The Larsen Lab: A Center for Nanotherapeutic Strategies in the Central Nervous System (CNS^2)

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Despite numerous attempts to universally develop central nervous system (CNS) treatments, a very limited number of pre-clinical studies have led to clinically available drugs. The field of CNS treatment is plagued by the complexities of the encompassing organs, with biologic barriers in place like the blood-brain barrier (BBB) and the blood-cerebrospinal fluid barrier preventing the passage of greater than 98% of small molecule drugs[1]. (Figure 1). Attempts to subvert these barriers have been narrow in their scope, focused looking for one approach to fully unlock the brain for all treatments, and thus have had limited translational success. BBB disruption has thus far demonstrated the most promise, with the uses of osmotic diuretic drugs[2–4] and focused ultrasound[5–7], but causes pathologies that are not yet understood and could cause long-term damage[8–10]. Because the BBB has not been able to be bypassed effectively, the global burden of neurologic disorders is one of the highest and will continue to increase with the increasing aging population[11–13]. Financially, neurologic illnesses and mental disorders are costing the United States around $760 billion a year[14]. Despite the financial gain possible with the development CNS treatments, pharmaceutical companies have begun to see failures once reaching clinical trials and major players have exited (Bristol-Myers Squibb, GlaxoSmithKline, Pfizer) or reduced (AstraZeneca, Merck&Co, and Sanofi) their research and development in the neurology space[15]. Recent "successes" in the neurologic pharmaceutical space, including aducanumab for Alzheimer's disease, have been plagued by concerns of limited therapeutic efficacy that could provide false hope for patients and families[16]. The suffering of pharmaceutical companies in CNS disease treatment opens an opportunity for academic researchers to zoom out on the field as a whole and come up with more creative solutions to these diseases. Clearly, the high throughput screening of lead compounds explored by pharmaceutical companies is not translating to medical advances concerning the brain and spinal cord.

To combat this key gap, the Larsen Lab focuses on pathology-driven approaches and rigorous materials design to identify best treatment practices. Identifying biomarkers of specific neurologic diseases drives stimuli-responsive materials design into self-assembled delivery vehicles that are tested in most-sensitive animal models. Through these disease-specific approaches, the Larsen Lab aims to create a library of tools with well-defined design rules to approach pathologic changes in the CNS and ultimately create patient-specific medicine for CNS disorders. Specifically, the Larsen Lab works with stimuli-responsive polymeric materials that can alter their structure in response to pathologic stimuli, specifically releasing drugs at the disease site[17]. Stimuli-responsive block co-polymers with a hydrophilic fraction between ~25 and 40% can create nanoparticle vesicles called polymersomes[18]. Polymersomes have major benefits associated with their potential for use in targeted therapies, highlighted in Figure 2 that can be harnessed to develop CNS therapies.

The CNS^2 team looks specifically at the following types of stimuli-responsive systems:

1. pH-responsive Delivery Systems

Enzyme Replacement Therapy. We have previously demonstrated that polymersomes (self-assembled polymer vesicles) can deliver enzymes to feline GM1 gangliosidosis cells (GM1SV3s), with β-galactosidase (βgal) payloads up to 0.01 mg/mg and using apolipoprotein E (ApoE) targeting[19]. Polymersome made from polyethylene glycol (PEG) blocked with polylactic acid (PLA) were selected to respond to the acidic environment of the lysosome, releasing encapsulated βgal where it is needed to ameliorate lysosomal storage products. ApoE targeting was selected due to upregulated low density lipoprotein receptors at the BBB in GM1 gangliosidosis-affected felines due to high levels of inflammation.

