Evaluating Physicochemical Properties of Poly-vinyl Pyrrolidone (PVP) Hydrogels for Local Delivery of Lipoproteins in Glioblastoma Multiforme (GBM)

Team NANO: Novel Advancements in Neuro-Oncology

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Abstract

Glioblastoma multiforme (GBM) is a fast growing, malignant tumor that develops from the glial cells that support the health of the nerve cells that affect the brain and spine. GBM has a low survival rate with minimal drugs/treatment options available. The purpose of our research was to seek potential innovative solutions to the challenges surrounding the treatment of GBM; particularly, we were interested in exploring a novel drug-delivery treatment that could be used in future GBM therapy. Through the use of Poly-vinyl Pyrrolidone (PVP), we aimed to develop a hydrogel with specific biomechanical properties that would contain lipoprotein-encased chemotherapeutic drugs; the idea being that these hydrogels would provide an ideal sustained released over a set amount of time. Using varying polymer concentration, molecular weights, and radiation doses, we tested the physical and chemical properties while simultaneously testing the kinetics of release of lipoproteins in each hydrogel.

CH1: Introduction

This project seeks to address the need for innovative solutions to the challenges surrounding treatment of glioblastoma multiforme (GBM). GBM is a fast-growing, malignant tumor that, like all gliomas, develops from the glial cells that support the health of the nerve cells and affects either the brain or spine. It is the most aggressive of the glioma types and is often very difficult to treat. GBM has an incidence of two to three per 100,000 adults per year, and accounts for 52% of all primary brain tumors and 80% of malignant brain tumors (Rajaratnam et al., 2020). GBM has a low survival rate as less than 5% of patients diagnosed with the tumor survive a length of five years (Tamimi et al., 2017). This research will expand on previous work to discover a cure for GBM by examining a nanomaterial based drug delivery system and improving its efficacy at treating GBM. The treatment will be evaluated in terms of its drug release properties, physical characteristics, and compatibility with the brain. Physical evaluation of synthesized materials will be supplemented with the use of mathematical modeling to better understand and predict the behavior of the nanomaterial.

The current standard of care for GBM involves chemotherapy, radiotherapy, and surgical resection. Common anticancer drugs used to treat GBM are Temozolomide (TMZ), Bevcizumab, and Carmustine. These chemotherapy drugs can be used in combination with other drugs and/or in addition to other forms of treatment (Lowe et. al, 2019). It is challenging to treat GBM as chemotherapy drugs have a difficult time crossing the blood brain barrier (BBB), a semipermeable membrane that prevents molecules above a certain size from crossing over into the brain (Ramachandran et al., 2017). GBMs likewise present a myriad of challenges to the use of surgical intervention. Tumor cells will widely disperse themselves in the parenchyma, making regrowth after surgery very likely. These cells are heterogeneous in nature and are able to rapidly

evolve in response to a change in their environment (Robertson et. al., 2019). This makes treatment that can be applied post resection of tumor both needed and useful, in order to properly address remnant tumor cells.

1.1 Drug Delivery in GBM

Drug delivery has been a hot topic in science and has been thoroughly researched by scholars, but most drug delivery systems developed for GBM have not been FDA approved due to their low efficacy and unpredictability (Norouzi et al., 2018). Specializing a drug delivery system for GBM therapy would represent a significant step forward for the field of medicine and, more importantly, for patients suffering from this disease.

Recently, a new drug delivery system has been introduced for the treatment of newly diagnosed or recurrent glioblastoma: the Gliadel wafer. This wafer has been FDA-approved for implantation after surgical resection of a GBM tumor so that the continual release of chemotherapeutic agents can suppress the proliferation and recurrence of cancer, thus negating the need for drugs to cross the blood brain barrier (BBB). However, the use of Gliadel is commonly associated with severe side effects, likely due to the lack of biocompatibility between the solid wafer and the extracellular matrix (ECM) of the patient's brain tissue.

In order to address the current challenges in GBM treatment, we were interested in exploring a novel drug-delivery treatment that could be used in future GBM therapy. Therefore, our project seeks to create an alternative to the Gliadel wafer using a hydrogel, a three-dimensional gel network with the unique characteristic ability of absorbing large quantities of water without dissolving. We aimed to develop a hydrogel with specific biomechanical properties that would allow it to be injected as a liquid into a post-surgical resection cavity of a patient with GBM, such that it would solidify into a gel and would slowly

undergo natural surface degradation in order to release its contents into the GBM microenvironment, killing any remaining cancer cells to reduce chance of recurrence. This system would capitalize on available methods for synthesizing injectable hydrogels from polymer solutions and the incorporation of lipoprotein-encased chemotherapeutics into the hydrogel in order to provide ideal sustained release over an extended period of time.

1.2 Research Question

Our project aims to solve the research question: Would a hydrogel loaded with lipoproteins for drug delivery be able to achieve gradual, prolonged release in order to suppress GBM recurrence? Our objectives were split into two categories that were worked on in conjunction with each other. We first synthesized the hydrogels, which are 3D networks of crosslinked poly-vinyl pyrrolidone using varying molecular weights, radiation doses, and polymer concentrations in order to evaluate the effect each had on the hydrogels' physical and chemical properties. The second portion of our project focused on integrating the prepared hydrogels with human plasma lipoproteins that allow for the incorporation of hydrophilic drugs inside them for prospective drug delivery in the local GBM environment. In order to answer the research question, we had to test the kinetics of the release of these lipoproteins from different hydrogel samples over an extended period of time. This can model the ideal slow release of chemotherapeutic agents from the hydrogel in order to induce apoptosis of the GBM cells and kill the cancer cells as they grow in the surgical cavity.

1.3 Experimental Design

As stated, one of the main focuses of our research for this project was the synthesis and characterization of hydrogels as the reservoir for the drug delivery system.

Crosslinking methods for hydrogel synthesis are categorized into physical or chemical. Physical crosslinks are usually caused by intermolecular bonds such as electrostatic interaction or attraction, hydrogen bonding, hydrophobic interactions, or crystallization. However, these intermolecular interactions are generally reversible in nature and are therefore weaker than the covalent bonds between linear polymeric chains formed through chemical crosslinking (Hu et al., 2019). Classic methods of chemical synthesis of hydrogels require crosslinking agents to catalyze these inter-chain branching reactions, but these agents are usually toxic compounds that must be carefully removed from the final product before implantation in subjects (An, 2007). Alternatively, advancements have been made in free-radical polymerization, where an initiator species produces active carbon radical centers among the polymer chains through homolytic dissociation of weak bonds or redox reactions. The initial radical is highly reactive and propagates a chain reaction of connecting non-radical carbons to reactive chain polymers in both intra- and inter-molecular fashions, eventually terminating when it produces a branched network of the hydrophilic polymers that can maintain a 3D structure while inside solution. Intermolecular cross-linking increases the average size and molecular weight of starting polymer chains, while intramolecular cross-links allow for retention of the molecular weight with reduced dimension (An, 2007).

Control of free-radical polymerization is possible through the use of light or ionizing radiation. This is dependent on the ability of the method to generate radicals in the form of small clusters or spurs while traversing a medium by the homolytic dissociation of weak bonds or by redox reactions (Ranganathan, 2018). Photopolymerization involves the use of a photoinitiator that is activated by a specific wavelength of light to initiate polymerization to form crosslinked hydrogels. High energy irradiation is another method used to synthesize hydrogels due to their

ability to ionize simple molecules, hence their categorization as ionizing radiation. These radiations include electron beams and gamma rays, both of which have been previously studied and verified in the use of hydrogel synthesis (Ranganathan, 2018). We chose to use electron beam irradiation, which is less powerful than gamma rays but reduces cost of production, which is an important design specification for our intentions of bringing this technology into easily accessible use throughout clinic settings.

We had the goal of optimizing the specific polymer as well as the concentration and molecular weight of that polymer to achieve successful integration of lipoproteins and gradual degradation in biological conditions (i.e. within the body). The chemotherapy drug would be encapsulated within lipoproteins, which have demonstrated better biocompatibility for effective drug delivery, and those lipoproteins would be physically entangled within the hydrogel polymer chains in order to form an entire injectable or moldable system that could be implanted in a cavity of the brain where a GBM has been removed.

The chosen polymer to design the hydrogel out of was polyvinylpyrrolidone (PVP). PVP has been used in clinical applications due to its solubility in water and generally inert properties which makes it have no toxicity effects when implanted in the body and allows it to be naturally dissolved over time. In addition, PVP is an incredibly versatile chemical in terms of the ability to tune its physical and chemical characteristics for different applications (Kurakula & Rao, 2020). One of these characteristics of particular interest to us is PVP's amphiphilic nature, meaning that it would accommodate both hydrophobic lipoproteins as well as the hydrophilic conditions within the GBM microenvironment. Such specifications made this polymer a very attractive option for our research purposes (Figure 1.3.1).

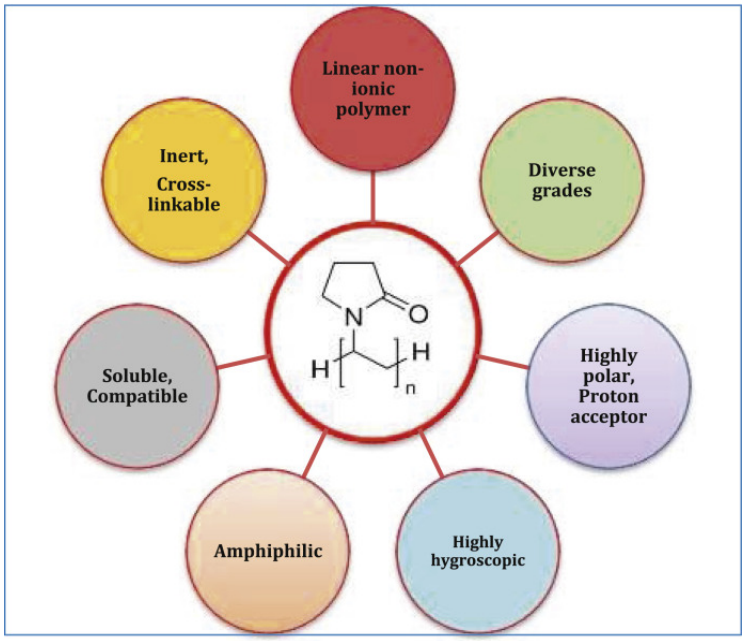

Figure 1.3.1. Polyvinylpyrrolidone properties. Figure reproduced from Kurakula et al., 2020.

