7-2023

Polymersomes as Chemotherapeutic Agents for Non-Invasive Treatment of Glioblastoma

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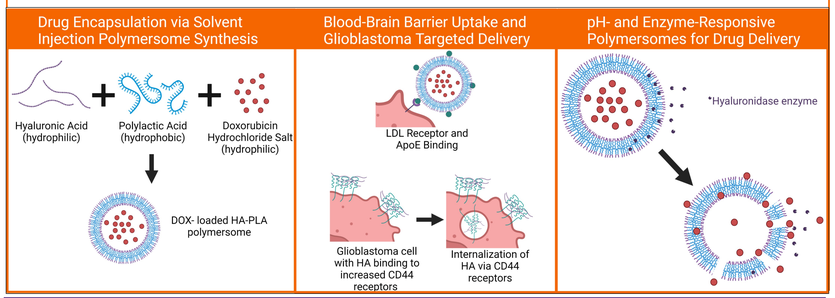
Chemical and Biomolecular Engineering

Abstract

Polymersomes (PS) are considered to be an intriguing and effective method for transporting drugs in a targeted manner throughout the body. The ability to encapsulate both hydrophilic and hydrophobic drugs, maintain biocompatibility, and release drugs in a tunable manner make polymersomes appealing candidates for the treatment of glioblastoma multiforme (GBM). However, there is still a need for a systemic delivery mechanism that yields increased polymersome uptake into the brain after noninvasive injections. Here, we report the encapsulation and release capability of hyaluronic acid (7 kDa)-b-polylactic acid (HA7-PLA) polymersomes. Specifically, we demonstrate high loading and controlled release of doxorubicin (DOX). We show that 5 mg of HA7-PLA polymersomes fully loaded 3 µg of DOX, then released approximately 3-fold the amount of DOX in a tumorigenic environment versus a neutral environment. Dynamic light scattering (DLS) confirmed an average particle size of 101.8 ± 16.50 nm while transmission emission spectroscopy (TEM) confirmed there was no change in HA7-PLA polymersome shape after loading DOX. Our results motivate further study into using HA7-PLA polymersomes as a nanoparticle drug delivery system.



Motivation

The World Health Organization (WHO) estimated an annual incidence of GBM to be ~5 cases per 100,000 people in the United States.[1]

- High-grade tumors are deemed malignant
- Glioblastoma multiforme (GBM) makes up 60-70% of all tumors

Table 1: WHO classification of gliomas and the percentage breakdown.

WHO Classification			
Low-grade	Grade I	Pilocytic astrocytoma	< 10%
	Grade II	Diffuse astrocytoma	< 10%
High-grade	Grade III	Anaplastic astrocytoma	10-15%
	Grade IV	Glioblastoma multiforme (GBM)	60-70%

Current "gold standard" treatment consists of surgical resection followed by radiation therapy and chemotherapy after the surgical site has fully healed.[2]

- Without treatment, average 2-year survival rate of 5%
- With the current optimum treatment, average 2-year survival rate of 30%
- The 5-year survival rate post optimum treatment is only 7.2%

Despite the existence of a treatment, glioblastoma multiforme remains a public health concern.

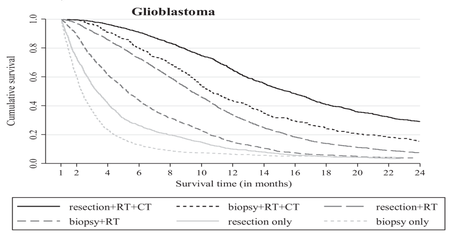


Figure 1: Cumulative survival of GBM with various treatment plans.

The blood-brain barrier is a semi-permeable, extremely selective barrier that blocks 98% of small molecules from transporting through

- Tight junctions tie endothelial cells together and prevents water-soluble agents in blood from crossing
- End-feet astrocytes maintain water balance and buffers extracellular ion concentrations
- More than 7000 drugs in CMC database but only 5% treat central nervous system[3]

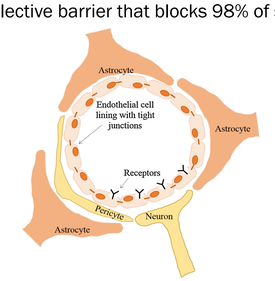


Figure 2: Diagram of the blood-brain barrier.

