Characterization of Diclofenac-Loaded Lipid Nanoparticles for Neutrophil Modulation

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Introduction

- Traumatic brain injury (TBI) results in acute and chronic neuroinflammation that can lead to long term disability and even death [1].
- Increased blood-brain barrier permeability following TBI leads to greater neutrophil infiltration to the injured site and an opportunity to deliver therapeutic payloads to the injured brain [2,3].
- L-selectin is an adhesion molecule on the surface of neutrophils which enables migration to sites of inflammation [4].
- Lipid Nanoparticles (LNPs) are promising for drug delivery and can be used to encapsulate diclofenac, an anti-inflammatory drug, which enhances L-selectin shedding in neutrophils [4].
- Targeting peptides can be conjugated to LNPs to enhance drug delivery to the injured brain.

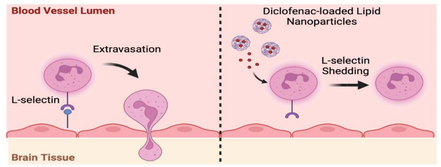


Figure 1. Diclofenac-loaded LNPs inducing the shedding of L-selectin on neutrophils.

Objectives

- Diclofenac-loaded LNPs, in comparison to free diclofenac, will have a comparable effect on enhancing the shedding of L-selectin for neutrophils *in vitro*.
- Targeting peptides can be successfully conjugated onto LNPs through copper-catalyzed azide-alkyne click chemistry (CuAAC).

Nanoparticle Formulation

LNPs were fabricated via nanoprecipitation utilizing a low-cost, open-source syringe pump. Fabrication variables such as: Aqueous:organic (A:O) ratio, injection rate (IR), and stirring rate (SR) were manipulated to obtain LNPs with desired physical characteristics.

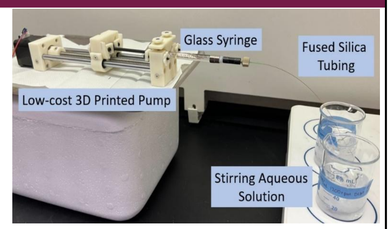


Figure 2. Nanoprecipitation syringe pump set up.

Nanoparticle Characterization

LNPs fabricated with an A:O ratio of 5:1, IR 3 ml/min, 30% drug load, and a SR of 400 rpms yielded the smallest LNPs (55.58 nm) and were used in subsequent drug encapsulation experiments.

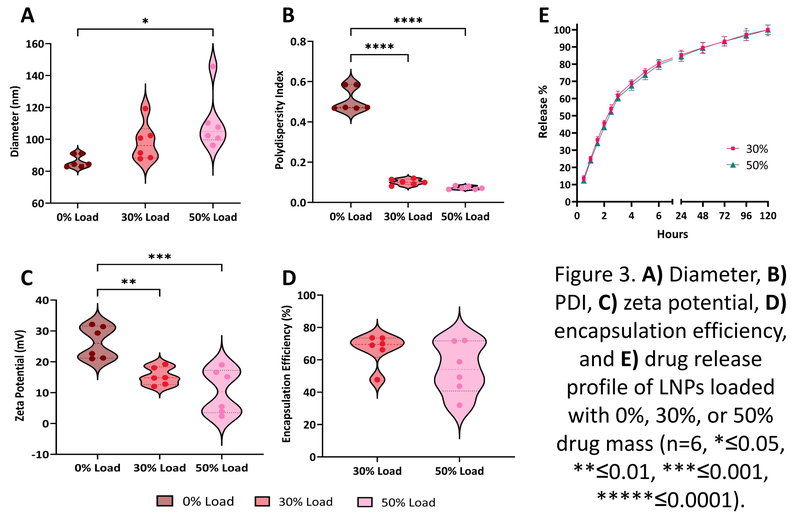
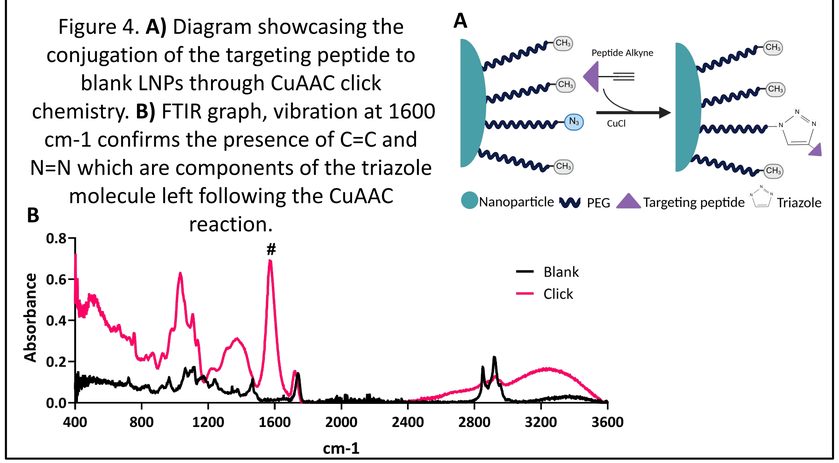