Although PVP-based hydrogels are well-studied for many scaffolding and tissue engineering applications (Kuźmińska et al., 2020), we have identified a gap in research on the specific mechanisms behind changing the mechanical and physical properties of PVP hydrogels. This was a key component of our research design because we wanted to optimize the parameters with which we synthesized our hydrogels in order to create the hydrogel with the most sustained, prolonged, and effective drug release over time. Therefore, our initial experiments revolved mostly around investigating the effects of varying different aspects of hydrogel synthesis, including the dose rate of the electron-beam irradiation for PVP crosslinking to form the gel, the molecular weight of the PVP used, and the amount of PVP used measured by weight % in aqueous solution.

Next, we would evaluate the parameters of these varied hydrogels in functional studies, by incorporating low density lipoproteins (LDL) from human plasma, the drug carrier in the

system, and performing kinetic analyses of their release over time from the hydrogel. By creating numerous samples of PVP hydrogels with these altered synthesis methodologies, we were then able to perform characterization of the resulting gels under conditions mimicking those of the GBM microenvironment to determine what the best form of hydrogel would be for our overall drug delivery system. The characterization methods used were:

1. Fourier-Transform Infrared Spectroscopy (FTIR) to confirm the composition of our PVP hydrogels synthesized using electron-beam irradiation,
2. Thermo-Mechanical Analysis (TMA) to determine the crosslinking density and therefore the relative strength of the PVP hydrogels, and
3. Ultraviolet-Visible (UV-Vis) Spectroscopy to study the time-dependent degradation of the PVP hydrogels as well as the release of integrated lipoproteins under biological conditions.

CH2: Literature Review

2.1 Drug Delivery in the GBM Microenvironment using a Combinatorial Method of Hydrogel-Carried Lipoproteins

Drug delivery in the glioblastoma multiforme (GBM) microenvironment can be challenging due to the presence of a dense extracellular matrix and blood- brain barrier, which limit the transport of drugs into the tumor.

Low density Lipoproteins, or LDLs, are particles made up of fats and proteins. They are taken in by cells exhibiting LDL receptors. In the body, lipoproteins are used to transport cholesterol through the bloodstream to cells. They are different from the other cholesterol-carriers, high density lipoproteins, or HDLs, which carry cholesterol back to the liver for disposal as bile salts and acids. Given that they are biocompatible and they also have the ability to bind to specific receptors, they are opportune for drug delivery as they would both be compatible with the patient's internal environment as well as allow for the carrying of both hydrophobic or hydrophilic drugs. In addition, LDLs are metabolized faster by tumor cells than normal cells because of the increased need of the former for cholesterol in building new membranes, leading to LDL accretion in tumor cells (Grimes, 2016). That is why we chose LDLs to use in our development of a drug delivery construct for GBM as an optimal drug carrier for the tumor microenvironment.

Hydrogels also provide many solutions to these problems as a result of their mechanically strong but elastic characteristics that can be implanted in the cavity of a surgically resected GBM tumor such that they increase the bioavailability of chemotherapeutic or other anti-cancer drugs and gradually release them over a long period of time without causing side effects of toxicity to

healthy areas of the brain. Therefore, a combinatorial method of hydrogel-carried lipoproteins can be used to overcome barriers and improve drug delivery in the GBM microenvironment.

Hydrogels are hydrophilic, three-dimensional networks of crosslinked polymers that can be used as drug carriers. The use of these hydrogels as drug delivery systems is especially promising due to their ability to support continual drug release (Hu et al., 2019). The mechanical and biochemical properties of hydrogels are highly adjustable depending on the crosslinking of the individual polymer strands that produce the sol-to-gel phase transition from solution to structural network.

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intramolecular cross-links allow for retention of the molecular weight with reduced dimension (An, 2007).

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Furthermore, by incorporating lipoproteins into hydrogels, the resulting hydrogel-lipoprotein hybrid can mimic the structure and function of low-density lipoproteins (LDLs), which naturally transport drugs into cells by binding to a specific receptor on the cell surface and delivering its contents via receptor-mediated endocytosis (Low Density Lipoprotein, *ThermoFisher Scientific*).

This combinatorial approach has several advantages for drug delivery in the GBM microenvironment. First, hydrogels can protect the encapsulated drugs from degradation and improve their stability. Second, lipoproteins can bind to specific receptors on the tumor cells, facilitating the uptake of drugs into the tumor. Finally, the combination of hydrogels and lipoproteins can increase the size and stability of the amount of drug delivered to the tumor cells and prevent its growth.

2.2 Radiolysis of Water Through Electron Beam Irradiation for Hydrogel Synthesis

The generation of a 3-dimensional hydrogel network necessitates the forming of both intermolecular and intramolecular cross-links between individual polymer strands of PVP. This will occur through radical recombination using electron beam irradiation, where intermolecular covalent bonds form due to radical recombination happening between adjacent polymeric chains and intramolecular bonds out of recombination within a single chain.

Samples composed of different concentrations of PVP and sterile water are bombarded with high-energy electrons. This method is commonly used for sterilization of materials as well as strengthening of polymers. The number of intermolecular and intramolecular cross-links can be predicted and therefore controlled depending on the concentration of polymer and dosage rate of ionizing radiation, where at high concentration and low dose rate, there is a low probability of radicals existing simultaneously on a single polymer chain and intermolecular cross-linking will dominate the reaction, and vice versa (An, 2007). The control of the hydrogel synthesis is a critical part of the development of our drug delivery system for glioblastoma multiforme (GBM) because it will dictate the biocompatibility properties as well as the drug-release kinetics of the hydrogel implant.

Electrons fired at PVP samples lead to water radiolysis, or the formation of OH radicals (Figure 2.2.1). The highly reactive OH radicals are then able to spontaneously react with the backbone of the PVP polymer, creating carbon-centered free radicals and allowing intermolecular cross-linking between adjacent polymer molecules. This chain mechanism proceeds through the monomers such that they become radicalized and attach to other monomers for polymerization, eventually undergoing a solvent to gel (sol-to-gel) transformation characteristic for the hydrogel. The process of polymerization is terminated when two free

radicals form a covalent bond. In addition, purging the hydrogel solutions with nitrous oxide before irradiation allows for the OH yield to initiate, resulting in a doubling of the total radicals generated, following the chemical equation given below.

$$N_2O + e_{aq}^- \rightarrow N_2O^- \rightarrow N_2 + O^-(\rightarrow OH)$$

The bonds formed between the carbon-centered free radicals and the adjacent polymer molecules allow the resulting hydrogel to be stable even at room temperature for following characterization or experimentation.

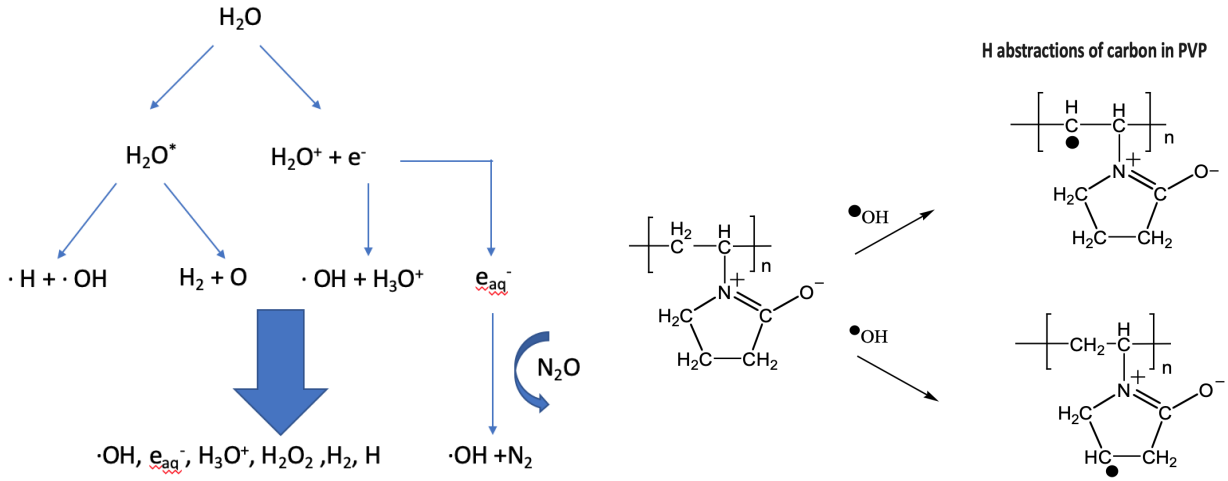


Figure 2.2.1. Radiolysis of water

2.3 Crosslinking Density as a Determining Factor of Hydrogel Fitness for Drug Delivery

In hydrogel synthesis for our experiments, polyvinyl pyrrolidone (PVP) in water solution underwent the formation of free OH radicals, which abstract hydrogen molecules from the polymer chain created carbon-centered free radicals, leading to intermolecular crosslinking. The crosslinking density of hydrogels influences the shape of the hydrogels, therefore affecting the swelling capacity and ratio. Hydrogels with lower crosslinking density have higher amounts of

polymer chains than that of high crosslinking, allowing for a greater expansion of the hydrogel in the presence of water.

Using the swelling ratio, the crosslinking density (v_d) can be calculated. The swelling ratio of a polymer is defined by the equation $q_s = (H_f / H_0)^3$. Using the molar volume of water, which is 18 cm^3/mol, and 0.225+(0.56/q_s) for the Flory polymer-solvent interaction parameter (χ_1), the crosslinking density can be calculated by the following equation: $v_d = (-\ln(1-q_s^{-1}) + q_s^{-1} + \chi_1 q_s^{-2}) / (q_s^{-1/3} - q_s^{-1}/2)$ (Chumakov, 2010).

Another method of measuring crosslinking density is the gravimetric method, in which the mass swelling factor is defined as the following, where m_t is the mass of the swelled hydrogel after equilibrium time is reached and m_0 is the original mass of the dry hydrogel.

$$SF_m = \frac{m_t - m_0}{m_0}.$$

The mass swelling factor can then be converted into the swelling ratio by volume, q_s, using the following equation, where p_p is the density of the dry hydrogel, which in this case is 1.2 g/cm^3, and p_L is the density of the solvent, which in this case is water with a density of 1 g/cm^3 (Sievers et al., 2020) . The q_s can then be used for the crosslinking density formula.

$$SF_V = \frac{V_t}{V_0} = \frac{V_{t,L} + V_0}{V_0} = \frac{\rho_p \cdot m_{t,L}}{\rho_L \cdot m_0} + 1 = \frac{\rho_p}{\rho_L} \cdot \frac{m_{t,L} + m_0 - m_0}{m_0} + 1 = \frac{\rho_p}{\rho_L} \cdot \frac{m_t - m_0}{m_0} + 1 = \frac{\rho_p}{\rho_L} \cdot SF_m + 1.$$

By adjusting the crosslinking density in hydrogels, swelling capacity can be adjusted (Figure 2.3.1). This property of hydrogels can be particularly useful in the use of drug delivery.

