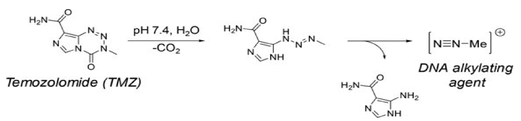
In Vitro Evaluation of a Drug-Loaded Self-Assembling Peptide Hydrogel for Treating Glioblastoma Multiforme

Alexandra Nukovic, Megan Pitz, Margaret Elpers, Arica Gregory, Angela Alexander-Bryant, Ph.D.

Department of Bioengineering, Clemson University, Clemson, SC

INTRODUCTION

- Each year, an estimated over 25,000 adults and children in the United States will be diagnosed with cancerous tumors of the brain or spinal cord.[1]
- Glioblastoma multiforme (GBM) is the most common and aggressive primary brain tumor.
- The median survival rate for GBM is about 15 months.[2] Chemotherapy with the slightly hydrophobic DNA alkylating agent, temozolomide (TMZ), is commonly used in treatment for GBM.
- TMZ is an alkylating prodrug that undergoes spontaneous hydrolysis at physiological pH (pH ~ 7.5) to its active form, a methyldiazonium cation.[3] However, studies have shown that full conversion of TMZ to its active form occurs at a more basic pH (pH>7.5).[4] Further, after TMZ is converted to its active form, it is unable to cross the blood brain barrier, thereby limiting availability in the brain and lowering the effectiveness of TMZ.[4]
- **We propose an innovative local delivery strategy using peptide hydrogels that will encapsulate TMZ and convert the drug to its active form upon degradation.**



References:

[1.] Ostrom, Q. T. *et al. Neuro. Oncol.* (2016)

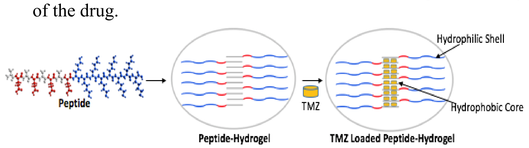
[2.] Friedman, Henry S., et al (2000) *American Association for Cancer Research*

[3.] Strobel H, Baisch T, Fitzel R, et al. *Biomedicines*. 2019;7(3):69

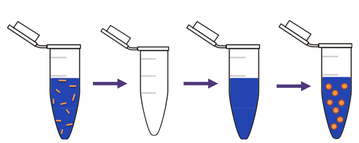
[4.] Andrasa, Melinda, et al (2010) *Journal of Chromatography B* 878(21):1802-1808.

METHODS

- Amino acid sequences were designed to promote self-assembly of a hydrogel.
- TMZ will load into the hydrophobic portion remain inactive until the hydrogel degrades.. As the hydrogel degrades, the hydrophilic shell will mediate activation of TMZ, thus increasing the efficacy of the drug.



- Hydrogels were formed via the Thin film Dehydration method.



RESULTS

1. Temozolomide displayed enhanced anticancer activity in glioblastoma cells when dissolved at a basic pH.

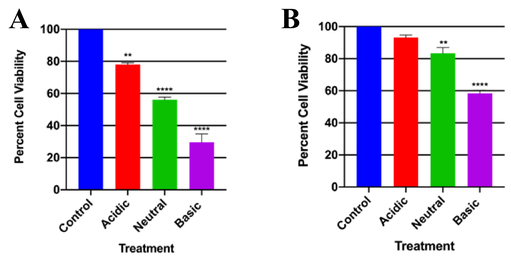


Figure 1. Cell viability of LN-18 cells (A) and T98G cells (B) 72 hours after treatment with TMZ dissolved in water at a pH of 2.5, 7.5, and 12. Data are mean ± SEM of three independent experiments performed in triplicate, where **P<0.01, and ***P<0.0001 compared to untreated cells (control, one-way ANOVA). Data courtesy of Arica Gregory.

