JNK-2 Gene Silencing Lipid Nanoparticles for Elastic Matrix Regenerative Repair in Abdominal Aortic Aneurysms (AAAs)

Shataakshi Dahal, PhD and Anand Ramamurthi, PhD

Department of Bioengineering, Lehigh University, Bethlehem, PA

Background

- AAAs : dilation of abdominal aorta due to loss of elasticity
- Primarily caused by upregulation of MMPs which breaks down an extracellular matrix protein called elastin
- MAPK signaling pathways are critical regulator of MMPs
- Major MAPKs are JNK, ERK 1/2 , ERK 5 and P38
- JNK is primarily involved in AAAs
- Silencing of JNK has shown to upregulate elastin and LOX expression and downregulate MMPs expression leading to AAA stabilization

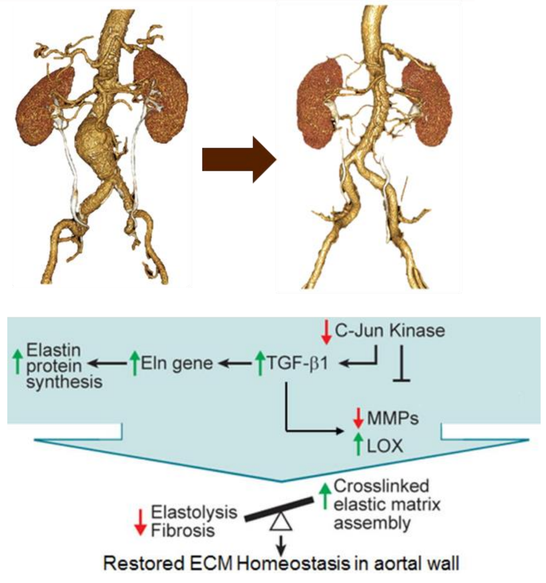


Figure 1. JNK regulation of MMPs via TGF-β signaling pathway.

LNPs : Mechanism and Methods

- LNPs advantages : excellent biocompatibility and biodegradability; low toxicity and immunity; structural flexibility; ease of large scale preparation
- Composition: cationic lipid DOTAP; cholesterol domain; fusogenic lipid DOPE ; DSPE-PEG2000 for escaping RES

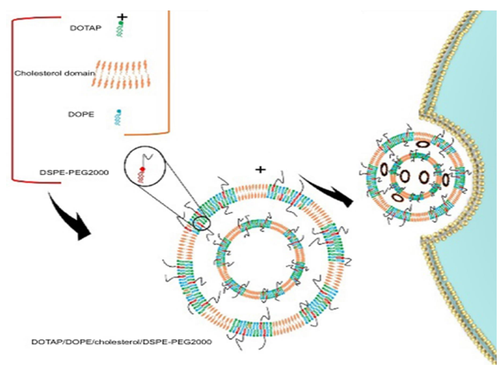


Figure 4. Lipid composition and mechanism of internalization[3]

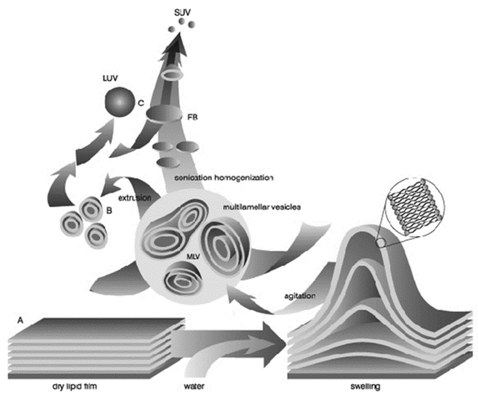


Figure 5. Hydration of lipid film method[4]

- Anionic siRNA interacts with cationic DOTAP; DOPE helps for cellular internalization by fusing with cell membrane
- Lipid film hydration method for preparation of LNPs ; DOTAP : DOPE : Cholesterol = 0.5 : 0.5 : 0.5 molar ratio to a final concentration of 5.6 mM ; PEG from 0 to 40 mol% of DOTAP
- siRNA used at N/P ratio 2.5; Encapsulation assessed my gel retardation assay

References:

1. Sevilla et.al, *Molecules* **2019**, *24*(14), 2570
2. https://en.wikipedia.org/wiki/Liposome
3. Hosseini ES et.al, *Int J Nanomedicine* **2019**;*14*:4353-4366
4. https://www.sigmaaldrich.com/US/en/technical-documents/protocol/cell-culture-and-cell-culture-analysis/transfection-and-gene-editing/liposome-preparation

Proposed Approach

- Common approaches : pharmacologic inhibition and gene silencing
- Pharmacologic inhibition : e.g. DOX; inhibition at protein level; is temporary and can have severe side effects
- Gene silencing using siRNA : silences target mRNA and protein expression ; applicable for targeting undruggable protein

