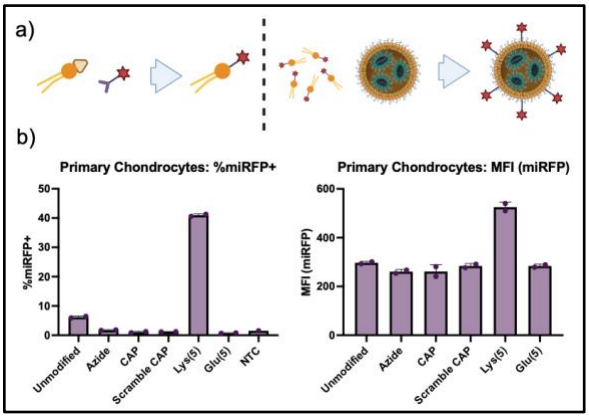
INTRODUCTION: Osteoarthritis (OA) is a degenerative disease affecting the articular cartilage of joints and afflicts over 200 million people worldwide, with increasing prevalence.[1] It is a progressive disease characterized by joint pain, stiffness, and loss of mobility as the cartilage tissue is degraded. Existing OA treatments are largely palliative, do not treat the underlying causes of disease pathology, and cannot regenerate lost tissue. Gene therapies pose a promising strategy to treat the causal dysregulated catabolic processes, however, their delivery has proven challenging as nucleic acids are rapidly degraded enzymatically upon administration. In particular, delivery to resident cartilage cells for the treatment of OA—sequestered within the dense, negatively-charged, avascular extracellular matrix (ECM) of articular cartilage—is significantly limited. Lipid nanoparticles (LNPs) are the leading vehicle for nucleic acid-based therapies and can be directly administered to the joint space via injection. LNPs serve as an ideal candidate for targeting to chondrocytes as they are agnostic of the gene payload, protect the cargo from endogenous extracellular nuclease activity while facilitating delivery to the to the cell cytoplasm, elicit minimal immunogenicity, and exhibit robust stability in physiologic conditions. Investigations into the exploitation of LNPs' unique properties for the delivery of gene therapies to the joint space have thus far been very limited.[2] Modification of the exterior surface of LNPs can further increase their delivery efficiency by targeting the unique extracellular properties of the resident chondrocytes. Incorporation of PEG lipids covalently modified with customizable peptide sequences allows us to decorate LNPs with unique epitopes modulating their physical properties and cell interaction. We therefore **hypothesize** that modification of LNPs with poly-lysine peptides will facilitate their entry into the cartilage ECM and modulate the cell interaction with chondrocytes resulting in increased transfection efficiency.

METHODS: The harvesting of live bovine joint cartilage explants as well as the isolation of resident chondrocytes provide a valuable *in-vitro* model for studying both the infiltration ability and transfection efficiency of modified LNPs. Specifically, cartilage tissue explants were harvested from the lateral and medial condyles of immature bovine knee joints using a 4 mm biopsy punch. The explants were cultured at 37C in chondrogenic medium. Primary bovine chondrocytes were harvested from immature bovine carpometacarpal joints and isolated from the cartilage ECM using collagenase type VII treatment. LNPs were formulated by mixing an aqueous nucleic acid solution in a pH = 5 sodium acetate buffer with a solution of the respective lipids dissolved in ethanol via a T-junction mixer. The two solutions were combined at a volume ratio of 3:1 aqueous:ethanol and a mass ratio of 0.025 nucleic acids:total lipids. The ethanol lipid mix was comprised of d-lin-MC3-DMA, DSPC, cholesterol, and DMG-PEG at a 50:10:38.5:1.5 mol% ratio, respectively. Upon mixing, LNPs spontaneously precipitate and the ethanol is subsequently removed by dialysis in PBS overnight and sterile filtered. The LNPs are characterized for particle size using dynamic light scattering and for encapsulation efficiency using Quantifluor nucleic acid dye. To generate modified LNPs, peptides were synthesized with a terminal cysteine and reacted with maleimide-DBCO. The resultant peptide-DBCO was then mixed with the lipid DSPE-PEG$_{2k}$-azide, utilizing copper-free click chemistry to generate a peptide-lipid conjugate. Both reactions were performed at room temperature overnight in DMSO. Peptide-lipid conjugates were then mixed with native LNPs at 0.5 mol% excess for 2 hours and unincorporated peptide-lipid removed via dialysis (**Fig. 1A**). To evaluate LNP infiltration ability into cartilage ECM, cartilage explants were incubated with fluorescently labeled LNPs overnight at a concentration of 1 µg/mL of cargo nucleic acid in the culture media. The explants were then rinsed with PBS, sectioned in half from the superficial surface through the depth of the cartilage to the subchondral bone, and stained with either a live/dead stain kit or Hoechst. Cartilage sections were then analyzed via confocal microscopy to determine tissue viability and the extent of labeled LNP infiltration into the tissue. *In-vitro* transfection efficiency was determined on primary chondrocytes by treatment with modified or unmodified LNPs delivering mRNA encoding for the reporter protein miRFP670 and the relative fluorescence analyzed via flow cytometry. The chondrocytes were cultured in 24-well plate format and treated with an LNP dose of 400 ng of mRNA per well for 24 hours prior to analysis.



RESULTS SECTION: Flow cytometry analyses of LNP transfection in chondrocytes show the greatest transfection efficiency in chondrocytes using poly-lysine -modified LNPs, with a roughly fourfold increase compared to unmodified LNPs (**Fig. 1B**). Confocal imaging shows that both native and poly-lysine -modified LNPs penetrate throughout the cartilage ECM depth.

DISCUSSION: The positively charged poly-lysine peptide modification may allow for increased uptake and residence time within the cartilage ECM due to charge interactions in addition to also facilitating increased transfection in the resident chondrocytes. Interestingly, incorporation of chondrocyte-associating peptide (CAP) on to the LNPs did not increase transfection and contrasts the results originally observed in viral vectors—suggesting cellular uptake mechanisms of modifications may be vehicle-specific.

SIGNIFICANCE/CLINICAL RELEVANCE: All promising new gene therapies aimed at treating the underlying dysregulated systems causing OA must first be successfully delivered into the tissue's resident cells. This work not only demonstrates the potential of LNPs as a cartilage delivery vehicle for clinical OA gene therapies, but also reveals a targeting strategy that potentiates the efficacy of delivery in chondrocytes fourfold over native LNPs.

REFERENCES:

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Figure 1: a) A schematic illustrating the LNP modification strategy using click chemistry to modify the hydrophilic end of a DSPE-PEG with peptide targeting moieties and their subsequent post-addition into LNPs. **b)** Flow cytometry analysis of the percent miRFP-positive cells (left) and the median fluorescence intensity of those miRFP+ cells (right) following 24 hours treatment with unmodified and peptide-modified LNPs *in-vitro*.