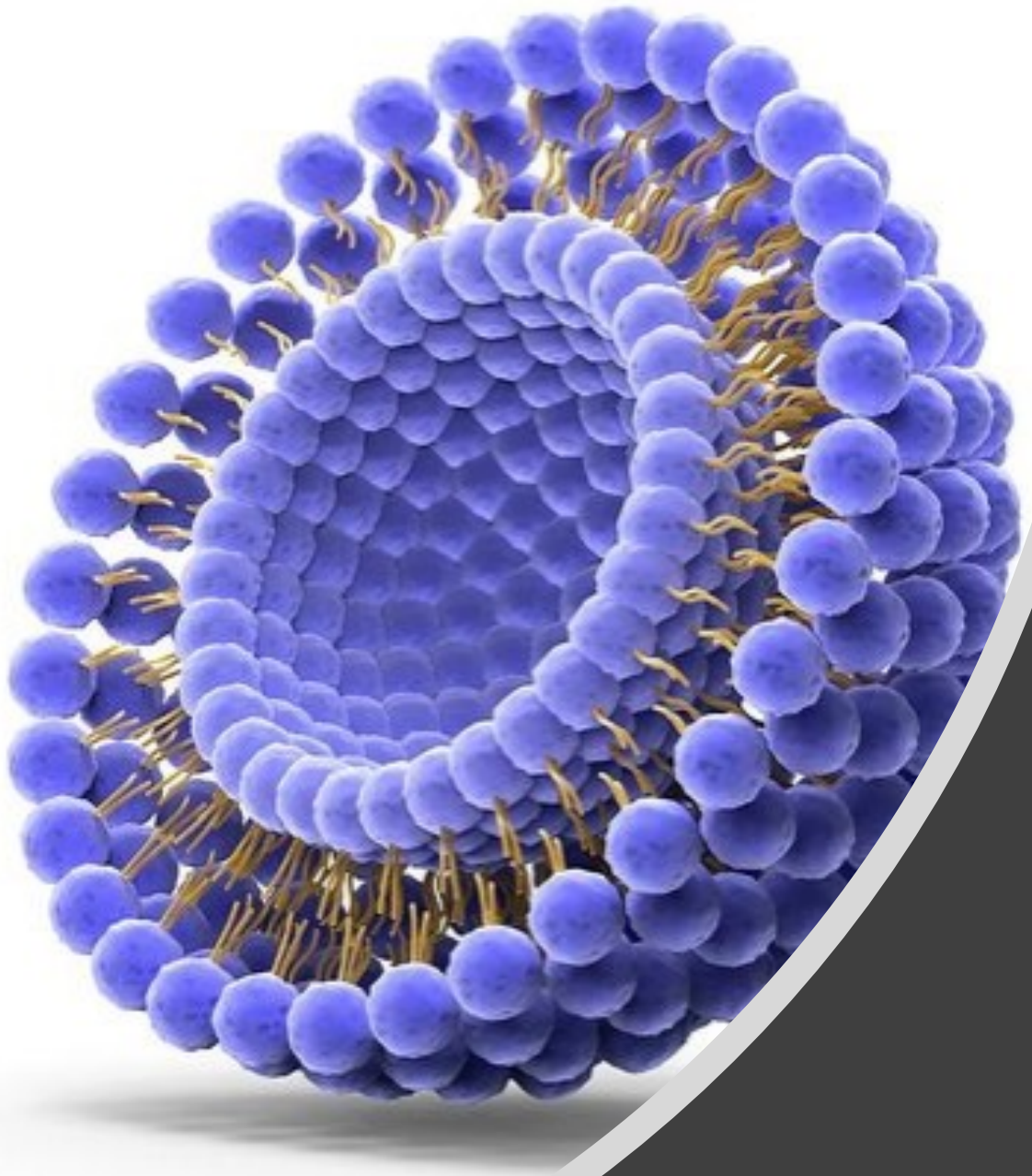

https://fineartamerica.com/shop/posters/nanomedicine

Optimization of Liposomal Encapsulation Efficiency

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https://universitymedicalcentergunden.blogspot.com/2015/06/university-medical-center-knoxville-tn.html

Abstract

Introduction:

My project was a continuation of the Vascular Research Lab's (VRL) ongoing research at the University of Tennessee Medical Center Knoxville (UTMCK) aimed at optimizing liposomal encapsulation efficiency of small interfering RNA (siRNA) which can be used to silence genes to prevent a variety of disease pathologies.

