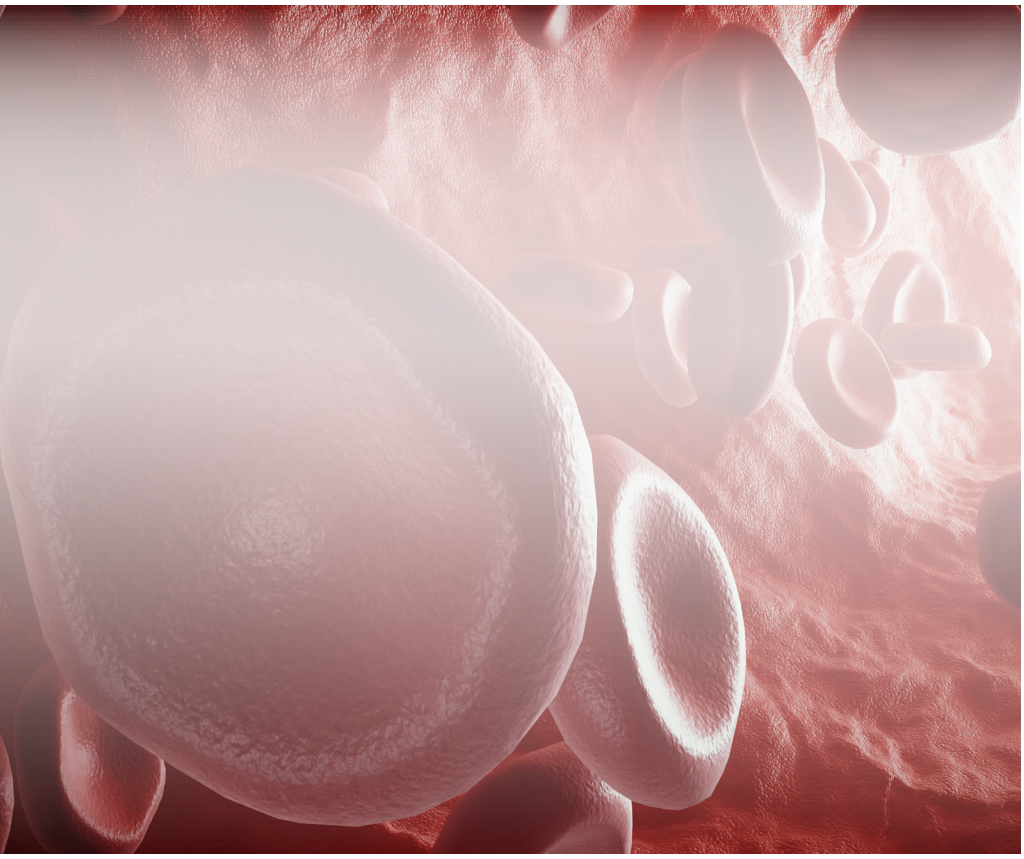

Challenge

Tracking targeted drug delivery systems

Solution

Ex vivo fluorescent imaging

Liposomal Nanocarriers Designed for Sub-Endothelial Matrix Targeting under Vascular Flow Conditions

Introduction

Peripheral vascular disease (PVD) is the most common disease affecting the arteries. Endovascular therapy is the primary route taken to treat PVD in a minimally invasive fashion, but mechanical injury to the diseased vessel is unavoidable. This damage results in a multitude of cellular disruptions that oftentimes contributes to the development of secondary vascular pathologies, such as intimal hyperplasia-induced restenosis. Although there are approved therapeutic interventions available that are aimed at the inhibition of this dysfunctional remodeling, there are currently no benchmark therapeutic options with proven long-term success.