In our experiments, we tested several different crosslinking densities that could potentially be used in delivery of carmustine to the area of treatment for glioblastoma.

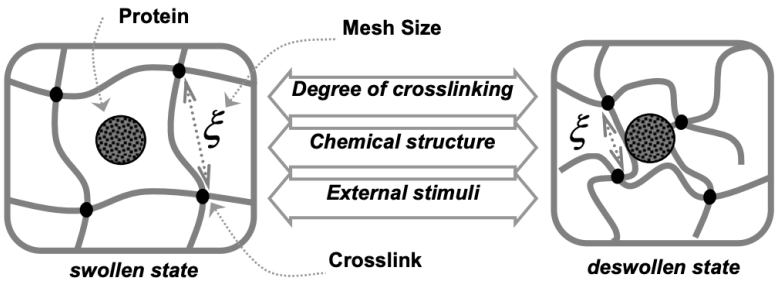


Figure 2.3.1. Schematic of mesh size in hydrogels at swollen or shrunken states in relation to release of loaded compounds (e.g. proteins). Figure reproduced from Lin, 2007.

2.4 Integration of Lipoprotein into PVP Hydrogels

There are three types of drug-network interactions that can be used for controlled drug delivery: covalent conjunction, electrostatic interactions, and hydrophobic association (Li & Mooney, 2016). In covalent conjunction, there is a covalent link between polymer and drug. A covalent link is a chemical link that is formed when two atoms share electrons. Under covalent conjunction there are two types of covalent linkages, a highly stable covalent linkage and a cleavable covalent linkage (Li & Mooney, 2016). A highly stable covalent linkage is able to hold and immobilize the drug within the polymer network until the network degrades (Li & Mooney, 2016). A cleavable covalent linkage is designed to cleave the link between the drug and the network over a period of time or in response to environmental cues including enzyme activity and hydrolysis (Li & Mooney, 2016). Another drug-network interaction is electrostatic interaction; electrostatic interaction occurs between drugs and polymers that carry charges (Li & Mooney, 2016). In this drug-polymer interaction, negatively charged polymer chains interact with positively charged drugs. In electrostatic interaction, drug release occurs when the polymer

network degrades or mobile ions cause a shielding effect (Li & Mooney, 2016). The third type of drug-network interaction is hydrophobic association. Hydrophobic association occurs when the hydrophilic hydrogel network is made to contain hydrophobic polymers to serve as binding sites for hydrophobic drugs.

Lipoproteins can be added to the hydrogel network for drug delivery as they can be used to carry and transport chemotherapeutic drugs. The outside region of the lipoproteins is hydrophilic and the inner region is hydrophobic; these characteristics allow it to be a suitable transporter for the anticancer drugs in the PVP hydrogel. The drug will remain stable within the lipoprotein and as the hydrogel degrades the lipoproteins will diffuse out with the drug. The encapsulation of chemotherapy drugs inside the nanocarriers is outside the scope of our drug delivery design but the results can be used for future work for cancer treatments.

Lipoproteins are added to the gel solution prior to the irradiation process; once the gel is irradiated with the electron beam the drug carriers will be integrated into the gel network. The concentration of the lipoproteins in the hydrogel will depend on the dose needed to be administered to the tumor region. The hydrogel size will depend on the tumor environment and the amount of nanoparticles it needs to contain in its network. The lipoprotein release kinetics will depend on the crosslinking density of the hydrogel (Yi et al., 2020).

CH3: Methodology

3.1 Hydrogel Synthesis

Hydrogels were made of varying weight % in water out of both 360 kDa and 1300 kDa polyvinylpyrrolidone (PVP). The calculated amount of PVP powder was weighed out and added to 40 mL of ddH_2O. Solutions were stirred overnight at 25 ˚C before being aliquoted into glass 40 mL vials with needle-perforated lids.

Following preparation of the polymer solution and preceding the gel formation step, the solution was bubbled through with nitrous oxide (N_2O) in order to start the conversion process of hydrated electrons in solution to hydroxyl radicals. The hydrogel samples were purged for irradiation in small 7 mL sample vials (Sigma-Aldrich) fitted with septum caps that allow for the purging of oxygen from the sample vial and replacement with N_2O. Samples were purged for 15-20 min at 20 psi.

The electron-beam irradiation of the samples was conducted at the Medical-Industrial Radiation Facility (MIRF) at the National Institute of Standards and Technology (NIST) using an electron accelerator with a pulse width of 6 μs and a pulse repetition of 120 pulses/sec at 10 MeV and room temperature. The dosage level (J/kg=Gy), or energy absorbed per mass of target material, is highly unpredictable but easy to modify since allowing for the electron beam machine to run for shorter or longer times will modify dose.

3.2 Confirmation of Hydrogel Synthesis with FTIR Spectroscopy

Fourier Transform Infrared (FTIR) spectroscopy is an ideal characterization technique to analyze changes to the molecular structure undergone during the polymerization process of PVP hydrogel synthesis. IR takes advantage of the molecular absorption at specific wavelengths

corresponding to vibrational transitions, including symmetrical and anti-symmetrical stretching, rocking, wagging, and scissoring of the bond between two atoms (Chumakov, 2010). Expected species of interest identifiable in PVP are described in the table below (reproduced from Ammar et al., 2020).

Table 1 Principal vibration bands observed by FTIR

Sample	λ (cm^{-1})	vibration type
PVP	1279	C–N: bending vibration of amide
	1439	C–N: stretching vibration of amide
	1650	C=O: stretching vibration of amide
	2880	C–H: symmetric stretching vibration
	2980	C–H: asymmetric stretching vibration
	3550	O–H: stretching vibration of humidity

3.3 Characterization of Crosslinking Density with TMA

The thermomechanical analyzer (TMA) was used in this experiment to determine the mechanical properties and crosslinking density of the PVP hydrogels. To begin, a sharp blade was used to cut a rectangular prism of hydrogel measuring around 5 mm x 2 mm. The final hydrogel sample was weighed for consistency.

Adapting from ASTM Standard F2214, Standard Test Method for In Situ Determination of Network Parameters of Crosslinked Ultra High Molecular Weight Polyethylene (UHMWPE), the samples were swelled in an aluminum crucible in a Mettler Toledo TMA/SDTA841e Thermomechanical Analyzer until equilibrium was reached within 30 minutes in order to calculate for swelling ratio, crosslink density, molecular weight between crosslinks, and number of repeat units between crosslinks.

To load the sample onto the TMA, the hydrogel was transferred onto an aluminum crucible pan. The crucible was placed on the measurement platform of the TMA module, and the

glass measurement probe was gently rested on top of the hydrogel cylinder. At this point, enough distilled water was added to the measuring crucible so that the hydrogel was completely submerged. The crucible was placed on the platform of the TMA and the quartz probe was rested directly on the hydrogel sample. A schematic of how the initial and final height due to swelling is measured by the TMA module as well as a picture of the probe of the device is shown below.

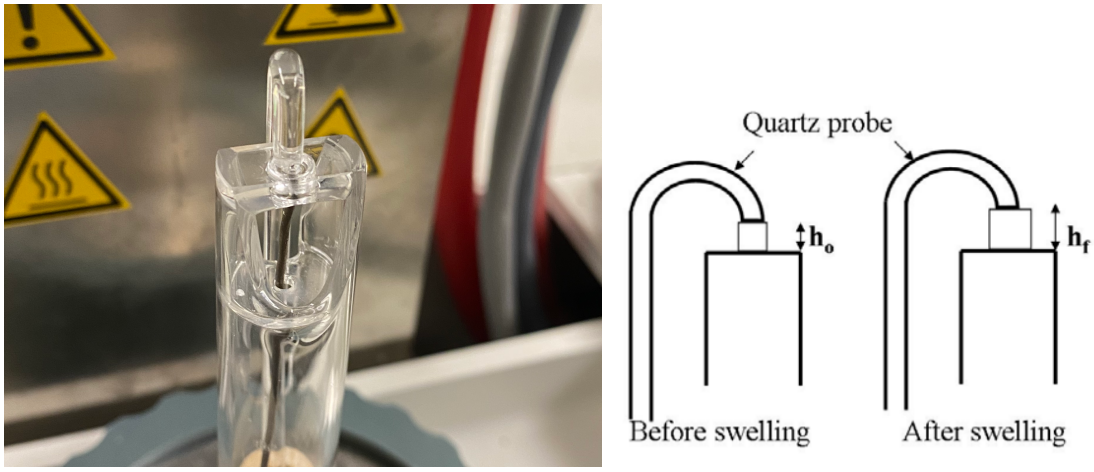


Figure 3.3.1. A photograph of the quartz probe on the TMA machine (a) and a schematic of specimen height as measured by the device (b).

Once the module finished calibration, the samples were run on the program such that the TMA furnace heats from 20 ˚C to 65 ˚C, with a heating rate of 10 ˚C per minute. The samples ran for 30 minutes to measure the displacement of the glass probe caused by swelling of the hydrogel over time. The displacement curve was qualitatively analyzed for the important characteristics of an initial steep incline and a gradual decrease in slope that levels off to a flat plateau by the end of the 30 minute run. The displacement caused by hydrogel swelling determined the volume swelling ratio, which was then converted into crosslinking density and other properties using the formulas discussed in section 2.3.

3.4 Characterization of Crosslinking Density by Gravimetric Method

Furthermore, for samples that were not measured by TMA, the crosslinking density was calculated based on mass swelling ratio by the gravimetric method. First, a 5 mm x 2 mm section of hydrogel was cut and weighed on a weighing boat. Next, ddH_2O was added to cover the hydrogel sample in the weighing boat and the sample was left at room temperature for 30 minutes while waiting for equilibrium. After the incubation period, the hydrogel was transferred to a new weighing boat to record its mass after swelling, and the mass swelling factor (SF_m) was determined using the formula in section 2.3. The SF_m was then converted into the volume swelling ratio that can be put into the equation that returns the crosslinking density.

3.5 Lipoprotein Integration into Hydrogel

A simple and efficient method for integrating low density lipoproteins (LDLs) into a hydrogel polymer was used to create a functionalized drug-delivery system. For the first round of lipoprotein integration, the 30 wt% PVP and 360kDa molecular weight with a 100kGy dose rate was used. Initially, 5 grams of hydrogel were added to a larger beaker. A graduated cylinder was then filled with 5 mL of water, and 1 uL of LDLs was added using a micropipette. The water-LDL mixture was subsequently poured into the hydrogel in the larger beaker.

To ensure complete mixing, a homogenizer was utilized to mix the hydrogel-LDL solution at 1200 rpm for 5 minutes. The homogenizer allowed for efficient and uniform mixing of the hydrogels and LDLs. Once mixed, the solution was packed into a smaller beaker and refrigerated to allow the hydrogel to set.