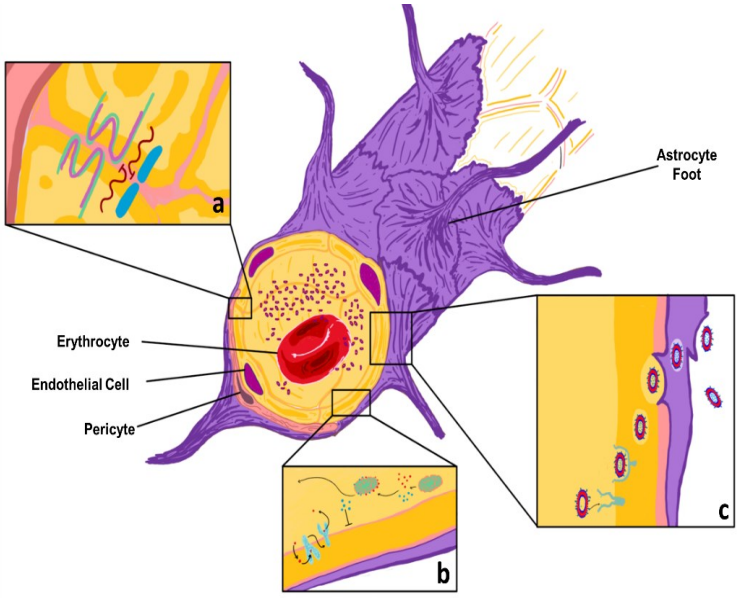

Figure 1: The Blood-Brain Barrier (BBB). (a) Endothelial cells in blood brain barrier are continuous as a result of junction complexes sealing the paracellular space. (b) Uptake of free drug molecules, both hydrophobic (red) and hydrophilic (blue), is negligible across the BBB. Small hydrophobic molecules that are able to diffuse across the apical membrane of endothelial cells are excised back into blood by P -Glycoproteins. Hydrophilic drugs are unable to passively diffuse across the BBB. Freely distributed drugs face protein absorption in the blood, significantly reducing the amount of drug available for cell uptake. (c) Theoretical representation of polymersomes conjugated with targeting ligands crossing the BBB by receptor mediated transcytosis (Figure drawn by Larsen Lab alumni, Nicholas L'Amoreaux)

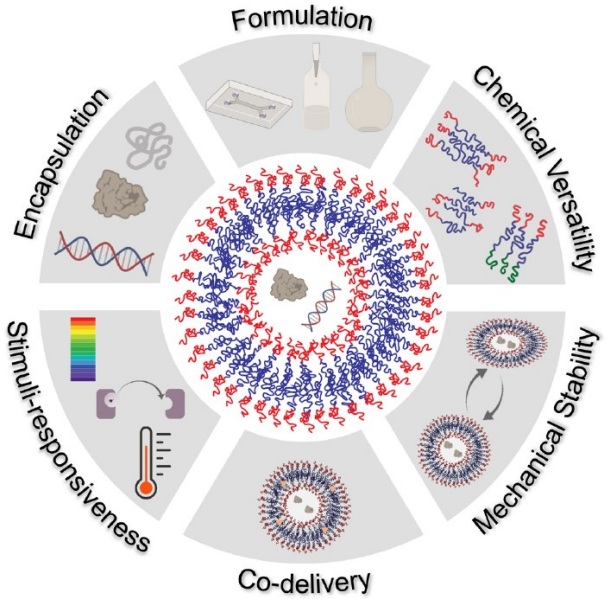

Figure 2. Polymersome Schematic. Advantages associated with the use of polymersomes in targeted drug delivery applications[17].

GM1SV3 cells do not have the complexities of the BBB but have the genetic manifestations of GM1 gangliosidosis. Similarly, although free βgal cannot cross the BBB and treat the brain, polymersome-mediated delivery of βgal occurred to the full brain of GM1 gangliosidosis affected felines in a dose-dependent manner, with higher doses leading to enzyme activities up to 80% normal in the cerebellum, a nine-fold increase compared to untreated cohorts (Figure 3)[19]. Preliminary data demonstrates potential transport across the BBB using the same targeting mechanism[20–23].

Gene Editing. Because of our success with encapsulating and maintaining the activity of high molecular weight enzyme βgal in polymersomes, we decided to address the challenges of encapsulating gene editing tool, CRISPR-associated protein 9 (Cas9). To date, no one has yet demonstrated that polymersomes can deliver Cas9[24]. The Larsen Lab is working to encapsulate Cas9 complexed with guide RNA in its ribonucleoprotein (RNP) form. Using electroporation, the RNP achieves ~40% knockdown of fluorescence in HEK293-GFP cells. Cas9 RNP is encapsulated in PEG-poly(lactic-co-glycolic) acid polymersomes, which has a decreased bio-degradation rate in the lysosome compared to PEGPLA, allowing time for lysosomal escape via positively charged peptides. Encapsulation efficiency of 53.8 ± 7.4% is achieved, and polymersomes maintain their size of 184.1 ± 13.0 nm in diameter. Work is ongoing to determine the therapeutic impact of Cas9 RNP loaded polymersomes *in vitro*.