Methods:

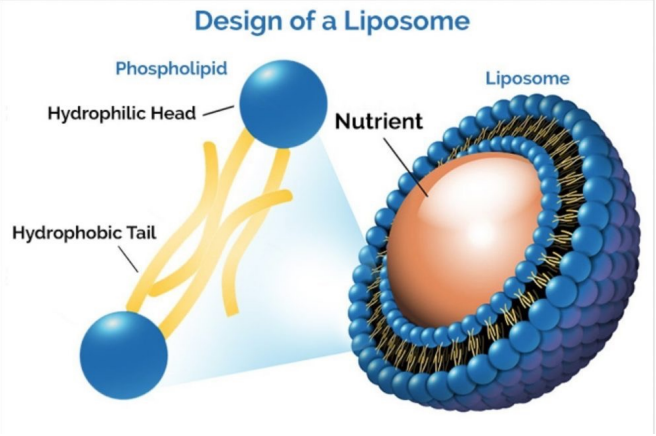
- Assay siRNA loading capacity of liposomes based on lipid concentration
- Development of a method for liposome purification
  - HPLC
  - HiTRAP Column

Results & Conclusion:

- siRNA loading capacity
  - Higher lipid:siRNA resulted in increased encapsulation efficiency
- HPLC – did not work as expected
- HiTRAP Column – currently being optimized to be used as part of standard operating procedures

Introduction

- Small interfering RNA (siRNA)
  - Used to silence genes to prevent disease pathologies
    - i.e. Intimal hyperplasia development & atherosclerotic plaque formation
- Liposomal delivery of siRNA
  - Emerging field
  - many approaches: utilize cationic synthetic lipids to promote siRNA encapsulation within the liposomal nanoparticles
    - Immunogenicity & cytotoxicity hinders translational success
- VRL is working to develop an assembly technique to optimize siRNA encapsulation into liposomal nanoparticle
  - Comprised of neutral, naturally occurring phospholipids
- **Goal**: to create a biocompatible nanoparticle with downstream translational potential



https://www.yourwellness4life.com/liposomal-products-changing-drug-delivery-cellular-level/

siRNA Loading Capacity of Liposomes

- Analysis was based on lipid concentration
- Varying lipid-to-siRNA weight-to-weight ratios were used at assembly from 2.5:1 to 100:1
- The resulting siRNA encapsulation was quantified by RiboGreen
  - A fluorescent assay
  - nucleotide residues are fluorescently labeled for detection
- By denaturing formed liposomes and labeling entrapped siRNA, this would give a linear output so that the unknown sample's fluorescence could be easily related to a concentration along a standard curve.

Liposomal Purification

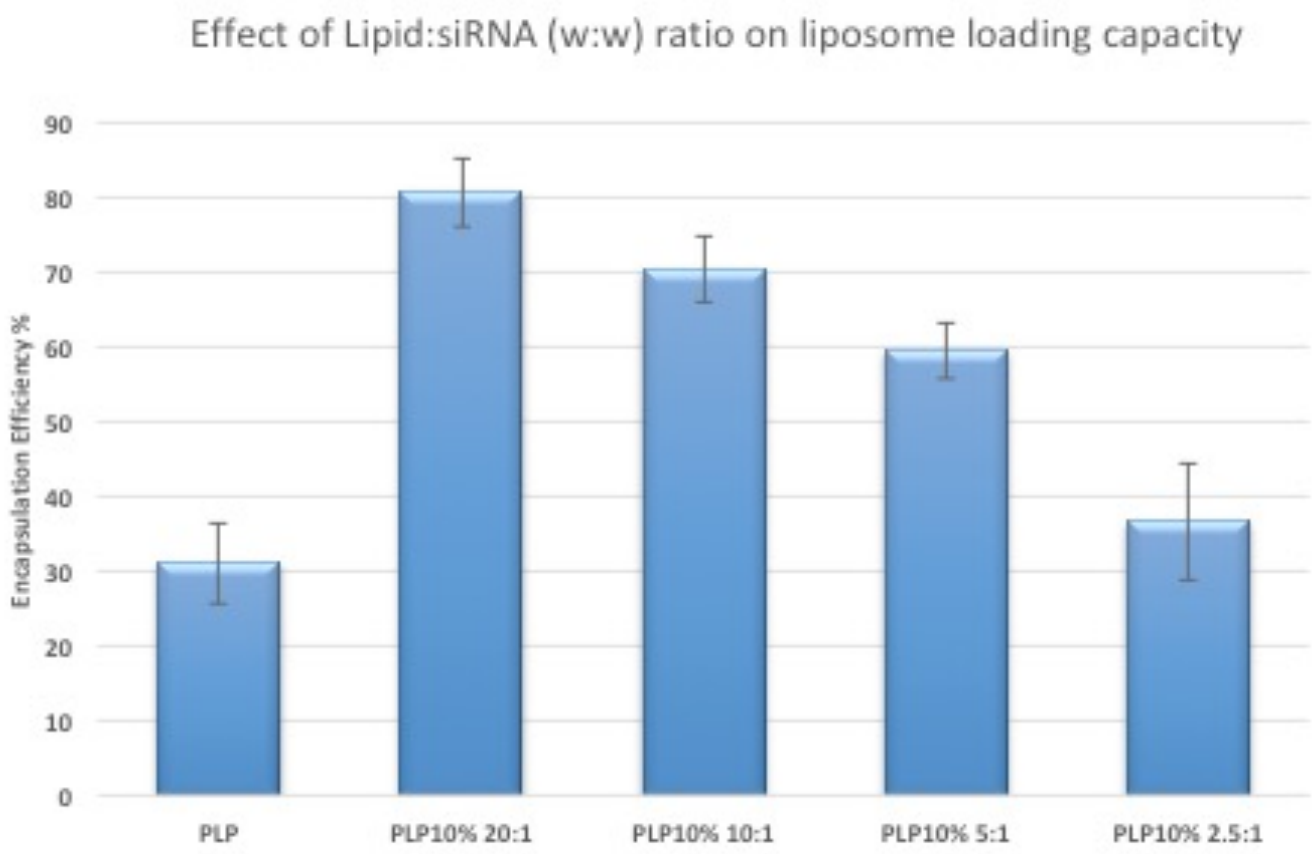
https://en.wikipedia.org/wiki/High-performance_liquid_chromatography

