INTRODUCTION

With liposomal nanoparticles (LNPs) becoming a greater part of the biomedical landscape, including in the development of cutting-edge mRNA vaccines such as the COVID-19 vaccines, the increasing availability and breadth of component lipids is essential[13]. Polysciences' expanding range of lipid reagents, including DOTAP chloride (DOTAP-Cl), DOPC, and DSPC, provide key components for the creation of neutral, anionic, and cationic nanoparticles. These diverse and cost-effective tools allow scientists to engineer precise means of targeted drug delivery and unlock mechanisms behind cellular uptake[4].

MATERIALS AND METHODS

REAGENTS

Lipids including DOTAP-Cl, DOPC, DSPC, and Cholesterol were synthesized and provided by Polysciences. Additional lipids DSPG and POPC were purchased from Echelon Biosciences and DOPG was purchased from Sigma Aldrich. The mini extruder with heating block, extrusion membranes, and syringes were purchased from Avanti Polar Lipids. Cascade blue dextran and fluorescein dextran were purchased through Thermo Fisher Scientific.

LIPOSOME PREPARATION

Liposomes were prepared as previously described.[2 5 6] Lipids were thawed to room temperature for approximately 10 mins. Mixtures of DSPC, DSPG, and cholesterol (molar ratio 53:21:26), DOTAP-Cl and DOPC (51.1:48.5) and POPC and cholesterol(55:45) were prepared. Single-lipid preparations of DOPC, DOPG, DSPC, and DSPG were also created. All lipid preparations were dissolved in chloroform in a glass tube, to which dissolved clodronic acid (5 mg/ml) was added. The chloroform was then evaporated under dry N2 (Middlesex Gases, Everett, MA) and further desiccated under vacuum for three hours, forming a lipid film. Following desiccation, the lipid film was resuspended in 5% D-glucose solution and vortexed until no lipid precipitate remained. If chunks remained after vigorous vortexing, the solution was sonicated in the capped glass vial for 5 mins (42 kHz, 100 W) and left overnight for liposomal swelling. If precipitates reformed overnight, the solution was sonicated again prior to extrusion.

EXTRUSION AND PURIFICATION

The liposomes were filtered through 0.45 µM filters, 0.22 µm filters, and then extruded via a mini-extruder with heating block (Avanti Polar Lipids, Alabaster, US) 11 times through a 100 nm PC membrane and transferred to a microcentrifuge tube. If the liposomes contained dye, they were centrifuged as previously described[7]. The pellet was resuspended in Milli-Q water and spun twice more. Following the final wash, the pellet was resuspended in Milli-Q water (0.5 ml) for a final lipid concentration of 1 mM. A few microliters of the liposome solution were taken for injection into the zebrafish while the remaining solution was retained for analysis.

ZEBRAFISH LINES AND EMBRYOS

Zebrafish (*Dania rerio*) were maintained in accordance with the Boston University Medical Center Animal Care facility. The zebrafish line used was AB. Embryos were collected, incubated at 28°C, and kept in embryo medium until 48 hpf.

ZEBRAFISH INTRAVENOUS INJECTIONS

Clodronic acid-loaded liposomal formulations were co-injected into zebrafish (48-54 hpf) alongside Cascade blue dextran and fluorescein dextran at a 1:1:1 volume ratio. An injection solution of free clodornic acid Cascade blue dextran and fluorescein dextran in a 1:1:1 volume ratio was used in control embryos. Embryos were anesthetized in tricaine and placed on agarose prior to injection. Into each embryo, 3 nl of this solution was injected into the duct of Cuvier. Successful injections were identified by observation of cell dispersion at the injection site and an undamaged yolk sac. All previously described LNP formulations were injected into 5-10 embryos each that met the criteria for successful injection.

ZEBRAFISH IMAGING AND FLUORESCENCE QUANTIFICATION

Microscopy was performed using a Yokogawa CSU-W1 spinning disk mounted on an inverted Nikon Eclipse Ti microscope equipped with a PCO Edge CMOS camera. Images were acquired via NIS-Elements (Nikon), processed with Imaris (Bitplane), and quantified with Fiji. Quantifications of fluorescence intensity in the caudal hematopoietic niche (CHT) were acquired by subtracting the background fluorescence intensity from the CHT fluorescence intensity. Additionally, the particle size of uptake fluorescence was measured.

STATISTICAL ANALYSES

Statistical analysis was performed in RStudio. Analysis of variance (ANOVA) coupled with post hoc Tukey multiple comparisons of means were used to assess statistical significance between treatment groups and the control.

Experiments were designed by Justine Wagaman in the laboratory of Dr. Elliott Hagedorn at the Section of Hematology and Medical Oncology, Boston University Medical Center.

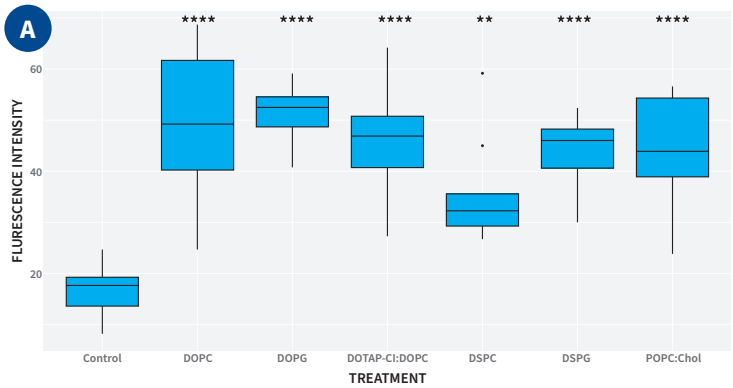
RESULTS

LNPs were synthesized using Polysciences lipids (along with other lipids) and loaded with clodronic acid. The synthesis of the nanoparticles was confirmed via dynamic light scattering (DLS) (Malvern Panalytical, Westborough, USA) analysis (Table 1).

