Team NANO: Biodegradable Hydrogel Drug Delivery System to Attack Glioblastoma Multiforme (GBM) Cells

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Research Problem and Hypothesis

Glioblastoma multiforme (GBMs) are fast-growing, aggressive tumors that can occur in the brain or spine. Despite treatments such as ionizing radiation, chemotherapy, and surgery, the median survival rate is 15 months. This is due to the cancer cells that are left behind post-operation leading to GBM recurrence.

Research Question: How can a polyvinyl pyrrolidone (PVP)-based biodegradable hydrogel be optimized for delivery of lipoprotein-encapsulated Carmustine to achieve gradual and prolonged drug release in the glioblastoma multiforme (GBM) microenvironment?

Two principle facets to the optimization of hydrogel material properties:

- The stability of the drug delivery system, with regards to both the hydrogel-enclosed lipoproteins encapsulating the Carmustine chemotherapeutic as well as the hydrogel itself to the GBM environment
- The controlled release of lipoproteins from the hydrogels as the hydrogel swells due to contact with the aqueous environment in vivo

Methodology

Hydrogel synthesis:

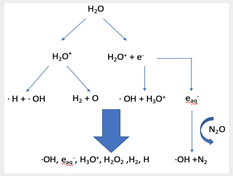

Figure 1: Example of Electrons fired at PVP samples leading to water radiolysis, OH radicals .

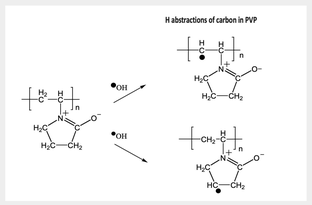

Figure 2: Example reaction of OH radicals creating carbon-centered free radicals, allowing intermolecular cross-linking between adjacent polymer molecules.

Lipoprotein integration and testing:

- Sonication is used to agitate Carmustine and load it into cholesterol lipoproteins.
- The hydrophilic interactions between the lipoproteins and the gel will stabilize the Carmustine and allow for their release from the gel as it degrades.
- The hydrogel is put into a saline solution, imitating the GBM environment to test the lipoprotein release kinetics.
- UV-Vis spectroscopy will be used to determine lipoprotein concentration under different conditions

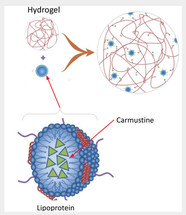

Figure 3. Schematic of hydrogel construct loaded with lipoprotein-encapsulated carmustine.

Thermo-mechanical analyzer (TMA) Methodology:

1. **Prepare Sample:** Cut gels into thin sheets then punch out cylindrical sample. Sample tested had height and diameter of 1.6mm.
2. **Load Sample:** Place sample into aluminum crucible and hydrate with DI water.
3. **Set Temperature:** Set TMA module to 20 ˚C (68 ˚F)
4. **Run Sample:** Place probe on sample, take measurements for 15 min.

UV-Vis Spectroscopy Methodology:

1. **Prepare Sample:** Fill 25 mL cuvette with 15 mL of distilled water. Cut and weigh approximately 2 grams of the hydrogel. Place hydrogel into water beaker and mix well to create the hydrogel sample.
2. **Load Sample:** Blank the UV spectrophotometer with distilled water. Place approximately 2 mL of hydrogel sample into cuvette.
3. **Collect data:** Measure and record the absorbance of each sample in 10 minute intervals after immersion in distilled water for 1 Hr total..

Data Collection and Analysis

Hydrogel Gross Characteristics

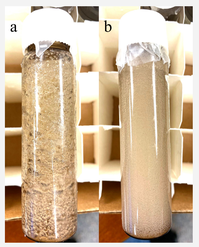

Figure 4a. PVP hydrogel with a molecular weight of 1300 kDa. **Figure 4b.** PVP hydrogel with a molecular weight of 360 kDa.

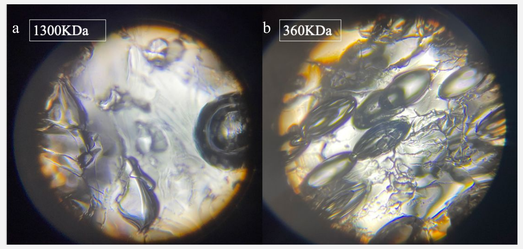

Figure 5a. PVP hydrogel under 40x magnification. Hydrogel with molecular weight of 1300 kDa possesses large round bubbles that are sparsely and unevenly distributed. This gel fragments easily. **Figure 5b.** 360 kDa hydrogel possesses elliptic bubbles of mostly uniform size, distribution and shape. Bubble density is higher in 360 kDa.

The material properties discussed are influenced by the molecular weight of PVP and radiation dose administered to the PVP to cause crosslinking of the gel. We predict that a higher molecular weight and longer irradiation dose will result in a denser, less elastic, and less porous gel resulting in slower drug escape.

