Self Assembled Microparticles for Targeted Protein Delivery to Sites of Internal Hemorrhage

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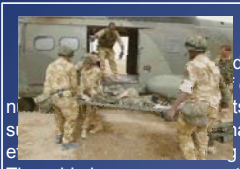
Introduction



Uncontrolled hemorrhage is the leading cause of preventable deaths in the
d and is also the second leading
death in civilian traumas. A
ts have been
al hemorrhage, but an
internal hemorrhage is yet to be found.

Thrombin is an enzyme central to the coagulation cascade. The ability to safely deliver thrombin specifically to sites of internal injury could be very effective at controlling internal hemorrhage. Therefore, we are interested in developing a biocompatible, synthetic microparticle that can be targeted to deliver thrombin to the site of injury where it can initiate rapid blood clotting. We have conducted a fundamental investigation into the development of spontaneously assembled, protein-encapsulating microparticles using poly-L-glutamate (PLE) with pentalysine, and the dependence of this phenomenon on solution pH, ionic strength, and polyelectrolyte concentration.

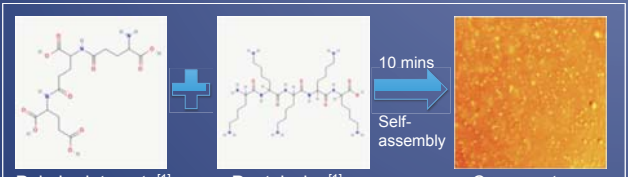
Research Goals

- Synthesize poly-L-glutamate (PLE) microparticles via simple coacervation
- Investigate the effects of polyelectrolyte concentration, pH, and ionic strength
- Produce stable, solid-like microparticles by applying a cross-linking agent

Significance

- Determine optimal conditions for microparticle formation
- Optimize microparticle porosity, size, quantity
- Microparticle properties = carrier effectiveness

Coacervate Synthesis



Poly-L-glutamate[1] Pentalysine[1] Coacervates

Assembly Conditions:

- Polyelectrolyte Concentrations
- pH
- Ionic Strength

[1] http://pubchem.ncbi.nlm.nih.gov

Optical Microscopy

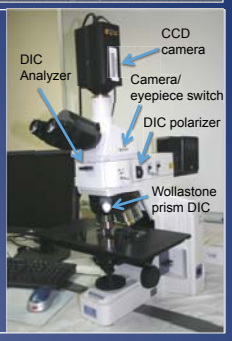
Optical Microscopy was used to verify the absence or presence of coacervates within the solution mixture.

Advantages

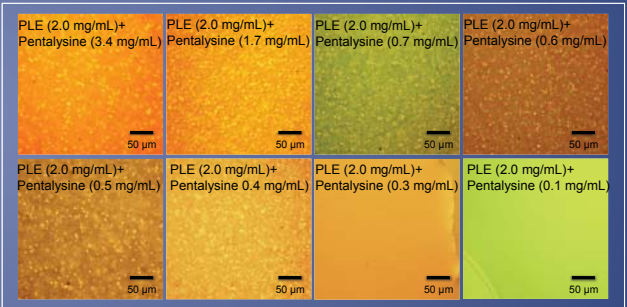
- Qualitative and quantitative observations (size, quantity, shape, etc)
- Visually identify the type of substance in solution

Disadvantages

- Time-consuming
- Wrong adjustments → miss coacervate formation

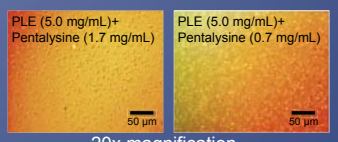


Coacervates Optical Microscopy Images

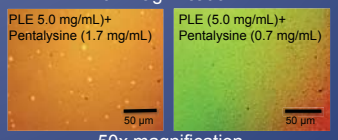


2.0 mg/mL PLE with varied pentalysine concentrations

➢No coacervate formation at pentalysine concentrations below 0.3 mg/mL



20x magnification



50x magnification

5.0 mg/mL PLE with two different pentalysine concentrations

Summary and Future Plans

We demonstrated the first investigation into the development of spontaneously assembled, protein-encapsulating microparticles using PLE and pentalysine. A trend in size and quantity of coacervates synthesized under varying polyelectrolyte concentrations was observed, where the size of coacervates increased and the quantity slightly decreased with increasing pentalysine concentration.

In the immediate future, we plan to test more pentalysine concentrations with 5mg/mL PLE to verify our conclusion, analyze images with ImageJ software for quantitative results, explore effects of pH and ionic strength, and finally cross-link coacervates under different conditions to determine how solution conditions influence microparticle properties. These results will serve as a guide for the synthesis of microparticles for protein encapsulation and for the optimization of microparticle properties.

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