Figure 3. **A)** Diameter, **B)** PDI, **C)** zeta potential, **D)** encapsulation efficiency, and **E)** drug release profile of LNPs loaded with 0%, 30%, or 50% drug mass (n=6, *≤0.05, **≤0.01, ***≤0.001, *****≤0.0001).

Enhanced Targeting via Peptide Bioconjugation

Figure 4. **A)** Diagram showcasing the conjugation of the targeting peptide to blank LNPs through CuAAC click chemistry. **B)** FTIR graph, vibration at 1600 cm-1 confirms the presence of C=C and N=N which are components of the triazole molecule left following the CuAAC reaction.



Diclofenac-loaded LNPs Induce Increased Shedding of L-Selectin

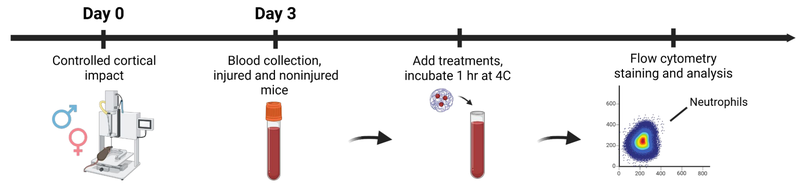


Figure 5. Flow cytometry experimental timeline.

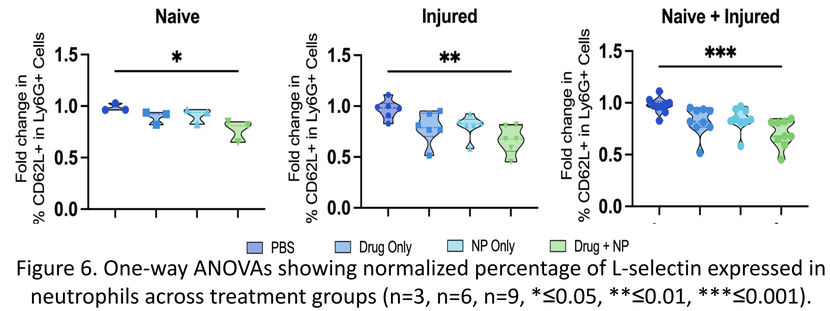


Figure 6. One-way ANOVAs showing normalized percentage of L-selectin expressed in neutrophils across treatment groups (n=3, n=6, n=9, *≤0.05, **≤0.01, ***≤0.001).

Conclusions

- LNP formula was optimized to produce sub-100 nm LNPs and successfully encapsulate diclofenac.
- Treatment with diclofenac-loaded LNPs significantly decreases L-selectin expression in neutrophils compared to treatment with PBS.
- Results will be be used to inform future in vivo experiments focused on decreasing the inflammatory response in the brain after injury.
- Future studies will explore the efficacy of the targeted drug-loaded LNPs in vivo.

Acknowledgements

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References

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