2. Peptide hydrogels mediated delivery of Coumarin-6 into glioblastoma cells.

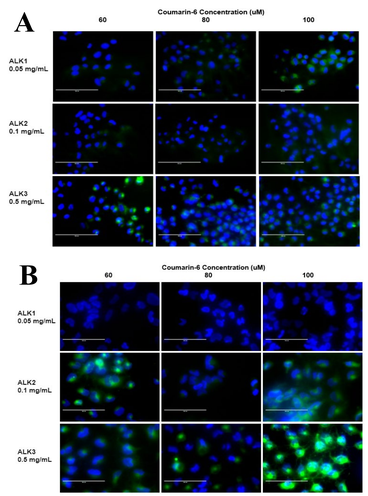


Figure 2. ALK1, ALK2, and ALK3 peptide hydrogels were each synthesized in dd-H2O at peptide concentrations of 0.05 mg/mL, 0.1 mg/mL, and 0.5 mg/mL. respectively. LN-18 (A) and T98G (B) glioblastoma cells were treated for 4 hours at 37°C with the hydrogels loaded with 60, 80, and 100µM of Coumarin-6 (green). Nuclear components of cells were counterstained with NucBlue (blue).

3. Peptide hydrogels formed nano- and microstructures.

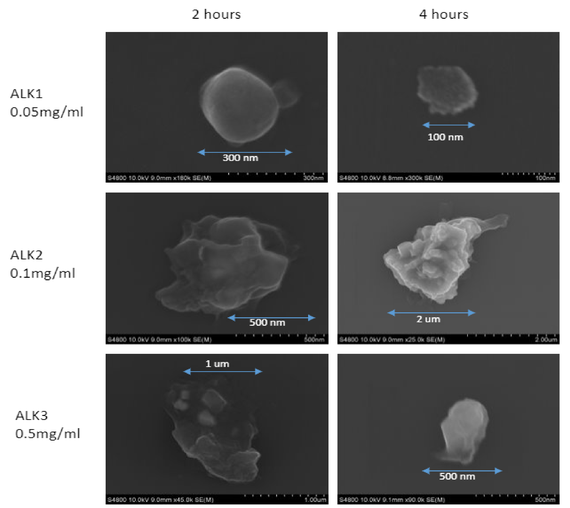


Figure 3. Scanning electron microscopy images of peptides formed via film hydration, rehydrated with water, and shaken on a shake plate for 2hr and 4hr after rehydration.

4. Unassembled peptides are not cytotoxic

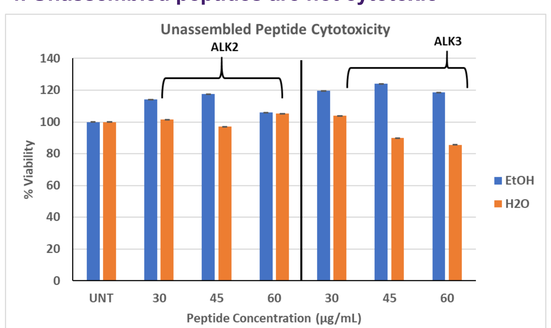


Figure 4. ALK2 and ALK3 peptides were dissolved in ethanol and water and applied to T98G glioblastoma cells in increasing concentrations of 30, 45, and 60 µg/mL. Cells were treated for 48 hours and cell viability was analyzed via MTT assay.

CONCLUSIONS

- TMZ dissolved in a basic pH solution exhibits greater cytotoxicity in glioblastoma cells.
- Peptide hydrogels were able to mediate uptake of hydrophobic drugs into glioblastoma cells.
- SEM imaging indicated micellar nanoparticle formation for ALK1 and microstructure hydrogels for ALK2 and ALK3.
- Hydrogels formed with 4-hour shake time resulted in greater nanoparticle formation.
- Unassembled peptides dissolved in solution are not cytotoxic to glioblastoma cells.
- **These results demonstrate the potential of the peptide-hydrogels as delivery vehicles for TMZ.**

FUTURE WORK

- Further characterize our peptide hydrogel delivery system through circular dichroism and SEM.
- Obtain flow cytometry results to quantify hydrogel mediated uptake of Coumarin-6 in glioblastoma cells.
- Further evaluate ability of peptide hydrogels to mediate conversion of TMZ.
- Further pursue micellar structure of ALK1 through DLS and SEM.
- Evaluate the cell viability of glioblastoma cells treated with the TMZ-loaded peptide hydrogel compared to TMZ alone by performing MTT cell viability assays.

ACKNOWLEDGEMENTS

Advisor: Dr. Angela Alexander-Bryant, Ph.D.

Lab Members: Megan Pitz, Lora Alatise, Jessie Boulos, Timmy Samec, Samantha Gardner, Anthony Hazelton, Serena Gilmore, Aaron Williams, Sarah Wilde

This work was supported by:

NSF EPSCoR Program under Award # OIA-1655740

NIH COBRE under Award# P30GM131959