Control Release Anesthetics to Enable an Integrated Anesthetic-MSC Therapeutic

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Background

Local Anesthetics (LA):

- Commonly employed procedure to minimize pain and discomfort
- Act directly on voltage gated sodium channels and reversibly block the conductance in neurons [1].
- Common local anesthetics include bupivacaine, lidocaine, and ropivacaine.

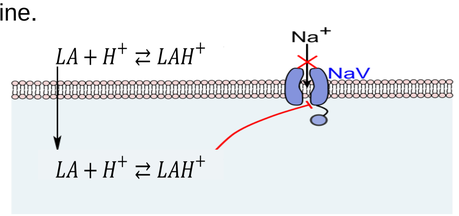


Figure 1: Mechanism of Action for Local Anesthetics. Ionized LA blocks sodium from entering the cell. This inhibits action potentials from being propagated, which halts signal conductance. Figure modified from [2]

Mesenchymal Stromal Cells (MSCs):

- MSCs are an attractive option for tissue engineering and regenerative medicine applications because:
  - Multi-lineage differentiation potential
  - Immunomodulatory functions
  - Generally non-immunogenic [3]

Effect of Local Anesthetics on MSCs:

- LAs affect the MSC:
  - Proliferation capacity
  - Differentiation potential
  - Adherence phenotype
  - Secretome
  - Immunomodulatory function
  - Viability
- In a potency and time dependent manner [4,5]

A cell therapy must be developed that can avoid compromising the integrity and potency of an MSC therapy and still deliver the necessary level of comfort to the patient.

Bupivacaine-loaded Liposomes

- A bilayer of lipids surrounding bupivacaine
- Bupivacaine slowly leaks through the bilayer
  - Slower rate than bolus dose [6]

Hydrogel-Liposome Construct

- Liposome slows down drug release but it is still too fast for clinical use.
- Liposomes are encapsulated in alginate hydrogel to further slow down the drug release.

Bupivacaine-Loaded Liposome → Hydrogel-Liposome System

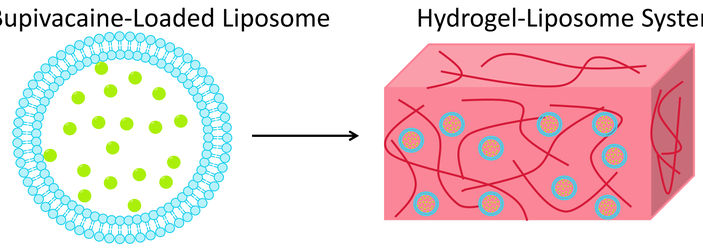


Figure 2: LA delivery model utilizing alginate encapsulated liposomes.

Objectives

Create a LA delivery model that can enable co-administration of LAs and MSCs without decreasing their anti-inflammatory or regenerative properties.

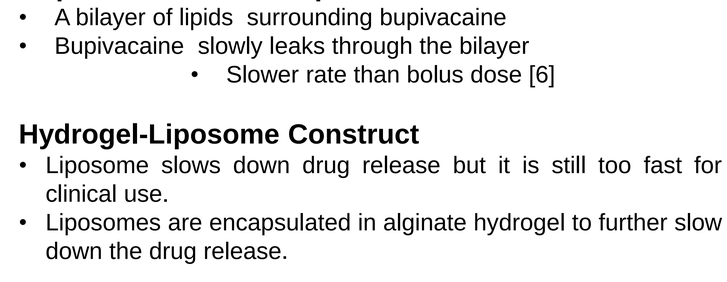
To do this, we aim to:

- Design tunable hydrogel encapsulated liposome structure that will allow for control of the degradation and drug release profiles of LA
- Create a system that can release sufficient and sustainable LA levels to minimize pain without harming therapeutic cell functions