Nanoparticles designed to bind to these exposed matrices could provide targeted drug delivery systems. Liposomal nanoparticles are promising drug delivery vehicles for potential translation. Naturally occurring phospholipids can be arranged in bilayers that form spherical nanoparticles mimicking the cell membrane, and surface-linked polyethylene glycol (PEG) can be incorporated to increase membrane stability. PEGylated liposomes (PLPs) are particularly promising in the field of targeted drug development. The goal is to further develop these PLPs via surface modifications designed to preferentially target collagen type IV, a primary component of the vascular basement membrane. Here, investigators present the development of PLPs conjugated with short peptide fragments previously shown to bind collagen IV, and describe the optimization of assembly and modification parameters for functional collagen-targeting PLP nanoparticles (CT-PLPs). The UVP iBox Studio is sophistication and ease-of-use in one system for high resolution fluorescence imaging. From

in depth in vivo studies to quick screening, researchers can use this compact yet powerful imaging system to perform their choice of in/ex vivo fluorescence applications. Camera and light source options provide maximum flexibility in system configuration. The UVP iBox Studio imaging system was used in this study, ex vivo, to determine where these CT-PLPs bind within the dysfunctional vascular matrices.

Materials and Methods

Samples and reagents

- N-(Cyanine 7)-1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (Cy7-DOPE)
- 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[dibenzocyclooctyl(polyethylene glycol)-2000] (DSPE-PEG-DBCO)
- KLWVLPKK(N3)-NH2 (collagen-targeting peptide (CTP))
- 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC)
- Ovine cholesterol (Chol)
- 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] (DSPE-PEG)
- N-(lissamine rhodamine B sulfonyl)-1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine (Rho-DPPE)
- Ethanol alcohol (EtOH)
- Chloroform
- Tris-HCl
- Phosphate-buffered saline (PBS)
- 100nm polycarbonate extruder

Samples preparation

Collagen-targeting peptide (CTP) modified lipid synthesis: DSPE-PEG-DBCO, a form of lipid derivative with a modification that can be used in azide-alkyne cycloaddition reactions, was used to form collagen-targeting peptide modified lipids (CTLs) via click chemistry reaction. Briefly, at equal moles, CTP and DSPE-PEG-DBCO were combined at room temperature under constant agitation for 2 h.

PEGylated liposome (PLP) assembly: Base PLPs were formed with bulk lipid DOPC and Chol at a mole ratio of 7:3 plus 10 mol% DSPE-PEG and 0.5 mol% Cy7-DOPE for fluorescent labeling. PLP nanoparticles were assembled using an EtOH injection technique previously described in literature. Briefly, lipids were dissolved in chloroform, combined as indicated, and dried under N2 gas and vacuum to remove the remaining solvent. Dried lipid films were then resuspended in 100% molecular grade EtOH and injected dropwise into 10 mM Tris-HCl at pH 8.0 and a 2:3 EtOH:aqueous volume ratio, under constant vortexing at room temperature. Liposomes were purified from EtOH via 24 h dialysis against PBS at 4 °C and extruded using a 100 nm polycarbonate extruder.

Collagen-targeting PLP nanoparticles (CT-PLP) assembly: CT-PLPs were formed by inserting 0.5–15 mol% CTL into base PLPs, via lipid hydration. CTLs were combined with base PLP lipid constituents at the time of lipid drying under N2 gas. Lipid hydration with EtOH was performed in one step, and all lipid constituents were incorporated at the time of the initial liposome assembly. Liposomes were purified from EtOH via 24 h dialysis against PBS at 4 °C and extruded as described.

Human vessel explant binding under ex vivo perfusion: Saphenous veins were procured from lower extremities amputated in an operating room at the University of Tennessee Medical Center as a standard of care and transported to the research laboratory to maintain tissue viability via bioreactor culture. Independent vessel segments were perfused for 30 min with 50 µM total lipid using a closed perfusion bioreactor system.

Imaging

Following perfusion, vessel explants were fluorescently imaged via a UVP iBox Studio In Vivo Imaging System with near-infrared (NIR) filters for the fluorometric detection of Cy7-DOPE. Binding was quantified as the mean intensity of bound Cy7-labeled lipid per vessel area, normalized to the background of non-perfused vessels using VisionWorks® software.