Confirmation of Hydrogel Molecular Structure with Fourier-Transform Infrared Spectroscopy (FTIR)

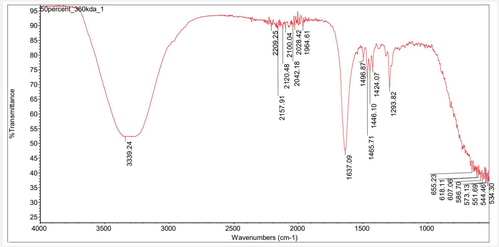

Figure 6. FTIR spectroscopic analysis of hydrogel sample (360 kDa) after 20 kGy irradiation at dose rate 120 Hz.

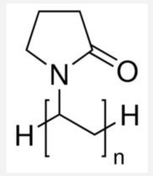

Figure 7. Molecular structure of polyvinyl pyrrolidone monomer.

FTIR spectroscopy shows evidence of the following functional groups confirming presence of polyvinyl pyrrolidone (PVP) in irradiated hydrogel sample: OH peak at ~3339 cm^{-1}, carbonyl peak at ~1637 cm^{-1}, and alkyl chain C-H vibrations in fingerprint region. Therefore, the irradiated hydrogel is of pure PVP composition.

TMA Data Analysis of Hydrogel Swelling Ratio

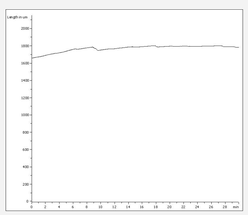

Figure 8. Swelling of hydrogel with a height and diameter of 1.6mm using TMA.

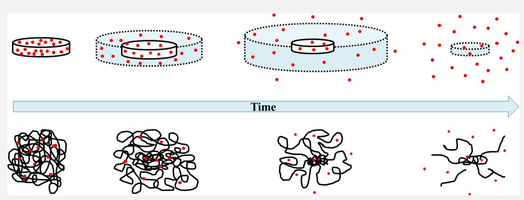

Figure 9. Swelling of a hydrogel network caused by a stimulus leading to the release of the encapsulated drugs over a duration of time. Figure reproduced from Caccavo et al., 2016.

Swelling Ratio Calculation
q_s = (Vf/Vo) = (Hf/Ho)^3
q_s = (1798.51µm/1654.5µm)^3

The hydrogel has swelling capabilities with a swelling ratio of **1.28022.**

As swelling ratio of hydrogels may affect drug delivery rate, it will be necessary find the optimal swelling ratio for our drug delivery system. The higher the swelling ratio, the more it can swell under the experimental conditions. A higher swelling ratio also correlates with higher rates of drug release. Future testing will involve running swelling experiments with multiple hydrogel samples of different molecular weights and dose rates using TMA. This will allow for the comparison of their swelling ratios.

UV-Vis Spectroscopy for Lipoprotein Retention Time Determination

*The UV-Vis Spectroscopy experiments have yet to be performed, but will be done to ensure that the hydrogels do not degrade with or without lipoproteins & carmustine present. Shown below is example data of a similar UV-Vis Spec Experiment

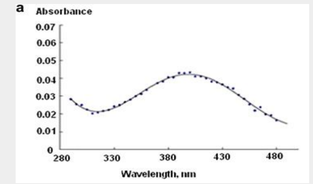

Figure 10a. Example of an absorbance spectrum of a freshly irradiated PVP Hydrogel (taken from al-Sheikhly et al. 2011)

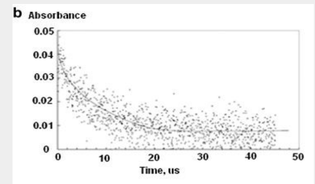

Figure 10b. Example of a decay profile of a transient PVP Hydrogel Species (also taken from al-Sheikhly et al. 2011)

High Performance Affinity Chromatography for Retention Times

*The HPLC experiments have yet to be completed, however these experiments can be used as a tool to characterize mixed mode interactions between the drugs and the lipoproteins. Shown below is the organization of the equipment and a diagram of the HPLC.

Figure 11a. Example of the setup for an HPLC in a laboratory setting

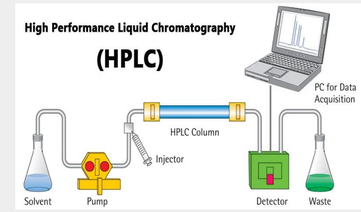

Figure 11b. A detailed diagram of the HPLC and the data acquisition method. Includes a collection beaker for waste from the column.

Future Research Goals

- Lipoprotein retention time and column experiments
- Calculate binding affinity of lipoprotein with hydrogel and drug
- Modeling to find swelling ratios
- Further experimentation to obtain more precise results
- Analyze data from characterization and Lipoprotein experiments
- Observe reactions with the drug and the hydrogel
- Observe reactions between drug and lipoproteins

Acknowledgements and References

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