Methods

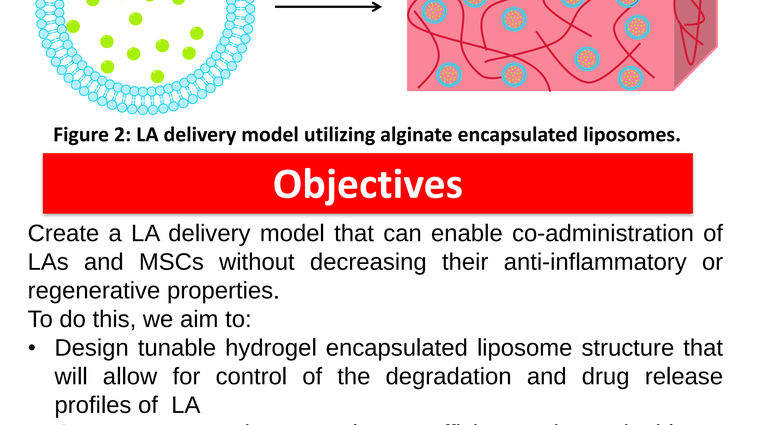
Bupivacaine-loaded Liposomes:

Day 1



Mix lipids in chloroform → Dried on Rotovap . Image from [7] → Suspended in water and incubated 2 hrs → Snap Frozen → Lyophilized Overnight

Day 2



Lipid suspended in Bupivacaine → Extruded through Polycarbonate membrane (200µm). Image from [8] → Eluted through Sephadex G-50

Methods (cont.)

Alginate-liposome Hydrogel:

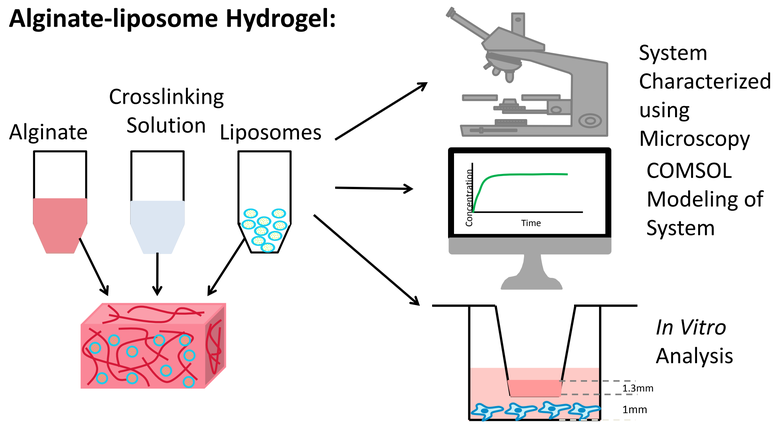


Figure 3: Experimental Setup for Liposome-Alginate Sustained Release Model

Results

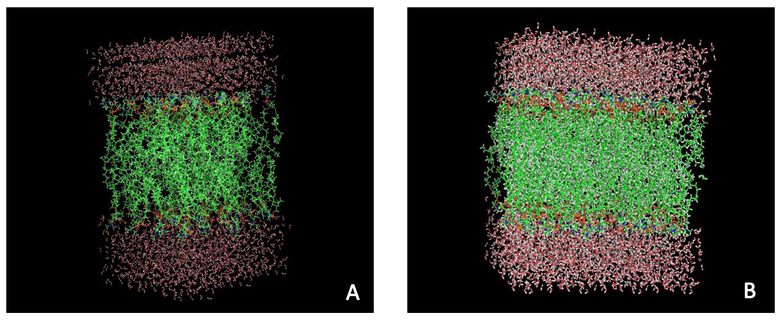


Figure 4: Liposome Characterization. A) Liposome layer folds correctly with hydrophobic and hydrophilic components. B) Water packed liposome model. Molecular dynamics performed using AMBER 14.