3.6 Release Kinetics of Integrated Lipoproteins Measured by UV-Vis spectroscopy

UltraViolet-Visible (UV-Vis) spectroscopy was used to find a range of concentrations of water soluble lipoproteins, measure their absorbance on the UV light spectrum and experimentally determine the molar extinction, ε. A calibration curve was first completed to determine the molar extinction coefficient for lipoproteins in order to later find the optimal concentrations of lipoproteins that can be used with the hydrogel.

The calibration curve began by performing five dilutions of the lipoprotein 0.00656 mg/uL stock solution with ddH_2O. Starting with 1 uL of the stock solution in 10 mL of ddH_2O, adding 2 uL of stock lipoprotein consecutively to create increased concentrations of five/six samples. Each sample concentration was then transferred into a silica cuvette using a glass pipette and the absorbance at the wavelength range of 200 nm to 300 nm was measured using the the UV-Vis spectrophotometer. The molar extinction coefficient of the lipoproteins was determined using the Beer-Lambert Law: $A = \varepsilon bc$. The calibration curve experiment that repeated 2 more times to accurately obtain a precise molar extinction coefficient.

UV-Vis spectroscopy was then used to determine the retention rate of the lipoprotein with the hydrogel in vitro. Immediately after homogenizing, the water solution was transferred into a silica cuvette using a glass pipette for absorbance measurement with the same wavelengths mentioned above with the UV-Vis Spectrophotometer. This was then repeated consecutively in increments of 10 minutes, 4 times, for a total of 5 measurements for the first trial and 7 times, for a total of 8 measurements for the second trial. We then used the calibration curve that was experimentally determined before to calculate the concentrations of lipoprotein that was released during each 30 minute interval to find the release kinetics of the lipoprotein from the hydrogel.

CH4: Results

4.1 Hydrogel Synthesis

The parameters varied in polymer preparation as well as electron-beam irradiation are summarized in the table below.

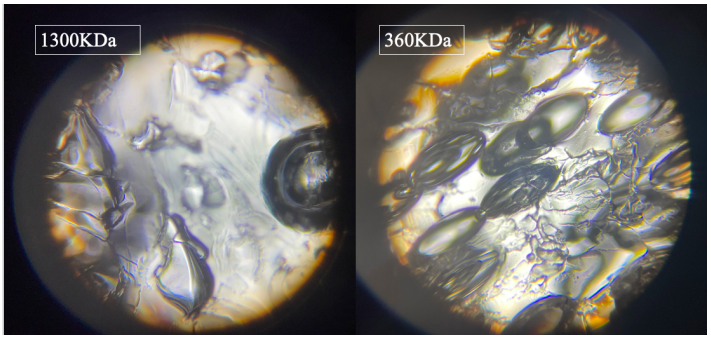
Table 4.1.1. Hydrogel sample synthesized and irradiated following procedures. n = 3 for all samples.

PVP Hydrogel Samples Synthesized		
Molecular Weight (kDa)	wt% PVP	Irradiation Dose (kGy)
360	25	50
360	30	50
360	30	100
360	30	150
360	45	50
360	50	50
1300	25	50
1300	45	50
1300	50	50
1300	30	50
1300	30	100
1300	30	150
1300	45	50
1300	50	50

Different molecular weights of polyvinyl pyrrolidone displayed different characteristics after samples were irradiated. Samples of 1300 KDa displayed larger pockets of air within the hydrogel while samples of 360 KDa had a more consistent pattern throughout. Microscopic

views at 40x were able to capture the different consistencies of the different molecular weight samples (Figure 4.1.1).

(a)



(b)

Figure 4.1.1. Representative images (a) and microscopic view at 40x (b) of hydrogels, 1300 kDa PVP on the left and 360 kDa PVP on the right at 50 wt% and an irradiation dose of 50 kGy.

To ensure polymerization of polyvinyl pyrrolidone after electron-beam irradiation, Fourier Transform Infrared (FTIR) spectroscopy was conducted to characterize the hydrogel samples. We were only able to conduct one round of FTIR that was done after the sample of 50% wt, 360 kDa PVP was irradiated at 50 kGy. As expected a large peak occurred around 3339 cm^{-1}

showcasing O-H stretching due to the presence of water. Along with additional peaks around 1446 cm^{-1}, and 1637 cm^{-1} showcasing C-N and C=O stretching, respectfully (Figure 4.1.2). Since we did not collect the data before irradiation to compare it to, this data can only serve as a proof-of-concept for our methodology of identifying certain bonds in PVP using FTIR. Therefore, in any future experimentation, we would look to collect comparison data before and after irradiation of the samples, specifically to identify if there are any changes to the molecular structure caused by crosslinking that can be seen through FTIR spectra, and if there are any impurities present in our samples.

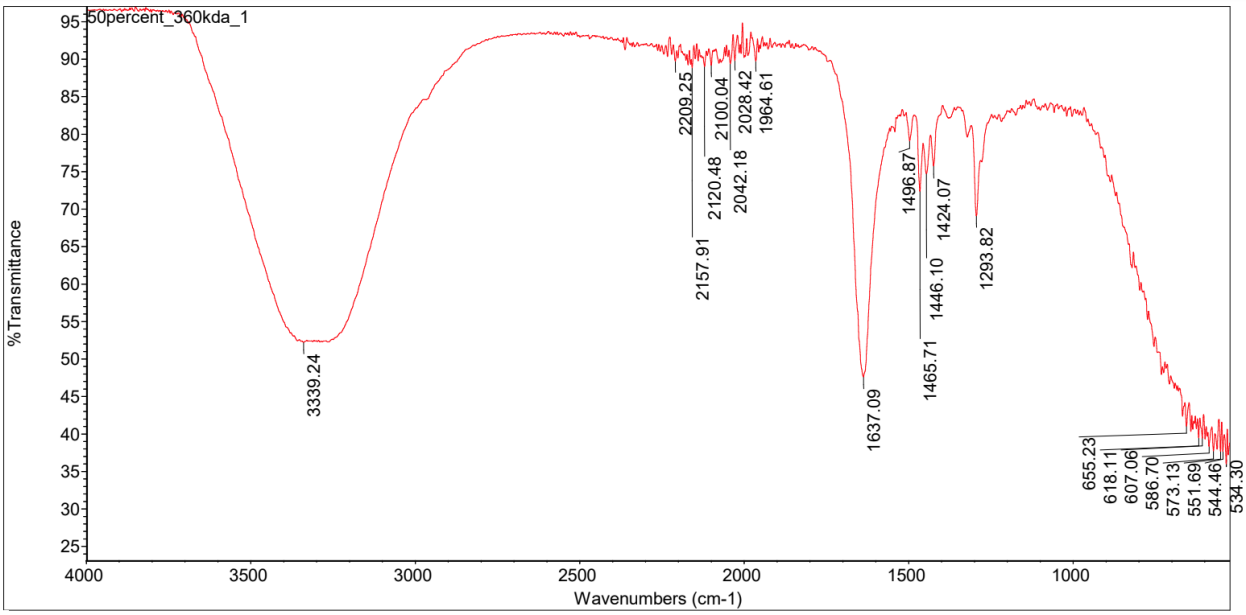


Figure 4.1.2. Representative FTIR spectrum from 50% PVP at 360 kDa and 50 kGy dose.

4.2 Hydrogel Characterization

The effect of molecular weight (MW) was first evaluated by taking swelling ratio measurements using the TMA method on hydrogels made of both 1300 kDa and 360 kDa PVP at 50 wt% and with an irradiation dose of 50 kGy. 5 samples of each were tested and the results are given below (Figure 4.2.1).

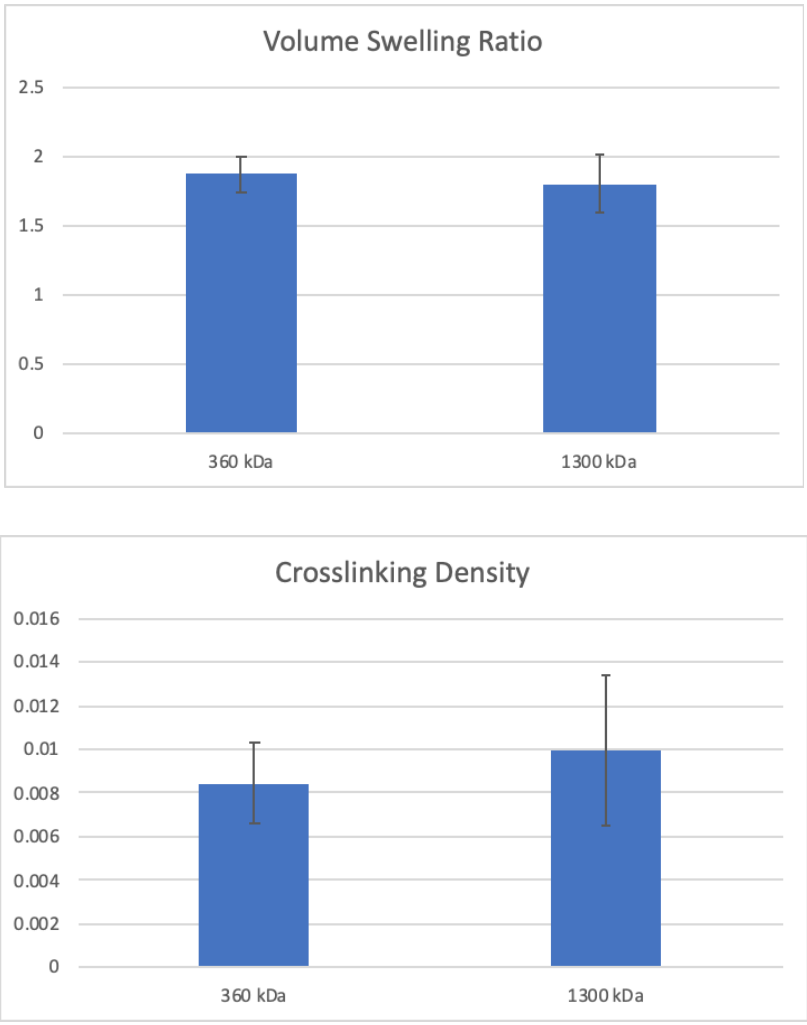


Figure 4.2.1. Swelling ratio and crosslinking density by TMA method for 360 kDa and 1300 kDa hydrogels at 50 wt% PVP and 50 kGy (n=5). Graph shows average +/- standard deviation (SD).

Two-tailed unpaired t-test on swelling ratios resulted in a p-value of 0.484, which was greater than the alpha-level of 0.05. Therefore, these results failed to substantiate any significant difference between swelling ratio as a function of MW. Similarly, crosslinking densities were not significantly different between the two MWs analyzed, with a p-value of 0.405.