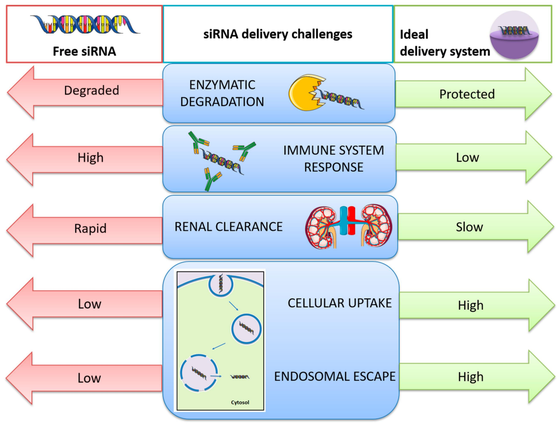


Figure 2. Free siRNA delivery vs delivery using carrier[1]

- We propose to use lipid nanoparticles (LNPs) as carrier for targeted delivery of JNK silencing siRNA

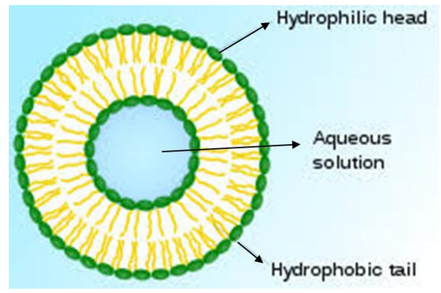
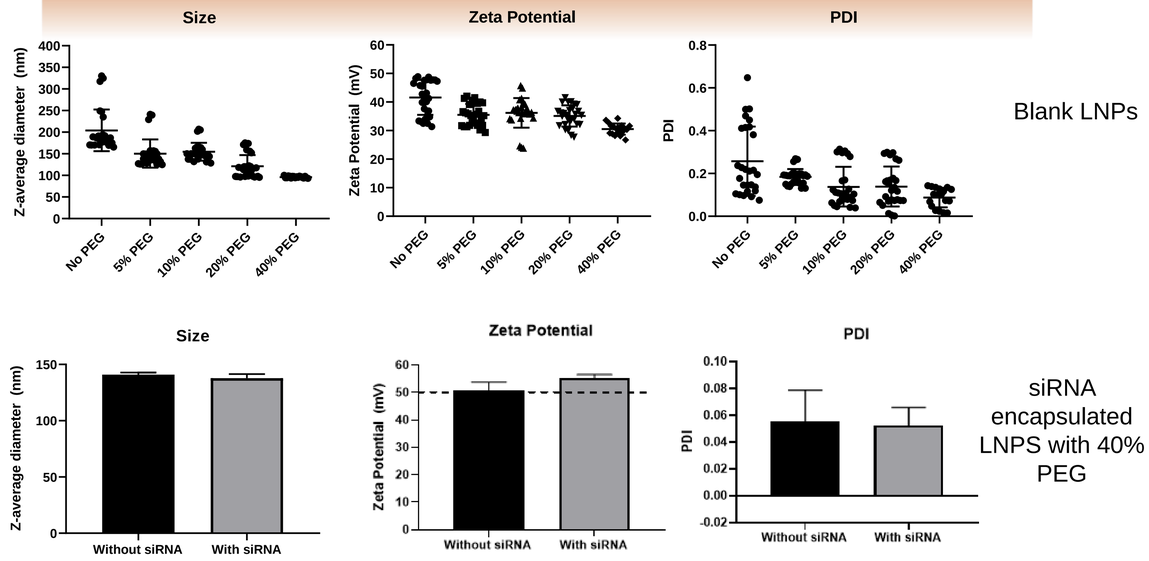
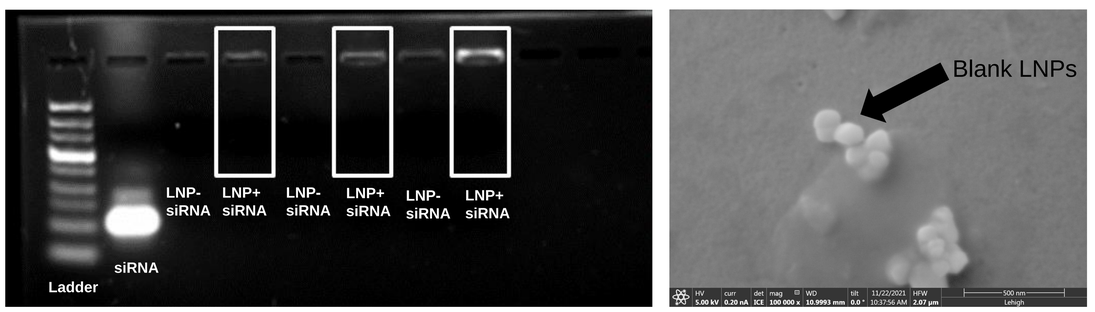


Figure 3. Lipid nanoparticle structure[2]

Key outcomes and Conclusion





Overall, the preliminary data shows that size, charge and PDI of LNPs decreases with increase in PEG percentage. siRNA encapsulation was verified using gel retardation assay however, the size and charge of LNPs seem to increase with siRNA encapsulation which requires further validation. LNPs can thus be a potential siRNA carrier for targeted delivery to aneurysm. Future work will surface modify the LNPs for targeted delivery.