Table 1: Size of LNPs loaded with clodronic acid (5 mg/ml)

LIPID NANOPARTICLE TYPE	SIZE
100% DOPC	100.39 ± 5.04
100% DOPG	108.05 ± 3.45
DOTAP-Cl:DOPC	132.55 ± 36.60
100% DSPC	172.97 ± 14.59
100% DSPG	160.70 ± 23.39
POPC:Chol	126.78 ± 5.61

Zebrafish embryos were injected with LNPs of various lipid compositions loaded with clodronic acid. Previous studies have reported that certain types of endothelial stress result in increases in uptake[8]. The uptake of these nanoparticles led to differences in uptake of co-injected fluorescein dextran, indicating the efficacy of the LNPs in delivering their clodronic acid payload. Although clodronic acid has not specifically been used before in this context, the endothelial stress from clodronic acid may explain the differences in dye uptake. All clodronic acid delivered via nanoparticle encapsulation showed an extremely significant increase in fluorescein expression (Figure 1), indicating that LNPs are a much more effective method of delivering drug payloads than injection of unencapsulated drugs.



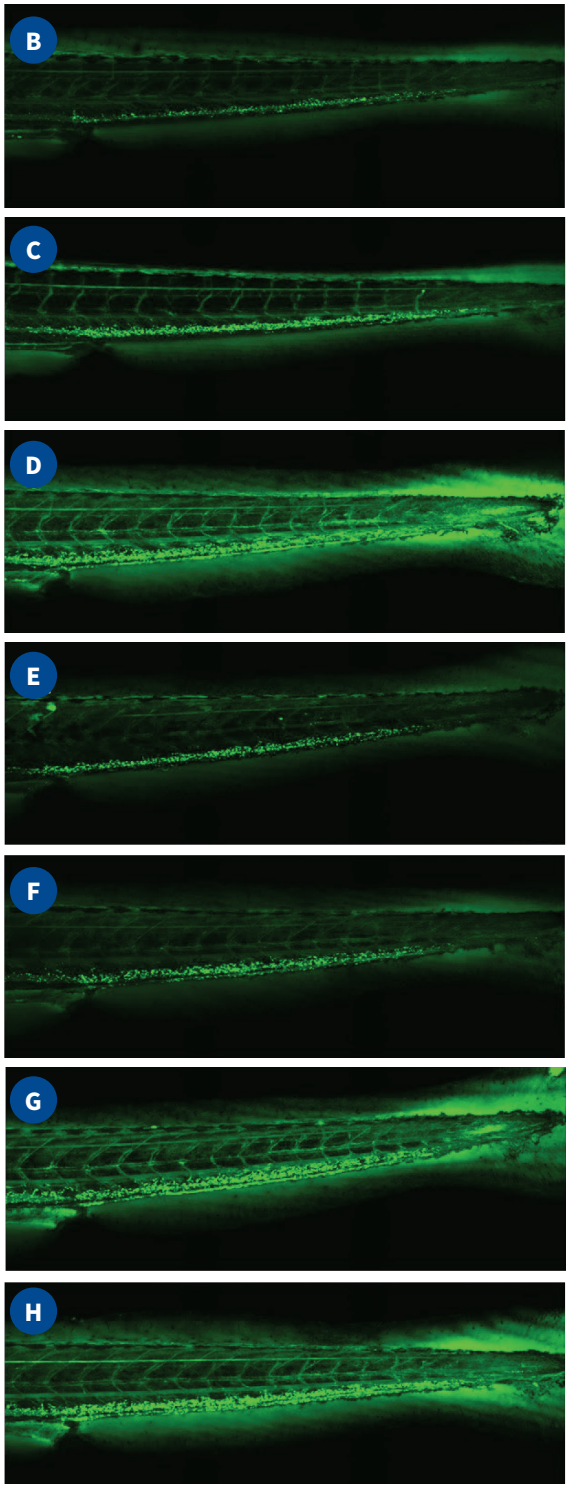


Figure 1: Fluorescence of fluorescein dextran co-injected with clodronic acid loaded LNPs in zebrafish 48hpi. A) Quantification of fluorescence. Graph represents average fluorescence + or - SD. Significance levels are compared to the control (**p ≤ 0.0, ****p ≤ 0.0001). B-H) Representative images of free clodronic acid and dyes, n=10 (B); DOPC, n=10 (C); DOPG, p=0.00, n=10 (D); DOTAP-Cl:DOPC, n=10 (E); DSPC, n=10 (F); DSPG, n=10 (G); POPC:Cholesterol, n=10(H) uptake in zebrafish. All images were normalized for background fluorescence.

CONCLUSION

The lipids DOTAP-CI, DOPC, and DSPC, among others, successfully facilitated increased uptake of drug payloads, placing them among the ever-growing list of lipids that increase efficacy and specificity of drug delivery via LNPs. Using these lipids in an in vivo setting reaffirms their potential in medical applications and the customizability of LNPs as a swiftly advancing platform for diverse payload delivery.

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