The TMA method was also used as a preliminary method to investigate the effect of concentration of PVP used to synthesize the hydrogel. In this experiment, 3 samples each of 360

kDa PVP hydrogels were synthesized at 30% and 50% weight and irradiated at 100 kGy before being run on the TMA to generate average swelling ratios and crosslinking densities (Figure 4.2.2).

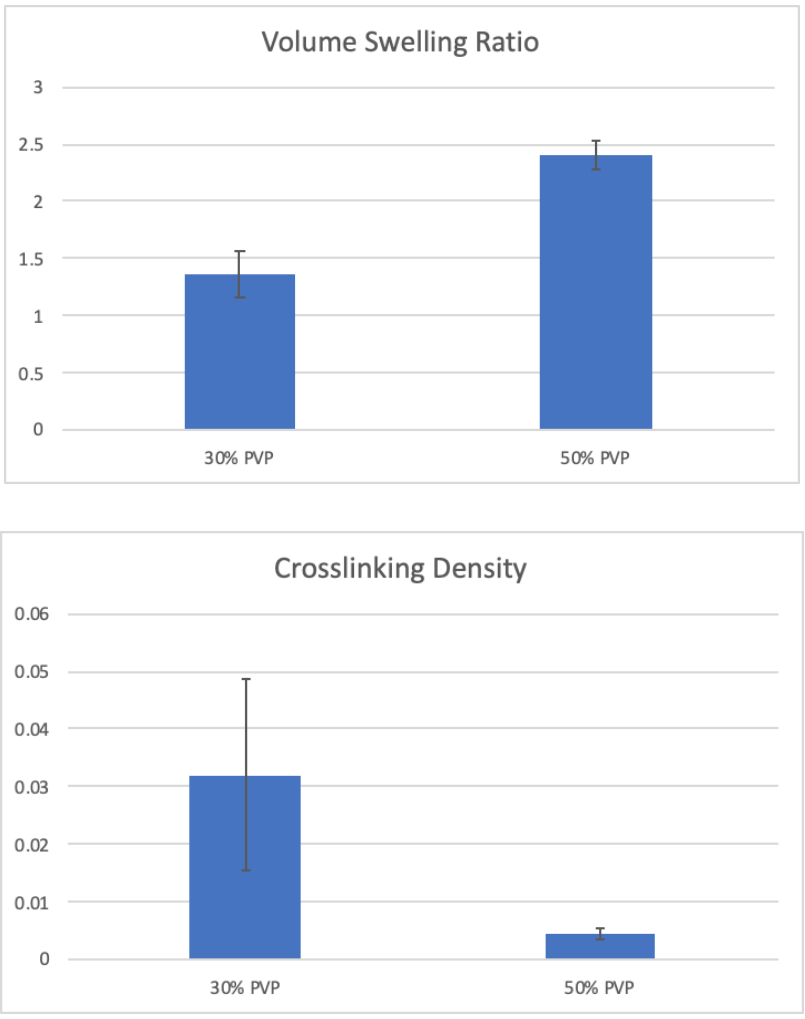
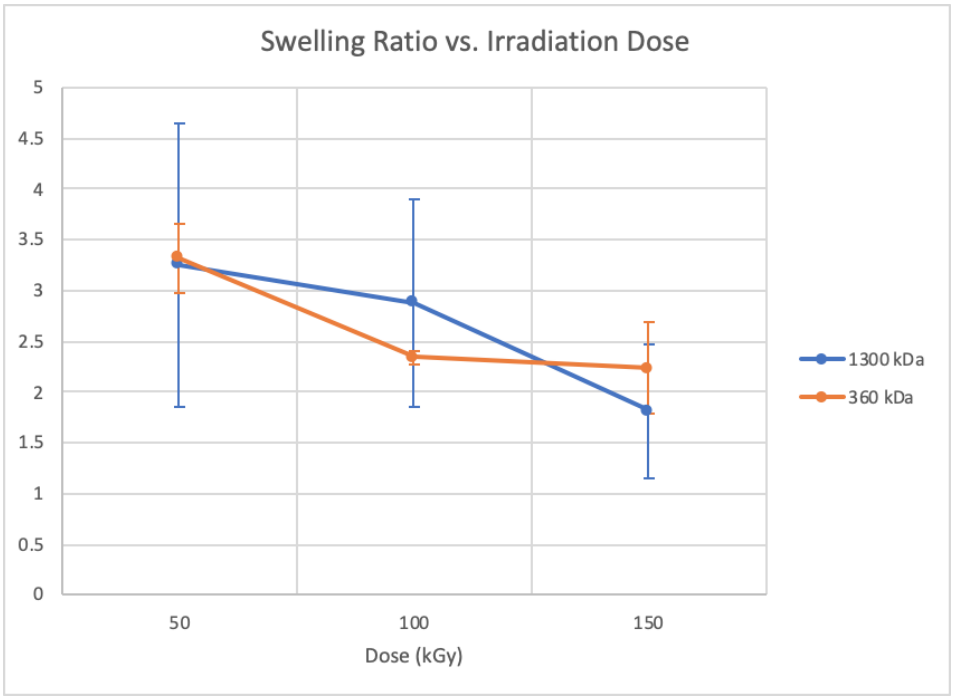


Figure 4.2.2. Swelling ratio and crosslinking density by TMA method for 360 kDa hydrogels at 30% and 50% PVP and 100 kGy (n=3). Graph shows average +/- standard deviation (SD).

The swelling ratios for 30% and 50% wt PVP samples produced a p-value of 0.0353 on the two-tailed unpaired t-test, which was smaller than the alpha-level of 0.05. Therefore, these results suggest a significant difference between the swelling ratios of the two levels of PVP

concentration. The crosslinking densities resulted in a p-value of 0.102, so those results were not statistically significant.

Next, the gravimetric method was used to analyze the effect of irradiation dose on the same parameters. 4 samples each of both the 1300 kDa and 360 kDa molecular weight hydrogels at 30 wt% PVP and varying irradiation doses of 50 kGy, 100 kGy, and 150 kGy were analyzed using the gravimetric method. The average volume swelling ratios and corresponding crosslinking densities were found for each experiment along with the standard deviation (Figure 4.2.3).



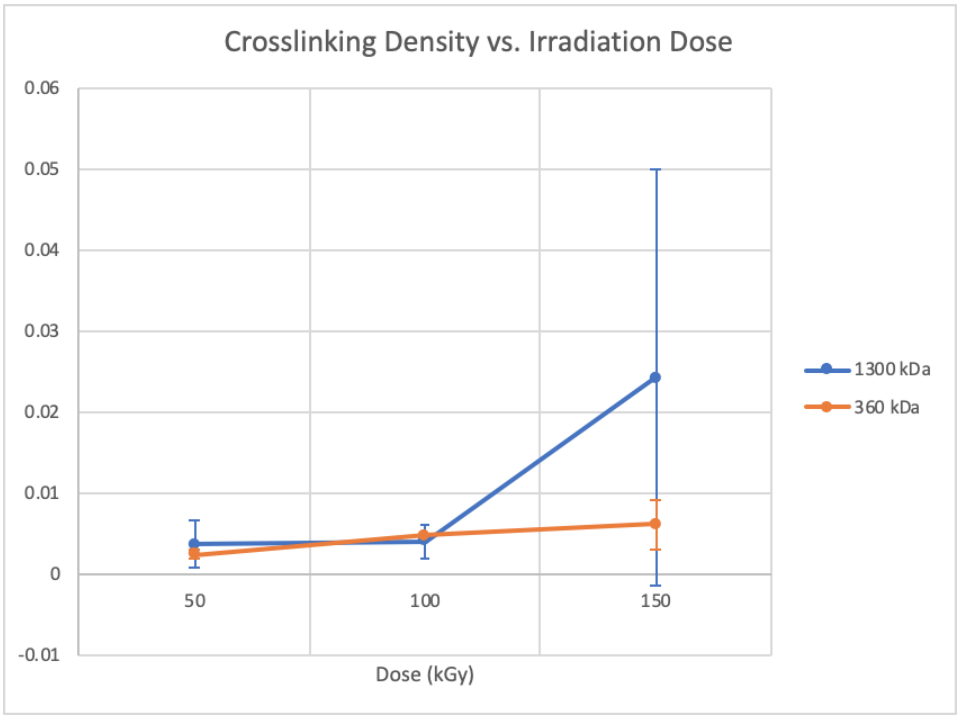


Figure 4.2.3. Swelling ratio and crosslinking density by gravimetric method for 360 kDa and 1300 kDa, 30 wt% PVP at varying doses: 50 kGy, 100 kGy, and 150 kGy (n=4). Graph shows average +/- standard deviation (SD).

One-way ANOVA was used to compare results between irradiation doses for both 360 kDa and 1300 kDa. For 360 kDa, the swelling ratios between groups (50 kGy, 100 kGy, and 150 kGy) were not found to be significantly different at a p-value of 0.199 while the crosslinking densities had a significant difference at a p-value of 0.002. Similarly, for 1300 kDa, p-value for swelling ratios was insignificant at 0.144 while difference in crosslinking densities between groups was statistically significant at a p-value of 0.0461 (See Appendix A).

4.3 Extinction Coefficient of Lipoproteins

A calibration curve was first completed to determine the molar extinction coefficient for lipoproteins in order to later find the optimal concentrations of lipoproteins that can be used with the hydrogel. The lipoprotein solution was measured at increasing concentrations and was found to absorb light at 227 nm and 270 nm. The extinction coefficients were determined at both wavelengths by plotting the absorbance by the lipoprotein concentration. For wavelengths of 227 nm and 270 nm, the extinction coefficients are determined from the rate of change as 88908 $(mg/uL)^{-1}$ cm^{-1} and 23403 $(mg/uL)^{-1}$ cm^{-1} respectively (Figure 4.3.1).

