

A Novel Porous Microcarrier for Extended Release of mRNA-Lipid Nanoparticles for Musculoskeletal Tissue Repair

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INTRODUCTION: Non-viral gene therapy is emerging as a safe and versatile strategy for repair and regeneration of musculoskeletal tissues such as articular cartilage and intervertebral disc [1], and lipid nanoparticles (LNPs) are a promising nanomaterial for delivering RNA-based therapeutics [2]. In recent work, we identified mRNA-LNP formulations capable of transfecting a range of skeletal cell types, including chondrocytes, with very high efficiency [3,4]. Challenges of local administration of mRNA-LNPs include transient expression limited to a few days and rapid clearance from the delivery site. In order to attain significant therapeutic benefits, multiple doses of mRNA-LNPs would likely be required, which is burdensome for patients and may cause inflammation at the site of injection. Therefore, the objective of this study was to develop a novel carrier for extended release of mRNA-LNPs to articular cartilage and intervertebral disc. We hypothesized that co-incubation of mRNA-LNPs with poly(lactic-co-glycolic acid) (PLGA) based porous microcarriers (PMCs) would enable loading of mRNA-LNPs within the pores of PMCs via electrostatic and hydrophobic interactions (Fig. 1A) and that the mRNA-LNPs would then exhibit extended release from PMCs via porous diffusion followed by degradation of PLGA over time (Fig. 1B).

METHODS: *Fabrication and Characterization of Microcarriers:* PLGA-based PMCs were fabricated using a modification of previously reported methods [5]. Pluronic F127 was used as a porogen. PMC size and porosity were determined using scanning electron microscopy (SEM). To visualize PMCs under confocal microscopy, Nile red (NR) was incorporated during fabrication. *Fabrication of mRNA-LNPs:* Green fluorescent protein (GFP) mRNA was encapsulated within LNPs, as previously reported [6]. LNP formulations containing 10% or 40% DOTAP (a permanently cationic lipid) producing LNPs that were overall anionic and cationic, respectively, were employed. Surface charge was measured using a Zetasizer. For visualization of mRNA-LNP loading under confocal microscopy, mRNA-LNPs were labeled with an infrared dye, DiR. *Loading and Release of mRNA-LNPs:* Freeze-dried PMCs were incubated with an mRNA-LNP suspension in PBS on a gentle shaker. After 24 h, the resulting suspension was filtered using a syringe filter and mRNA concentration was measured using the RiboGreen assay. Encapsulation efficiency of the PMCs was calculated as $(X-Y)/X \times 100$ where X and Y are the mRNA contents in the filtrate at the initial and final stage of incubation, respectively. Finally, release of mRNA-LNPs into PBS at time points up to 21 days was examined. All loading and release experiments were performed in triplicate. Statistical differences in mRNA-LNP loading and release over time were established using two-way ANOVA and one-way ANOVA with Tukey's test, respectively.

RESULTS: *Characterization of PMC Architecture:* SEM analysis of the unloaded PMCs (Fig. 2A) revealed formation of PMCs of average size of $30.35 \pm 4.54 \mu\text{m}$ and pore size of $1.26 \pm 0.76 \mu\text{m}$ (average of 10 images). Confocal imaging of PMCs loaded with fluorescent dye NR also revealed the porous architecture (Fig. 2B). In particular, the interconnectivity of the pores was clearly visible, maximizing permeation of LNPs even to the innermost section of the PMCs. *Loading and Release of mRNA-LNPs from PMCs:* Unloaded PMCs did not exhibit near infra-red fluorescence (Fig. 2C); however, when were incubated with DiR-labeled mRNA-LNPs, fluorescence was detected (Fig. 2D), confirming successful loading. The zeta potential values of PMCs, anionic LNPs and cationic LNPs are shown in the inset of Fig. 3A. As shown in Fig. 3A, almost 100% encapsulation efficiency was achieved within 24 h of incubation, with slightly faster loading observed for cationic LNPs ($n = 3$). Preliminary release kinetics demonstrated a linear release of anionic mRNA-LNPs beginning at day 3 and continuing until at least day 21 ($n = 3$, Fig. 3B).

DISCUSSION: In this study, we successfully developed a novel PLGA-based microcarrier for extended release of mRNA-LNPs in musculoskeletal tissues. Potential applications include targeting both catabolic and anabolic pathways to regenerate tissues such as cartilage and intervertebral disc. PLGA was chosen due to its excellent biocompatibility and biodegradability, which can be controlled by changing the proportion of lactic/glycolic acid [7], while porosity can be controlled by tuning the amount of porogen. As PMCs were slightly anionic in nature, conjugation with anionic LNPs was likely mediated by hydrophobic interactions, as both LNPs and PMCs were hydrophobic. Cationic LNPs adsorbed more quickly into PMC pores, likely due to additional electrostatic interactions, resulting in faster encapsulation. Ongoing studies will confirm bioactivity of released mRNA-LNPs on disc and cartilage cells *in vitro* and *in vivo*.

SIGNIFICANCE/CLINICAL RELEVANCE: The present study demonstrates a novel, robust, scalable and highly translatable technology for extended release of mRNA-LNPs for treatment of musculoskeletal disorders such as intervertebral disc degeneration and osteoarthritis.

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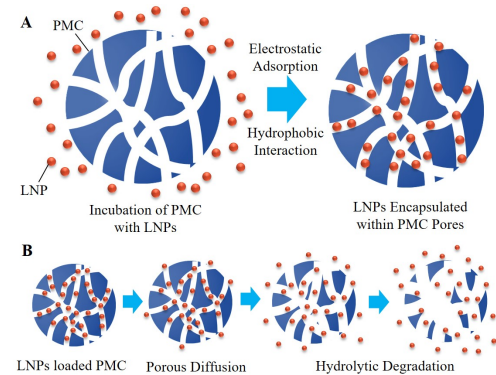


Fig. 1: (A) Schematic illustrating the principles and processes of loading LNPs within PMC pores. (B) Mechanism of extended release of LNPs from PMCs via porous diffusion and followed by hydrolytic degradation. The LNPs are representing bare LNPs, dye labeled LNPs and mRNA-LNPs.

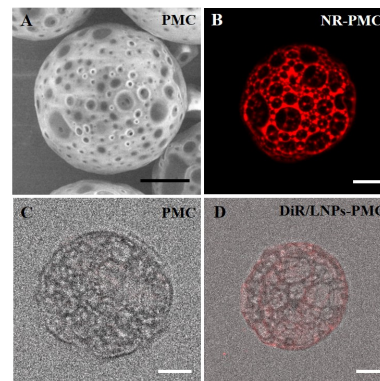


Fig. 2: (A) SEM image of the fabricated PMCs, depicting their morphology and porous nature. Confocal images of (B) NR loaded PMCs, (C) unloaded PMC and (D) PMC loaded with DiR dye labeled LNPs. Scale bar: 10 μm .

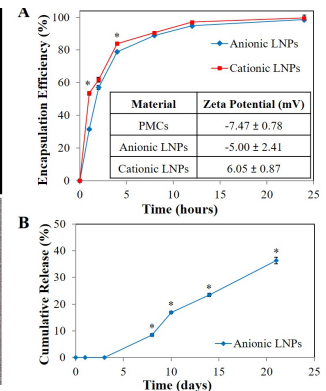


Fig. 3: Plots for (A) encapsulation efficiency of the PMCs (* $p < 0.05$ for anionic vs cationic) and (B) cumulative release of mRNA-LNPs from PMCs over time (* $p < 0.05$ for subsequent time points).