Temozolomide Delivery via Peptide Micelles in Glioblastoma Treatment

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Background: Glioblastoma multiforme, the most malignant and common brain tumor, has only a 9% survival rate after two years.[1] The high recurrence and low survival rate can be attributed to the invasiveness and infiltration of the tumor into healthy brain tissue.[2] Debulking surgery alone is not a viable treatment method; thus, it is often paired with chemotherapy and radiation. Temozolomide (TMZ) is a chemotherapeutic drug commonly delivered orally to treat glioblastomas. However, TMZ faces significant barriers to delivery, including conversion to its active form and insufficient intratumoral accumulation.[3] The purpose of this project is to evaluate peptide micelles as a drug delivery system for efficient delivery and conversion of TMZ to treat glioblastoma.

Materials and Methods: Peptides were dissolved in dimethyl sulfoxide (DMSO) at 1 mg/mL; afterward, micelles were formed via dialysis against water for 24 hours. The micelles were then extruded at 0.2 µm to decrease micelle size. Dynamic light scattering (DLS) was performed before and after extrusion. To observe loading, the peptide was dissolved in DMSO at 1 mg/mL and mixed in solution with TMZ dissolved in water at 1 mg/mL. The solution was sonicated for 30 minutes and then dialyzed against water. Loading was observed in the peptides over different time frames of dialysis, including 4, 8, and 24 hours. The solution was then spin filtered through a 0.65 µm pore size filter. TMZ absorbance was measured with a plate reader at 330 nm.

Results: DLS performed before extrusion resulted in an average size of 419.2 ± 36.9 with a PDI of 0.838 ± 0.115 and a zeta potential of -5.72 ± 0.267 mV. DLS performed after extrusion resulted in a bimodal distribution, with peak 1 showing an average size of 554.1 ± 70.29 nm and peak 2 showing an average size of 103.4 ± 13.26 nm. The PDI after extrusion was 0.621 ± 0.04, and the zeta potential was -0.246 ± 0.033 mV. The 4-hour dialysis resulted in 32.27% TMZ loaded into the peptides. The 8-hour dialysis resulted in 22.14% loaded TMZ, and the 24-hour dialysis resulted in 1.80% loaded TMZ.

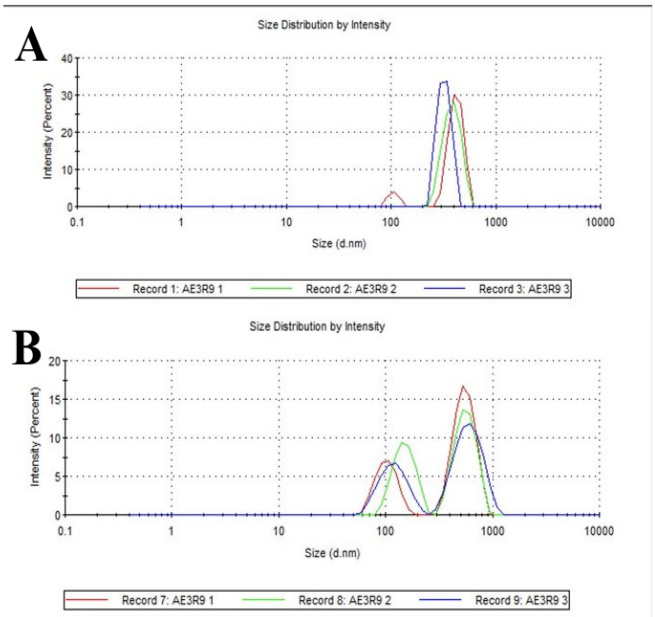

Figure 1: Size distribution of micelles A) before dialysis and B) after dialysis.

Conclusions: Results from DLS indicate that extrusion was successful in decreasing the micelle size; however, the distribution was bimodal. Micelle aggregation may justify the population of larger micelles observed after extrusion. Results indicate that for the loading of TMZ into micelles, the 4-hour dialysis was the most efficient. At time points beyond 4 hours, it is possible that the TMZ was released from the peptide, which explains the decreasing TMZ concentration observed after the 8-hour dialysis, and the almost negligible concentration of loaded TMZ observed after the 24-hour dialysis. Further loading experiments using a smaller spin filter size to remove unloaded TMZ, as well as observing micelle size via DLS after loading, will be completed next to further characterize the micelles.

References: [1] Jovčevska, Ivana. "Genetic secrets of long-term glioblastoma survivors." *Bosnian journal of basic medical sciences* vol. 19,2 116-124. 20 May. 2019, doi:10.17305/bjbms.2018.3717 [2] Hatoum, Adam et al. "The unique invasiveness of glioblastoma and possible drug targets on extracellular matrix." *Cancer management and research* vol. 11 1843-1855. 25 Feb. 2019, doi:10.2147/CMAR.S186142 [3] Bouzinab, Kaouthar et al. "Delivery of Temozolomide and N3-Propargyl Analog to Brain Tumors Using an Apoferritin Nanocage." *ACS applied materials & interfaces* vol. 12,11 (2020): 12609-12617. doi:10.1021/acsami.0c01514