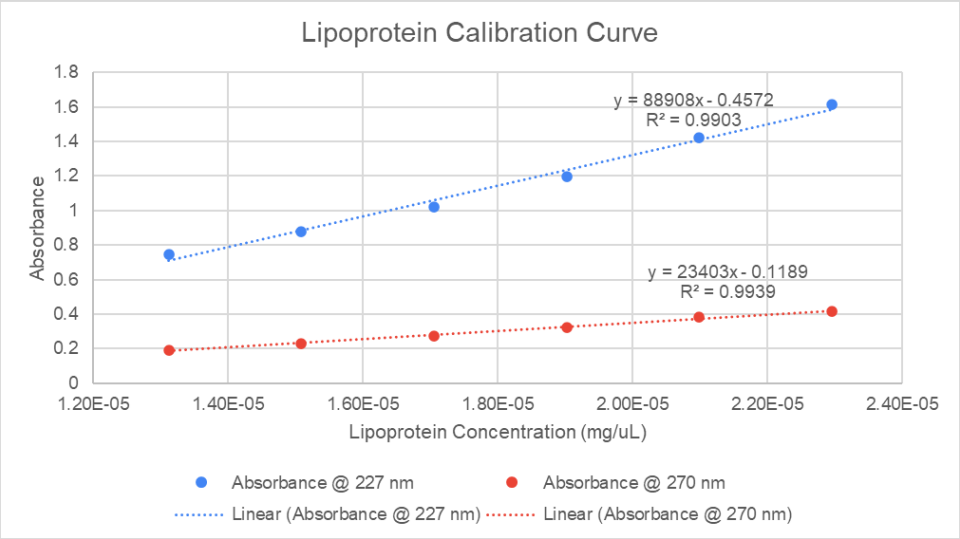


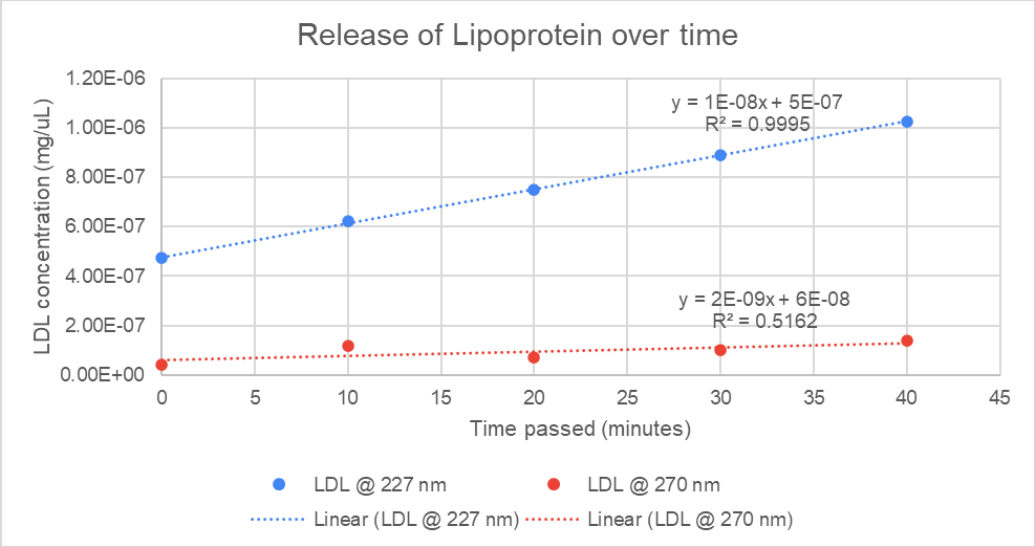
Figure 4.3.1. Calibration curve of 0.00656 mg/uL lipoprotein stock at 227 and 270 nanometers at five varying dilutions. Graph shows trendline and rate of change.

4.4 Kinetic Profile of Lipoprotein Release from Hydrogel

Based on the calculation of the extinction coefficient of lipoprotein from the calibration curve, we were able to characterize the kinetic profile of the lipoprotein. Five measurements of hydrogel-LDL in water were taken every 10 minutes with the UV-Vis spectrometer to determine the amount of lipoprotein released from the hydrogel. Using the Beer-Lambert Law equation: $A=\varepsilon lc$, from the absorbances we measured and the previously determined extinction coefficient, we calculated the concentrations of lipoprotein at each time interval. From there the release rate of the lipoprotein from the hydrogels was calculated.

Based on the absorbances measured at 227 nm, the release rate of the lipoprotein from the hydrogel was $1*10^{-8}$ (mg/uL)*min^{-1} and at 270 nm, the release rate was $2*10^{-9}$ (mg/uL)*min^{-1} (Figure 4.4.1). The experiment was repeated again, the release rates at wavelengths 227 nm and 270 nm were $2*10^{-8}$ (mg/uL)*min^{-1} and $2*10^{-13}$ (mg/uL)*min^{-1} , respectively.

Further data can be found in Appendix B.



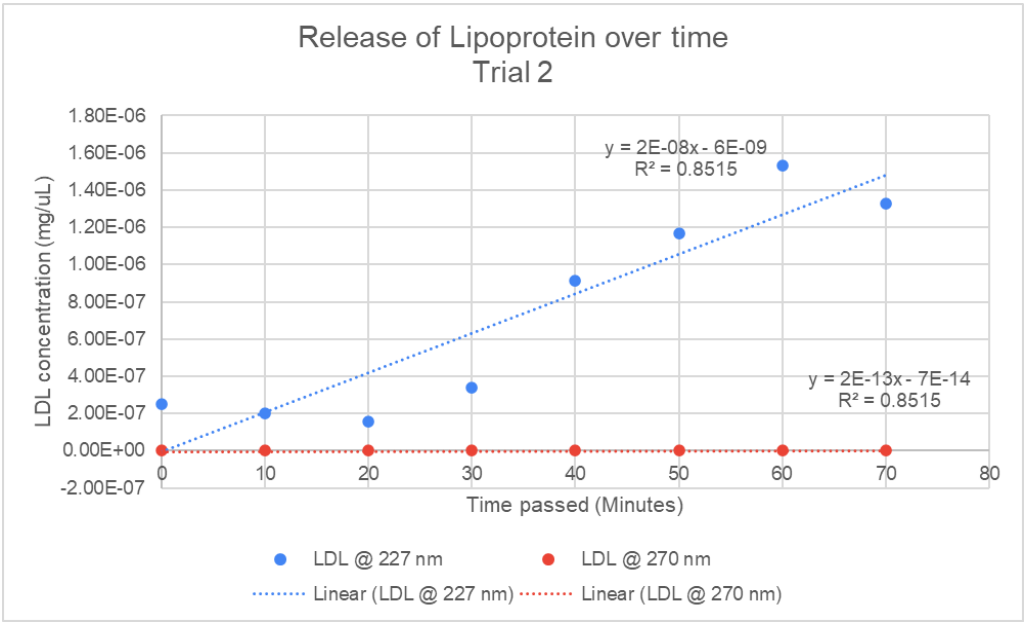


Figure 4.4.1. Graph concentration of lipoprotein vs minutes passed after initial mixing of LDL-hydrogel in water showing the release rate of the lipoprotein from the hydrogel at 227 and 270 nanometers, Graph shows trendline and rate of change.

CH5: Discussion

5.1 Effect of Hydrogel Synthesis Methods on Swelling Ratio & Crosslinking Density Parameters

Based on results in section 4.2, the effects of factors like molecular weight (MW), irradiation dose, and concentration of polyvinylpyrrolidone (PVP) in hydrogel could be evaluated qualitatively and quantitatively. With a higher degree of crosslinking it was expected that the volume swelling ratio would decrease because the hydrogel is less flexible and likely to take up water from the environment, and this can be measured both with TMA and the gravimetric method discussed earlier in this paper.

It should be noted that the difference in crosslinking can also be tactically and visually observed by inspecting the gel sample. Gel samples which were confirmed via TMA swelling measurement to have a higher degree of crosslinking were firmer and crumblier than gels with a lower degree of crosslinking, which exhibited a very sticky texture. The effect of crosslinking on gel textural properties must be considered, as this would inevitably affect processing and drug loading of the gel post-irradiation. The stickiness of gels with a low crosslinking density may pose a barrier to preparing the gel for drug delivery.

In understanding how molecular weight affects crosslinking density, there are two main theories which would result in opposite outcomes if correct. Firstly, a smaller molecular weight signifies a shorter chain of PVP. The smaller molecules are able to migrate faster through aqueous solution. This phenomena is similar to how the speed at which a DNA or protein fragment travels through a gel varies in accordance to size during gel electrophoresis. This migratory capacity theoretically increases the polymer's ability to collide and undergo a crosslinking reaction. The second theory, which our team hypothesized would be the case, is that an increase in molecular weight and thus PVP molecule size would cause an increase in free

radical generation per molecule, and an increased number of sites at which the cross linking reaction could occur. This would result in a higher degree of cross linking in samples with a heavier molecular weight.

Our experiments found no statistically significant data as evidence of the fact that MW affected the swelling ratio or crosslinking density based on the thermomechanical analysis (TMA). Further investigation using the gravimetric method also found no significant difference in crosslinking density between 360 and 1300 kDa PVP. This would indicate that either molecular weight in fact does not have a significant effect on crosslinking activity of the polymer perhaps due to a balance between the two proposed phenomena discussed in the previous paragraph, or the methods used in this investigation were not sensitive enough to detect a difference. Should either scenario be the case, this question of the impact of molecular weight on cross linking density has broad-spanning importance to the use of polymer hydrogels in medicine and other fields of technology and warrants continued investigation.

As stated, the effect of PVP concentration on crosslinking density was also investigated. The TMA experiments demonstrated that an increase in concentration of PVP resulted in significantly increased swelling ratio. From Figure 4.2.2, it can be seen qualitatively that this corresponded in a decrease in crosslinking density from 30% PVP to 50% PVP, although those results were not statistically significant due to the large standard deviation of the 30% PVP sample group. Interestingly, this went against our hypothesis, which was that a higher concentration of PVP would result in more crosslinking due to more probability of polymer radicals coming in contact with each other. A possible explanation for why this is that the concentration of PVP to water was too high for all of the polymer to become crosslinked, and the extra polymer may in fact be hindering the crosslinking process as there is not enough water to

react with it. This explanation should be confirmed or refuted with further experiments in which a lower concentration of PVP, between 5 and 15% is used.

In conjunction with PVP concentration, the impact of irradiation dose on crosslinking density and swelling ratio was also investigated. The gravimetric experiment demonstrated that increasing the irradiation dose led to an increase in crosslinking density and a decrease in swelling ratio doses. The doses that were used were 50, 100, 150 kGy, however these doses may also be too high, similar to the PVP concentration, for the purpose of our experiment. The data that was gathered may be further supported or disproved with lower irradiation doses of 10-25 kGy.

While we are not sure of the mechanism behind the relationship we observed in our data, it brings an interesting view of the synthesis of these hydrogel solutions as to what the optimal concentration of polymer is to generate higher swelling ratios vs. higher crosslinking densities and identifies a gap that can be filled with future research.

However, there were issues with collecting data on the TMA that could have influenced these results. Issues with the probe, the part of the TMA module that comes in contact with the hydrogel sample, slipping off of the sample were documented that disrupted the measurement of the displacement caused by hydrogel swelling. In such cases, the data was not reliable enough to be used. In some measurements, if the probe slipping occurred but not to a larger, more noticeable degree, the TMA results may not accurately represent the total amount of swelling that took place. Additionally, small variations and bumps upon the surface of the gel that the probe rested could have led to probe shifting during swelling as these bumps and irregularities expand. The machine used in these experiments also periodically demonstrated issues with calibrating its point of zero force, raising doubts as to if a shift may have occurred within data

collected due to machine error. Due to these concerns, TMA was not used to collect data on later rounds of synthesized PVP gels with the gravimetric method preferred.