Hyaluronic acid-b-polylactic acid Polymer and Polymersome Synthesis

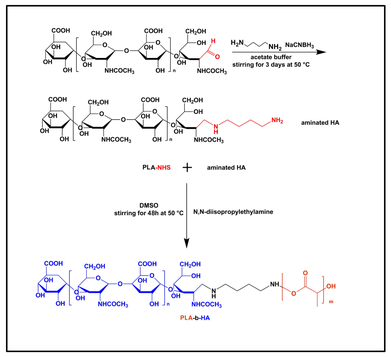


Figure 3: HA-PLA reaction scheme[4]

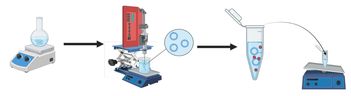


Figure 4: Polymersome solvent injection scheme[5]

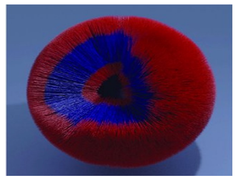


Figure 5: 3D polymersome structure[6]

HA-PLA block copolymer synthesis (**Figure 3**):

- Terminal reductive amination of HA
- $NaCNBH_3$ used as reducing agent
- Conjugation of PLA-NHS with aminated HA performed in one-pot synthesis

Solvent Injection (**Figure 4**):

- Polymersomes self-assemble due to thermodynamic stability
- Needle gauge and injection speed control polymersome size
- Structure and shape allows for passive drug loading

Polymersome Characterization

Table 2: Size and charge characterization of HA7-PLA polymersomes via dynamic light scattering

Sample Type	Unloaded			
	Run	PDI	Zeta Potential (mV)	Size (d.nm)
HA7-PLA	1	0.309	-10.687	91.500
	2	0.339	-10.173	92.970
	3	0.327	-13.043	120.833
	Average	**0.325**	**-11.3**	**101.8**
	St. Dev.	**0.015**	**1.5**	**16.5**

Successful polymersome synthesis and characterization was performed in triplicates.

- Low PDI indicates a highly monodisperse system[7]
- Particle size <200 nm required for diffusion through BBB via EPR effect[8,9]
- Negative zeta potential should result in increased uptake in cancer cells[10]

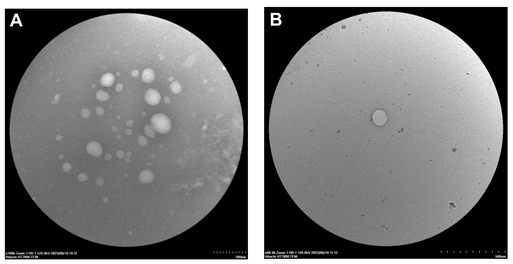


Figure 6: TEM images of (A) Unloaded HA7-PLA polymersomes and (B) DOX-loaded HA7-PLA polymersome.

TEM images were taken to confirm DLS data

- Confirmation of monodisperse, spherically shaped polymersomes
- Polymersomes maintained shape in both unloaded (**A**) and DOX-loaded (**B**) state
- Uptake kinetics and intracellular pathways are shown to be shape-dependent[11]

In Vitro Results

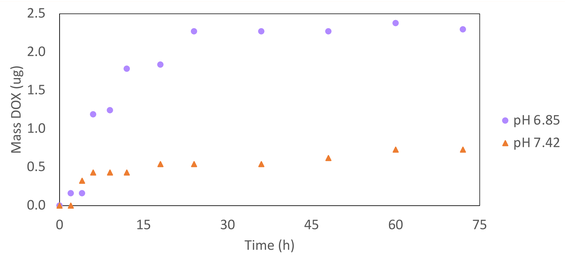


Figure 7: Release profile of DOX over time in acidic and neutral environments.

Designing pH-sensitive systems is of interest because they add a variable of control in drug release.

Tumor environments ≡ acidic pH
- Initial burst release of 40% DOX in first 6 hours
- Plateau around 24 hours with 75% DOX released

Healthy brain environments ≡ neutral pH
- Initial burst release of 11% DOX in first 4 hours
- Plateau around 18 hours with 18% DOX released

In Vivo Results

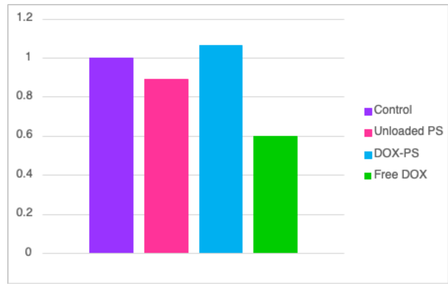


Figure 8: MTS Assay of U87 MG cells in PBS, unloaded HA7-PLA PS, DOX loaded HA7-PLA PS, and free DOX.

MTS Assay
- Compared cell survival to negative control (PBS)
- > 80% cell survival deemed nontoxic
- 40% cell death when treated with free DOX is expected
- Contact inhibition likely caused increased cell growth when treated with DOX-PS

Overall, polymersome deemed biocompatible while DOX is cytotoxic to U87 MG cells.

Future Work

- Cell Culture: determine efficacy of drug release *in vitro* for U-87 malignant glioma cells and HMC3 cells
- MTS Assay to determine the toxicity of the unloaded polymersomes, DOX loaded polymersomes, and free DOX
- Flow cytometry to determine nanotoxicity of the HA7-PLA polymersomes and DOX loaded polymersomes

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Acknowledgements

Thank you to the members of the Larsen Research Group.
Thank you to the Chemical and Biomolecular Engineering Department at Clemson University.
Thank you to the NSF and DMR #1950557 grant