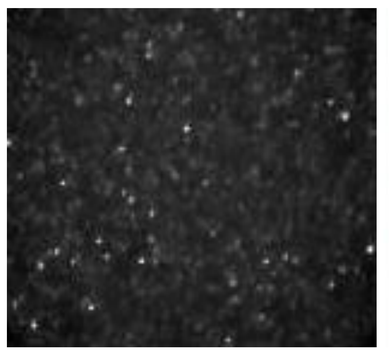


Figure 5: Fluorescent image of liposomes in alginate. The image is a representation of a z-section. As can be seen, a relatively homogenous distribution of liposomes is contained within alginate.

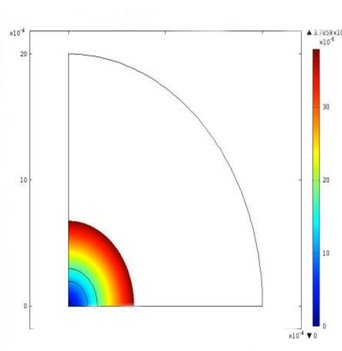


Figure6: CFD assessment of drug release from liposomal formulation. Figure demonstrates a CFD assessment of drug release from a liposomal formulation alone at 24 hours.

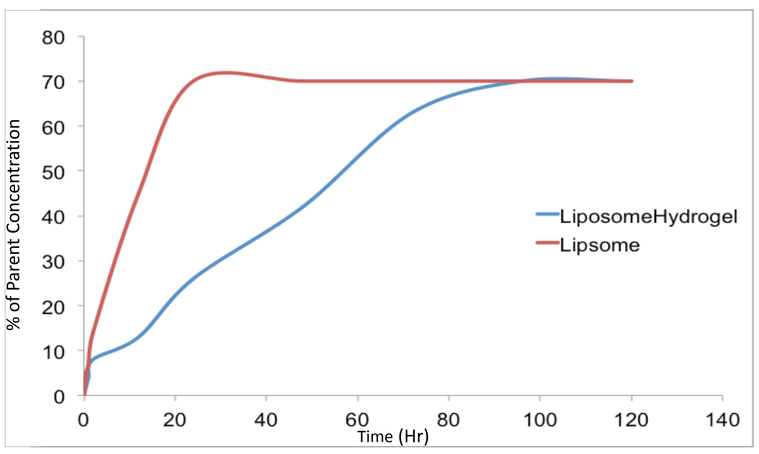


Figure 7: Control release of bupivacaine from liposome-hydrogel constructs. *In vitro* release of bupivacaine determined using LCMS.

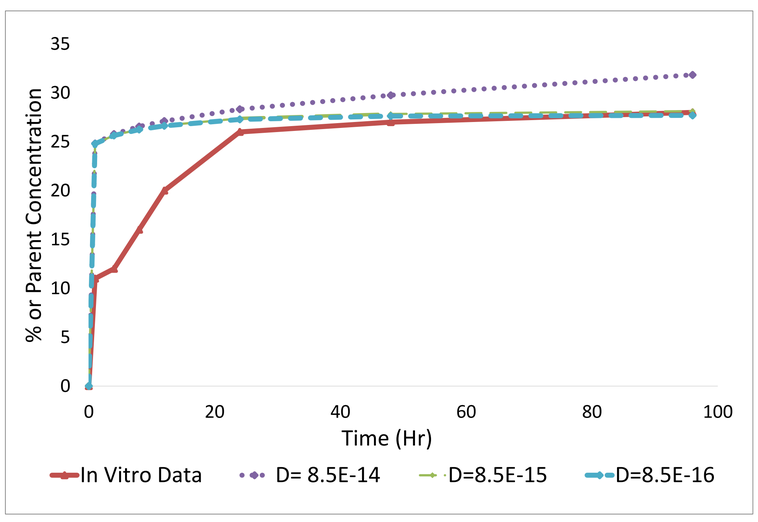


Figure 8: Diffusivity of Bupivacaine from Liposome-hydrogel Formulation. Comparing *in vitro* bupivacaine release data to model output at various diffusivity values.

Results (cont.)