- **High performance liquid chromatography (HPLC)**
  - size exclusion chromatography column (SEC) was used to separate formed liposomes from free siRNA based on their differential size.
    - Separation should take place over 30-45 min
  - By collecting the HPLC liposome fraction before siRNA elution, the liposomes could be denatured and assayed by RiboGreen as before.
- **HiTRAP Column**
  - packed with Phenyl Sepharose High Performance material
    - strong cation exchange resin
    - can selectively retain anionic nucleotide residues of the siRNA
  - liposomes could be manually pushed through the column
  - the siRNA was retained within the resin
  - The siRNA must then be manually eluted in a second step.
  - The two fractions were assayed by RiboGreen to quantify the encapsulated and free siRNA

https://www.gelifesciences.com/en/us/shop/chromatography/resins/affinity-tagged-protein/histrap-hp-histidine-tagged-protein-purification-columns-p-00250

siRNA Loading Capacity of Liposomes Results

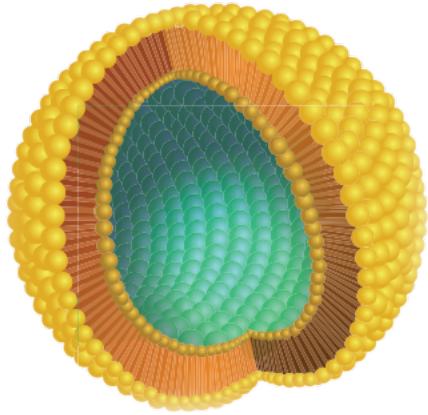
- loading capacity of siRNA is directly related to lipid content
  - not siRNA availability
- Higher lipid:siRNA resulted in increased encapsulation efficiency.



Liposomal Purification Results

- **HPLC**
  - did not work as planned
  - liposomal nanoparticles formed are <65nm in diameter
  - The SEC column resolution was not specific enough to accurately separate free siRNA from the nanoparticles
  - HPLC peaks were overlapped and the collection fractions could not be accurately resolved.
- **HiTRAP Column**
  - not an automated one-step system such as HPLC
  - manual process
  - Rapid purification method
  - can be performed in <60 minutes with accurate resolution
  - has the potential for scalability
  - is being further optimized in the VRL for implementation in their standard operating procedures.

Conclusions



https://thisisorenda.com/orenda-ultimate-pack/o-tropin/attachment/liposome-illustration/

My research project was a continuation of the VRLs ongoing research aimed at optimizing liposomal encapsulation efficiency of small interfering RNA (siRNA). siRNA can be used to silence genes to prevent a variety of disease pathologies, including intimal hyperplasia development and atherosclerotic plaque formation, the pathologies of primary interest in the VRL. Liposomal delivery of siRNA is an emerging field in biomedical engineering and research. The standardization of liposomal encapsulation could help other laboratories utilize liposomal siRNA delivery in their own areas of research and promote more research in the field of liposomal nanoparticle delivery.

Summary

Various methods were used to optimize liposomal encapsulation efficiency:

1. Assay siRNA loading capacity of liposomes based on lipid concentration
   - Higher lipid:siRNA resulted in increased encapsulation efficiency
2. Purification of liposomes
   a) HPLC
      - Did not work as expected
      - SEC column was not specific enough to separate free siRNA from nanoparticles
   b) HiTRAP Column
      - Successful
      - Now under further testing to optimize it to be used as part of standard operating procedures going forward

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