Modulation of polymersome shape to enhance neural cell uptake. Modulation of nanoparticle shape has long been explored to improve tissue penetration and allow for evasion from the immune system, with elongated and rod-like particles demonstrating the most significant improvement of therapeutic properties[25]. Although successful in solid NPs, combining membrane-bound particles with shape-enhanced uptake combines both benefits. Therefore, rod-like systems would be capable of enhanced cellular penetration, hydrophilic and hydrophobic drug loading, decreased payload loss, protection from the immune system, and stimuli-responsivity. Thus, achieving a persistent shape change in membrane-bound polymeric NPs should allow them greater efficacy as therapeutic delivery vehicles to the brain. Using osmotic pressure gradients, we have kinetically trapped self-assembled polymersomes into new elongated shapes of prolates[26], rods, and stomatocytes[27]. Spherical polymersomes formed from polyethylene glycol-b-polylactic acid (PEGPLA) and PEG-b-poly(lactic-co-glycolic) (PLGA) are dialyzed against various salt (NaCl) gradients (50 mM, 100 mM, and 200 mM) to create elongated shapes. The hydrophobicity of the polymer and the strength of the salt gradient dictate the primary shape formed[27]. Most importantly, elongated polymersomes (50 mM NaCl) have higher loading capacities (~2 times higher for hydrophobic Nile red (NR) and ~1.7 times higher for encapsulated protein BSA-fluorescein (FITC-BSA)) while maintaining their pH-responsive release behavior[26]. We recently published a paper demonstrating that through the slight elongation of polymersomes from an aspect ratio of one (spheres) to an aspect ratio of two (prolates), we dramatically increase uptake in neuroblastoma cells, SH-SY5Ys (Figure 4).

2. Enzyme-Responsive Delivery Systems

Improved Enzyme Replacement Therapy. Despite the significant promise of pH-responsive polymersomes as βgal delivery vehicles, only ~30% of enzyme is released upon degradation[28]. Because of this, we aimed to identify more appropriate stimuli-responsive materials based on GM1 gangliosidosis disease pathology. In GM1 gangliosidosis-affected felines, there is clear evidence for compensatory upregulation of lysosomal enzyme hexosaminidase A (HexA)[29]. We have increased our knowledge in the field of biomarkers discovery, identifying a direct relationship between autophagic behavior and enzyme upregulation in a cellular model of GM1 gangliosidosis[30]. In response, we have developed polymersomes from hyaluronic acid (HA) blocked with PLA formed at diameters of 135.9 ± 30 nm that degrade in HexA and release βgal. These polymersomes are taken up by GM1SV3 cells at high concentrations and restore healthy autophagic function. These results were very recently published[31], and this project is funded through the National Science Foundation's Early Career Award Program. This work has been expanded to include HA polymers of varying lengths, which have varying enzyme-responsivity. By altering the molecular weight of the HA block, we can fine tune release profiles in response to pathologically upregulated enzymes in GM1 gangliosidosis patients.

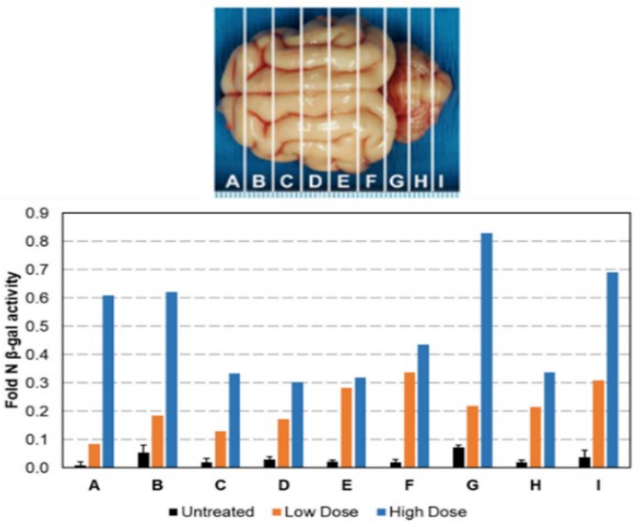

Figure 3. In Vivo Performance of ApoE tagged and βgal loaded polymersomes in GM1 gangliosidosis felines demonstrates increased enzyme activities throughout the entire brain, with higher doses reading near normal levels of βgal activity.

