Implantable peptide-hydrogel drug delivery system for treating glioblastoma multiforme

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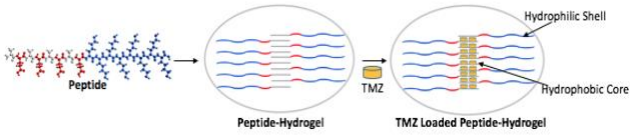
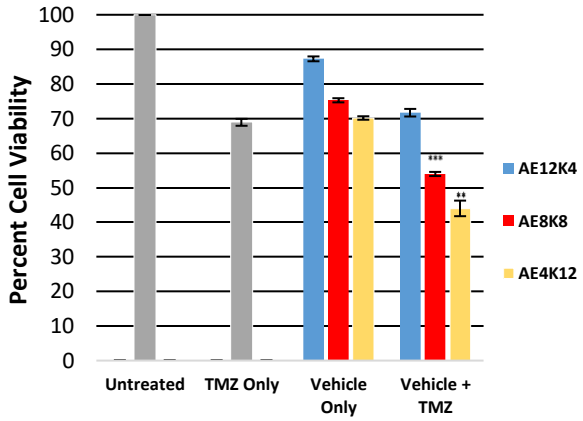
Introduction: Gliomas represent approximately 80% of all malignant brain tumors, and glioblastoma multiforme (GBM) accounts for nearly half of all gliomas [1]. Glioblastoma multiforme is the most common and aggressive primary brain tumor. Despite aggressive treatment including tumor resection followed by radiation and chemotherapy, the median survival rate for GBM is ~15 months [2]. Therefore, new therapeutic strategies are needed to improve the survival rate of those affected by glioblastoma multiforme. Chemotherapy with the DNA alkylating agent, Temozolomide (TMZ), is commonly used in treatment for glioblastoma multiforme. TMZ is an orally delivered drug that is be stable or inactive in the acidic pH environment of the stomach, and begins to convert to its active form at a neutral pH in the bloodstream (pH ~ 7.5). However, studies have shown that full conversion of TMZ to its active form occurs at a more basic pH (pH>7.5) [3]. 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 [3]. Therefore, we propose an innovative local delivery strategy using hydrophilic and alternating hydrophobic and acidic amino acids to form a hydrogel that will encapsulate TMZ and convert the drug to its active form. The hydrophobic residues of the peptide-hydrogel will load TMZ, and the hydrophilic residues will convert TMZ to its active form as the hydrogel degrades. This therapeutic strategy will allow for extended release of TMZ, thus increasing the efficacy of the drug.

Methods: Peptide Sequences were designed, synthesized, and formed into hydrogels for the delivery of temozolomide. TMZ was encapsulated into the hydrophobic core, which was surrounded by hydrophilic amino acid residues **(Figure 1A)**. TMZ was loaded into AE12K4, AE8K8, and AE4K12 peptide hydrogels at the concentration that exhibited the highest TMZ loading. Cells were treated with only TMZ, unloaded peptide hydrogel, TMZ loaded peptide hydrogel, or left untreated. Cytotoxicity of LN-18 cells was determined by MTT assay after 72-hour incubation. **(Figure 1B)**. For drug loading, 0.03mg of TMZ was combined with 0.5mg/mL, 0.1mg/mL, and 0.05mg/mL of peptide, and dehydrated to form a drug-loaded film. Water was added to rehydrate the film and it was shaken for 2 hours. The amount of TMZ loaded was then determined by spectrophotometry **(Figure 1C)**.

(A)



(B)



(C)

Concentration of Peptide [mg/mL]	AE12K4	AE8K8	AE4K12
0.05	0.02395	0.02755	0.02555
0.1	0.02507	0.02713	0.02513
0.5	0.02703	0.02131	0.01898

Figure 1. **(A)** Schematic of drug-loaded peptide - hydrogel. **(B)** Analysis of LN-18 cell viability after 72hrs treatment with peptide hydrogel loaded with TMZ or peptide hydrogel alone. Data are mean ± SEM of three independent experiments performed in triplicate, where **P<0.01 and ***P<0.001 compared to TMZ treated cells. **(C)** Concentration of drug loaded into the peptide-hydrogel. The highest concentration of loaded TMZ is identified for each peptide sequence.

Results: Our results demonstrated that delivery of TMZ via peptide hydrogels resulted in significant decreased cell viability in LN-18 cells (Figure 1B). We also demonstrated that at various concentrations, the proposed peptide-hydrogel efficiently loads TMZ (Figure 1C).

Conclusions: Our results demonstrate that the efficacy of temozolomide was enhanced when delivered in a peptide hydrogel system. Further, the peptide hydrogel was able to efficiently load TMZ. Future studies will further evaluate the potential of the TMZ-loaded peptide hydrogel for treatment of GBM.

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