Additionally, upon visual inspection of the PVP samples, which were irradiated in cylindrical vials, it was noticed that in some of the vials the PVP was somewhat stiffer along the outer few millimeters of the sample than within the core. This could indicate that the radiation was not penetrating the sample evenly and could result in a difference in swelling ratio from sample to sample. Thus, the degree of variation between different trials of the same type of hydrogel may not be as precise as hoped.

In addition, these samples were only tested at a specific irradiation dose of 50 kGy, which may affect the maximum efficiency of crosslinking that was possible. If that was the case, then the effect that we expected to see with higher weight % of PVP resulting in higher crosslinking densities would not be as evident.

Due to the issues described earlier with the TMA method, we made the decision after the rounds of TMA testing done on samples with varying MW of 360 kDa and 1300 kDa and concentrations of PVP that a different method should be used to test the swelling ratio of the hydrogels, which is why the gravimetric method was employed to evaluate our final parameter of interest, the effect of electron-beam irradiation dose on its swelling ratio and crosslinking density. As discussed in section 2.3, the crux of this experiment was the relationship established between the mass swelling ratio, which is the direct result of the gravimetric analysis, and the volume swelling ratio, which can be converted mathematically into crosslinking density and can be compared to the result garnered by TMA.

The benefit of this method was the ease of taking measurements, however possible error is introduced due to the protocol of transferring the swollen hydrogel from its original weighing

boat to a dry weighing boat for final measurement of weight. This process could have unintentionally released some of the water that was taken up by the hydrogel, thus affecting the final weight and the mass swelling ratio calculation.

The results of the gravimetric experiments at gradually increasing doses of 50, 100, and 150 kGy were not significantly different in regards to volume swelling ratio, but the one way ANOVA did produce statistical significance in regards to crosslinking density for both 1300 kDa or 360 kDa.

Specifically, looking at Figure 4.2.3 comparing the increase in crosslinking density due to increasing irradiation dosage, it is clear that the 1300 kDa group at 150 kGy resulted in a large amount of variation in results. This makes the data less trustworthy, and that could have been due to the error discussed earlier with the methodology. However, the overall trend still holds for both the 1300 kDa and the 360 kDa hydrogel groups that electron beam dose is positively correlated to crosslinking density. That result was as expected. An increase in irradiation dose means that more electrons will bombard the solution. This will increase the occurrence of the radiolysis of water and more free radicals are generated that can react with the polymer chains to cause the chain reaction of crosslinking.

A result of the crosslinking density of the hydrogel will be the physical textural characteristics of the hydrogel and this will in turn impact how the implant should be handled and placed inside the brain cavity. In our research we found the gels with a higher crosslinking density and thus firmness to be the easiest to work with. A gel implant with these characteristics could be shaped into tablets and placed inside the brain akin to how gliadel wafers are directly placed by surgeons using forceps. Should a more viscous gel texture be used, the method of injection of a set volume out of a syringe would be preferable for controlled placement of the gel.

5.2 Lipoprotein Integration

The goal of trying to integrate LDL (low-density lipoproteins) into hydrogels for drug delivery is to create a drug delivery system that can more efficiently and specifically target cells expressing LDL receptors. LDL is a type of lipoprotein that is naturally taken up by cells that have LDL receptors on their surface. By incorporating drugs into LDL particles, it may be possible to target these drugs to cancer cells, which express LDL receptors to achieve more efficient and targeted drug delivery. In addition, LDL is a biocompatible and biodegradable material, which makes it an attractive candidate for drug delivery. By using LDL as a drug carrier, it may be possible to reduce toxicity and improve the pharmacokinetics of drugs.

Furthermore, by incorporating LDL into hydrogels, it may be possible to create a more biologically relevant environment for drug delivery. Hydrogels can provide sustained drug release and protect drugs from degradation in the body, allowing for the improvement of the therapeutic efficacy of drugs. Overall, the goal of integrating LDL into hydrogels for drug delivery is to create a targeted drug delivery system that can improve efficiency while reducing off-target effects and toxicity.

In the lab, the primary goal was to thoroughly mix lipoproteins within the created hydrogel matrix. Then, to measure the retention time of the lipoproteins when submerged into water using UV VIS spectroscopy. Once the composite material was synthesized and characterized, it could be tested for drug delivery efficacy in vitro and/or in vivo. This involved loading the composite material with a drug and assessing the release kinetics and targeting efficiency. In theory, the retention time would be longer so that drugs can slowly enter the brain environment. Within the experiment, the concentration of the lipoprotein solution stayed the

same but the novelty of the hydrogel and its crosslinking density would be the determining factor in how fast or slow the retention time would be.

In the future, to improve upon this study different densities of hydrogels will be further assessed to evaluate which is best for lipoprotein release over time. In addition, it would be integral to perform studies on retention time in biological environments via cell models.

5.3 Kinetic Profile of Lipoprotein

The kinetic profile of the LDL-hydrogel complex was assessed to characterize the drug delivery system. As mentioned above, the goal is to find the optimal hydrogel composition, primarily dependent on the crosslinking density, and the optimal lipoprotein concentration that can be held in the hydrogel for drug delivery. The methodology included the use of UV-Vis spectrometry to measure the absorbance of the lipoprotein that is released from the hydrogel over time and using the calibration curve we were able to identity the precise concentration of the lipoprotein that was released to determine the release rate which is a critical characteristic of our desired delivery system.

The release rate of the lipoprotein from the hydrogel was measured with absorbance data at two different wavelengths in order to determine how well the hydrogel can retain/release the lipoprotein at an ideal rate. At 227 and 270 nanometers, the release rate of the lipoprotein was determined to be first $1*10^{-8}$ (mg/uL)*min^{-1} and at 270 nm, the release rate was $2*10^{-9}$ (mg/uL)*min^{-1} respectively and the second trial showed release rates of $2*10^{-8}$ (mg/uL)*min^{-1} and $2*10^{-13}$ (mg/uL)*min^{-1} respectively which determines the amount of lipoprotein that is released from the hydrogel per minute.

Although the methodology of the experiment was successful and these are promising primary results for the release rate, the coefficient of determination at 270 nm was very low (R^2=0.52) and in addition the coefficient of determination for both wavelengths in trial 2 decreased (R^2=0.85) which shows that the experiment should be repeated to obtain more consistent data with higher correlation (Figure 4.4.1). Theoretically the release rate should be the equal or very similar at both wavelengths (227 nm and 270 nm) since the LDL is released from the whole hydrogel in all areas at the same time. The disparity of the release rates was unexpected and we hypothesize that it is due to the fact that at such low concentrations of the lipoprotein, the solution is not able to efficiently absorb light and displays measurements of very low absorbance values and thus low concentrations for lipoprotein release. The ratio of concentration of LDL:hydrogel was also ideally proportional, as in order to create a workable LDL-hydrogel construct for testing, high amounts of hydrogel was used, but with limited resources of LDL, low amounts were used. Therefore, the ratio could be a factor of both the different release rates at the two wavelengths and the low release rates of LDL from the hydrogel. In order to get a more reliable result of the release rate, the concentration of the lipoprotein should be increased when first creating the LDL-hydrogel mixture in order to increase the absorbance when using UV-Vis spectroscopy for determining release rate.

The experiments are also to be replicated with hydrogels of different crosslinking densities to determine the optimal hydrogel composition that will serve as the drug delivery system, where we obtain a desired release rate. In addition, future directions will include replicating these experiments at body temperature (37 degrees Celsius). Our lipoprotein release rate experiments were performed at room temperature (25 degrees Celsius). We hypothesized that the lipoproteins were integrated into the hydrogel via hydrogen bonds. At higher

temperatures, the hydrogen bonds would be less stable, so the release rate of our drug delivery system may be faster in the environment of the body.

Similar methodologies have been previously used in other polymers such as Gliadel wafers. The Gliadel wafer is an FDA approved therapeutic option which releases Temozolomide (TMZ) to treat Glioblastoma Multiforme (Shapiro-Furman et al., 2018). In a previous methodology, Gliadel Wafers were loaded with TMZ and retention rate of the drug was measured. The TMZ molecules were encapsulated and coated with the polymer to form core-shell particles in which the coating shell served as a rate controlling membrane for the drug. Results suggested that there was reliable release of high dose TMZ for a period of 4 weeks. However, Gliadel wafers are not ideal as they tend to be stiff and lack specificity. Hydrogels counteract this as they enable local delivery and they are also a more gel-like consistency. More experimental needs to be done in order to increase the release rate of our LDL-hydrogel construct to ideally 1-2 weeks to continue to in vivo testing and eventually to 2-3 weeks with clinical trials.

5.4 Sterilization Considerations for Final Construct

Since the process of synthesizing the hydrogels requires high doses of irradiation, this would automatically sterilize the gel composite. However, the process of integrating lipoproteins by homogenization and the cutting of the final hydrogels for manufacture of implants in future clinical use could introduce possible contaminants. In addition, without the use of preservatives that are identified to not interact with the PVP or lipoproteins themselves, the shelf-life of the construct was not demonstrated or evaluated within the scope of this project. Therefore, future

work needs to be done to identify such a preservative that should be incorporated into the gel either during the initial making of the solution or the homogenization procedure.

Furthermore, all materials that are used need to be sterilized before introducing them into the sample. Therefore, the homogenizer components would need to be autoclaved, all pipetting should be done in a sterile environment like a cell culture hood, and the container that is used to hold the final gel would ideally be irradiated during the synthesis procedure but should also undergo autoclaving before use. In general, sterilization is an incredibly important consideration if our hope of one day translating this drug delivery system into clinical and surgical applications.

CH6: Conclusion

6.1 Equity Analysis

One of the main goals of this project was to extend the scope of this research beyond laboratory work and looking into how this research would be implemented fairly and equitably in a clinical fashion. As such, an equity analysis was performed to guide the extent of potential impact. Given that this research is preliminary at best, this equity analysis assesses current and future implementations of available drug delivery systems. Some suggest that the key to a properly equitable drug delivery system simply lies in lowering the cost of new treatments rather than having pharmaceutical companies prioritize lower cost generic versions of these therapeutics. To further this idea, the future direction of these nanotherapeutic treatment systems will likely favor patient specificity (Mitchell et al., 2020).

As of today, the cost of GBM treatment with Gliadel Wafers is roughly 37,000 USD (Drugs.com). As this disease strikes without regard to income level or insurance status, a continual effort to supply alternative methods of intracranial chemotherapy drug delivery is a necessary component to drive down costs.