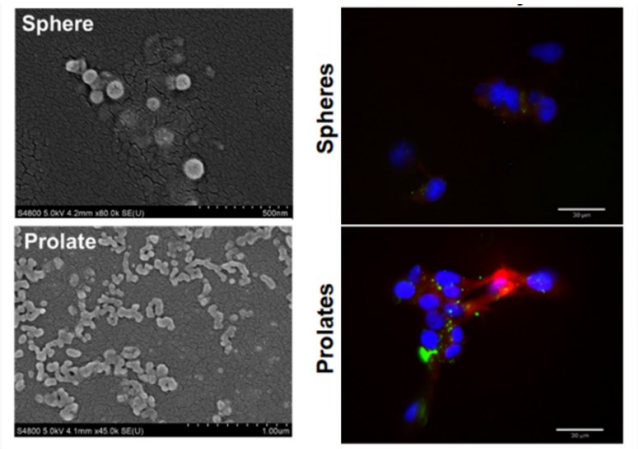

Figure 4. 50 mM of NaCl creates prolates (aspect ratio of 2) from spherical PEGPLA polymersomes. This is change was observed in TEM and SEM (pictured). PEGPLA polymersomes are also taken up by SH-SY5Y cells at higher concentrations as demonstrated by fluorescence imaging (red = NR, green = FITC BSA)

3. Temperature-Responsive Delivery Systems

Physically Cross-linked Hydrogels for Local Neural Administration. Recently, we have begun work on the development of thermally responsive hydrogels for local CNS injections. These polymers are physically cross-linked by changes in temperature, which controls the solubility of the polymer and therefore the hydrophobic interactions below and above a temperature called the lower critical solution temperature (LCST). Thermoresponsive biomaterials for noninvasive *in vivo* applications benefit from having an LCST between room temperature (25 ºC) and body temperature (37 ºC) to allow for injection of a liquid substance that does not transition into a gel until after entering the body (Figure 5). This also can improve drug encapsulation into the gel upon physical cross-linking. Specifically, we have begun to create hydrogels with the addition of citrate or citric acid to modulate local microenvironments in the CNS after injury, building on the current literature[32]. We aim to improve the use of these hydrogels in drug delivery applications through the inclusion of polymersomes simultaneously with the gel (Figure 6).

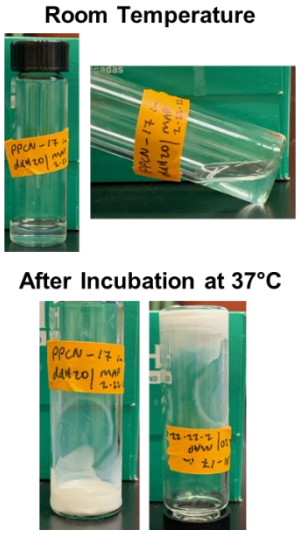

Figure 5. Thermally Responsive Behavior of Poly(PEG citrate-co -NIPAAm) (PPCN) hydrogel.

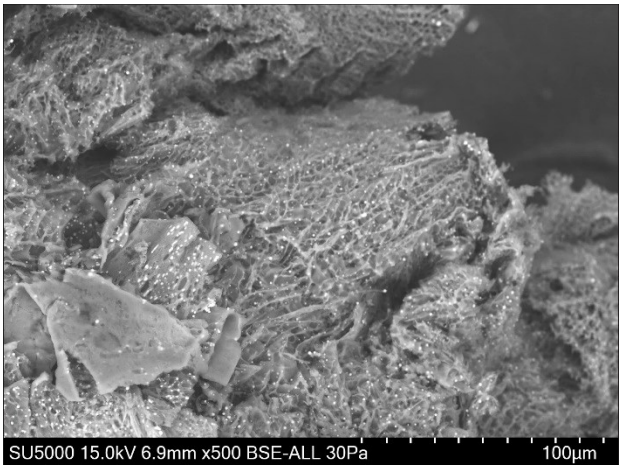

Figure 6. PPCN hydrogel with PEGPLA polymersomes in SEM.

Conclusions

Using pH, enzyme, and temperature-responsive polymers, the Larsen Lab develops pathology-relevant approaches to bypassing the BBB and treating the CNS. Using a pathology-driven approach requires interdisciplinary knowledge and unique approaches for each CNS disease. However, polymersomes are a versatile platform that can respond to various stimuli with alteration of materials chemistry and that can encapsulate small molecular weight, large molecular weight, and even multiple therapeutic payloads simultaneously. By combining the knowledge of each CNS disease with novel polymersome design, the Larsen Lab aims to finally unlock the brain to therapies.

Acknowledgements

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Notes and References

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