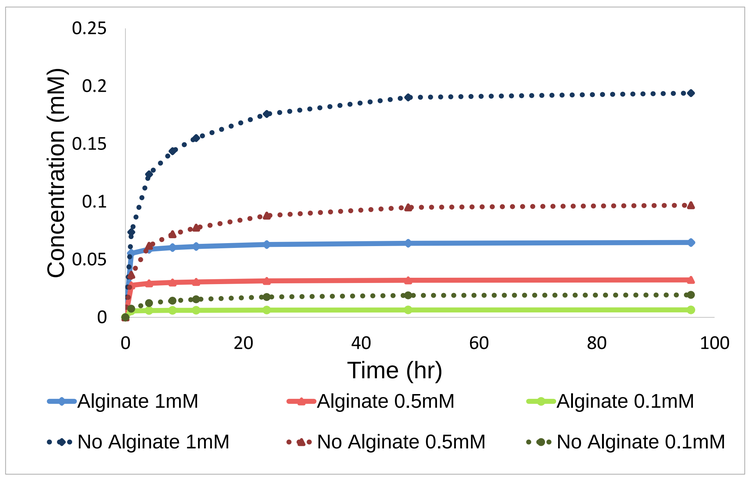


Figure 9: Simulated *in vitro* bupivacaine release profile over time. COMSOL Model output comparing the transwell alginate-liposome formulation with the transwell media-bolus concentration at different initial bupivacaine concentrations. The alginate-liposome system decreased the release profile of bupivacaine.

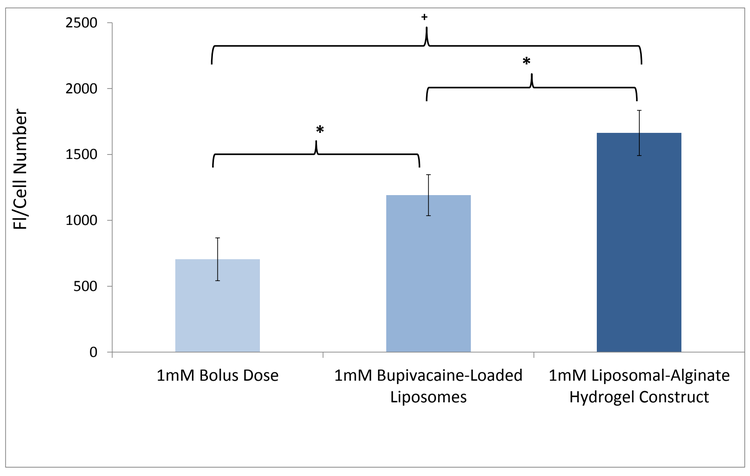


Figure 10. *In vitro* MSC Viability. After 96 hours in culture there is a significant protection of cell viability in the liposomal-alginate hydrogel construct conditions. MSCs treated with 1mM bupivacaine. Bars represent fluorescence intensities (FI) of reduced CellTiter-Blue reagent normalized by cell number. The data are the mean ± SEM of n=6 independent observations (N=2 experiments). *Statistically different ($p \leq 0.05$). +Statistically different ($p \leq 0.0001$).

Discussion and Conclusions

- COMSOL Modeling determined that our formulation could enable long term release of lower concentrations of bupivacaine to MSCs.
  - Starting dose of 1mM yielded a cell apparent dose of 0.1mM, enabling for 90% cell viability [5].
- Diffusivity of bupivacaine from liposome-hydrogel system is 8.5E-15mol/m^3 .
  - Discrepancy in bolus jump could be due to simplicity of model, which does not take into account binding and interactions between the drug and alginate and lipids in the system.
- This formulation provides multi-day pain-mitigation and can be co-administered with MSC therapies

Future Work

- The alginate-liposome formulation will be studied in conjunction with MSCs to determine the effect of the sustained release system has on the cells regarding functionaility.
- A cell uptake component will be added to the model to better simulate the *in vivo* experience.

References

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