Results and Discussion

In order to demonstrate the feasibility of this nanocarrier formulation for clinical translation, CT-PLP binding was assayed in human vascular tissue explants under ex vivo vascular perfusion conditions. CT-PLP demonstrated significant binding to human saphenous vein explants compared to PLP controls (Figure 1), indicating significant potential for clinical development towards a translational vascular therapeutic.

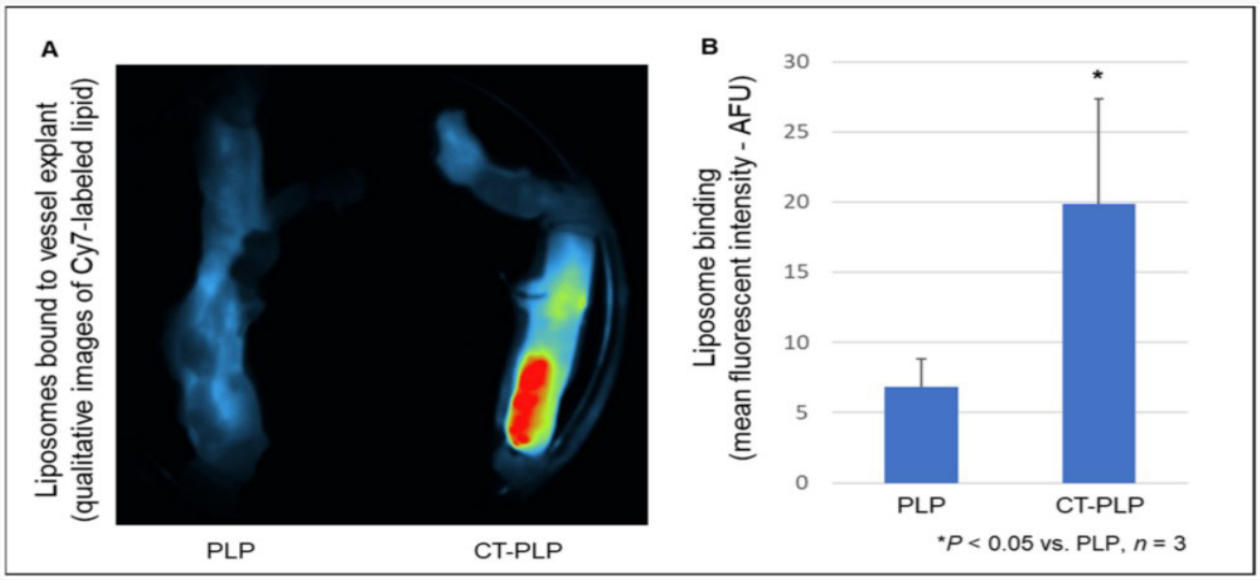

Figure 1 Figure 1 CT-PLP binding was significantly increased over PLP controls in human lower extremity vessel explants under ex vivo vascular perfusion. (A) Representative near-infrared (NIR) images showing Cy7-labeled liposomes bound to saphenous vein segments after 30 min continuous ex vivo perfusion. (B) After 30 min of a continuous flow, CT-PLP binding was significantly increased over PLP controls. Binding was detected using in vivo imaging system software to quantify fluorescent intensity per vessel area, and data were presented as the mean fluorescent intensity normalized to the fluorescent intensity of the background of non-perfused control vessels.

Conclusion

An efficient and safe gene delivery system was created by incorporating a PAMAM based dendriplexes system with an optimized liposomal formulation of DPPC:CH (85:15). The results revealed that the incorporation of liposome with naked dendriplexes has essentially increased the cellular uptake of the complexes, which was confirmed by ex vivo fluorescence imaging of the dissected organs. From the findings, it could be concluded that the development of such a non-viral nanocarrier system could be considered for an efficient gene transfection with a better safety profile, in both in vitro and in vivo delivery systems.

References

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