6.2 Economic and environmental impact analysis

Another goal of this project was to assess the importance of the environmental impact of using PVP-based hydrogels. As previously stated, PVP has many applications for hydrogels and nanoparticles. While PVP affects swelling ratio within hydrogels, it may also possess other unique properties such biodegradability when combined with other polymers. PVP in combination with pectin is known to be non-toxic and is undergoing use as a biodegradable food

packaging (Nešić et. al, 2017). Thus, the waste generated during the experimentation performed poses no environmental or health risk to the local community.

PVP is a low cost polymer that can be easily obtained, making it an ideal candidate for improving accessibility of novel drug delivery devices. As discussed in our equity analysis, treatment with the current standard of care, Gliadel Wafers, may pose an exorbitant cost for some patients that prevents them from seeking treatment or deeply burdens themselves and their families. Thus we expect the goals of this project, and research like it, to contribute to a positive economic effect on the stakeholders mentioned.

In the experiment, carmustine was loaded into a low density lipoprotein. The lipoproteins tend to be expensive, with 2 mg costing around $200.

6.3 Conclusion

This project serves as a step further in the characterization of PVP hydrogel, such that this versatile and biocompatible polymer can be utilized for the purpose of sustained localized drug delivery. Of critical importance to this project, and the broader context of hydrogel drug delivery systems, is an understanding of how the crosslinking density of the polymer is dependent on synthesis methods, as crosslinking density affects the swelling capacity of the polymer, and an understanding of how synthesis methods affect the drug or nanoparticle release capacity of the hydrogel. To accomplish the aim of this project, we synthesized a PVP hydrogel at two molecular weights, 360kDa and 1300kDa, and at a range of PVP concentrations (25-50%) and solidified the gel via e-beam irradiation to trigger crosslinking. Using the combined methods of TMA and gravimetric analysis we formed an understanding of how synthesis methods affect the crosslinking capacity of the gel. We found that the difference in PVP crosslinking density

between polymers of different molecular weights is non-significant. An increase in irradiation dose is associated with a decrease in swelling capacity and thus an increase in crosslinking density, and additionally, a higher concentration of PVP is associated with a higher swelling capacity, likely due to the hydrophilic nature of the polymer. These results indicate a rich opportunity for a continued and more refined study of the swelling kinetics of PVP.

An integral part of the project was development of a system in which lipoproteins are loaded into a hydrogel to comprise the prospective drug delivery system. Current systems, such as the Gliadel Wafer, have a stiff consistency and lack specificity. The hydrogel has a more gel-like consistency which is more compatible with a biological environment. In addition, the lipoprotein can be used to target specific cells expressing LDL receptors on their surfaces. To synthesize the system, lipoproteins were mixed into the hydrogels, and UV Vis was used to measure the retention time of the hydrogel. In the UV Vis experiments, at 227 and 270 nanometers, the release rate of the lipoprotein was determined to be $1*10^{-8}$ (mg/uL)*min^{-1} and at 270 nm, the release rate was $2*10^{-9}$ (mg/uL)*min^{-1} which determines the amount of lipoprotein that is released from the hydrogel per minute. Though these results are promising, further research will be done to increase the concentration of lipoprotein within the hydrogel itself and to also achieve more consistent results.

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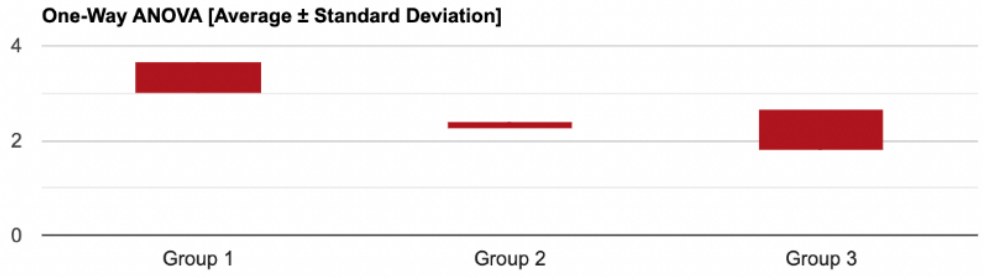
Appendices

Appendix A: ANOVA Results

One way ANOVA on 360 kDa samples for swelling ratio (Groups 1, 2, and 3 correspond to 50, 100, and 150 kGy, respectively).

Data Summary				
Groups	**N**	**Mean**	**Std. Dev.**	**Std. Error**
Group 1	4	3.3265	0.3427	0.1713
Group 2	4	2.346	0.0669	0.0334
Group 3	4	2.2318	0.4489	0.2244

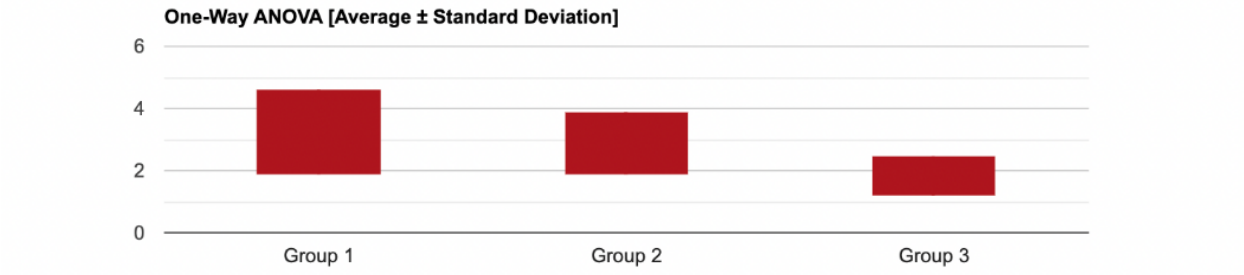
ANOVA Summary					
Source	**Degrees of Freedom** **DF**	**Sum of Squares** **SS**	**Mean Square** **MS**	**F-Stat**	**P-Value**
Between Groups	2	2.8971	1.4485	13.4359	0.002
Within Groups	9	0.9703	0.1078		
Total:	11	3.8673			



One way ANOVA on 1300 kDa samples for swelling ratio (Groups 1, 2, and 3 correspond to 50, 100, and 150 kGy, respectively).

Data Summary				
Groups	**N**	**Mean**	**Std. Dev.**	**Std. Error**
Group 1	4	3.2525	1.4015	0.7008
Group 2	4	2.883	1.0207	0.5103
Group 3	4	1.8163	0.6598	0.3299

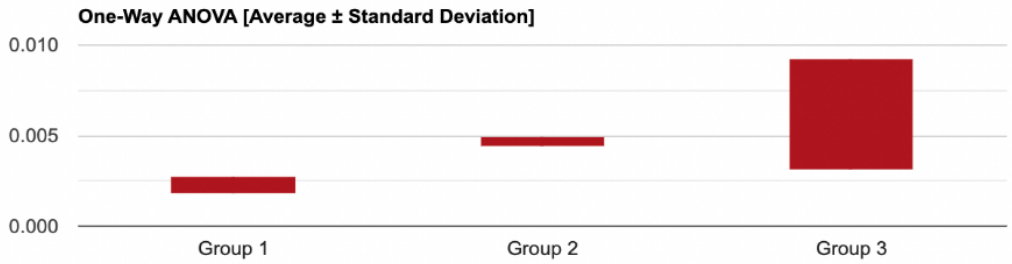
ANOVA Summary					
Source	**Degrees of Freedom** **DF**	**Sum of Squares** **SS**	**Mean Square** **MS**	**F-Stat**	**P-Value**
Between Groups	2	4.4494	2.2247	1.9394	0.1994
Within Groups	9	10.3241	1.1471		
Total:	11	14.7735			



One way ANOVA on 360 kDa samples for crosslinking density (Groups 1, 2, and 3 correspond to 50, 100, and 150 kGy, respectively).

Data Summary				
Groups	**N**	**Mean**	**Std. Dev.**	**Std. Error**
Group 1	4	0.0023	0.0005	0.0003
Group 2	4	0.0047	0.0003	0.0001
Group 3	4	0.0062	0.0031	0.0015

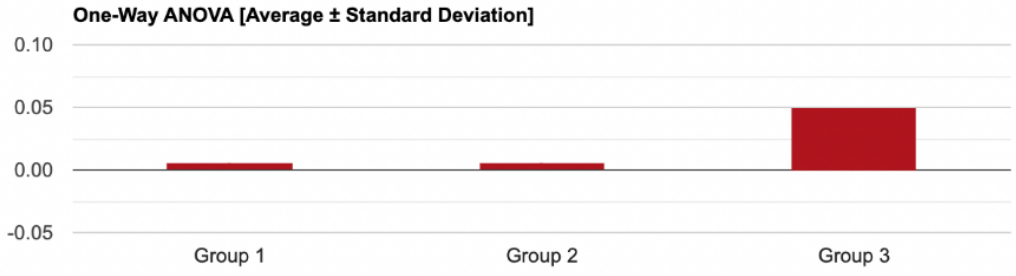
ANOVA Summary					
Source	**Degrees of Freedom** **DF**	**Sum of Squares** **SS**	**Mean Square** **MS**	**F-Stat**	**P-Value**
Between Groups	2	0	0	4.6673	0.0407
Within Groups	9	0	0		
Total:	11	0.0001			



One way ANOVA on 1300 kDa samples for crosslinking density (Groups 1, 2, and 3 correspond to 50, 100, and 150 kGy, respectively).

Data Summary				
Groups	**N**	**Mean**	**Std. Dev.**	**Std. Error**
Group 1	4	0.0037	0.0029	0.0014
Group 2	4	0.004	0.0022	0.0011
Group 3	4	0.0242	0.0259	0.0129

ANOVA Summary					
Source	**Degrees of Freedom DF**	**Sum of Squares SS**	**Mean Square MS**	**F-Stat**	**P-Value**
Between Groups	2	0.0011	0.0006	2.422	0.144
Within Groups	9	0.0021	0.0002		
Total:	11	0.0032			



Appendix B: Raw Data from UV-Vis Experiments

Absorbances of lipoprotein for calibration curve at varying concentrations by serial dilutions

Solution	[Lipoprotein] (mg/uL)	Peak Abs Value	Corresponding Wavelength (nm)
1	1.312*10^-5	0.748 0.194	227 270
2	1.5088*10^-5	0.879 0.231	227 270

3	1.7056*10^-5	1.023 0.273	227 270
4	1.9024*10^-5	1.196 0.324	227 270
5	2.0992*10^-5	1.421 0.383	227 270
6	2.296*10^-5	1.613 0.415	227 270

Trial 1 of absorbance and concentration of lipoprotein after five 10 minute intervals to determine release rate from hydrogel.

Time passed (mins)	**Absorbance @ 227 nm**	**Absorbance @ 270 nm**	**LDL Concentration @ 227 nm (mg/uL)**	**LDL Concentration @ 270 nm (mg/uL)**
0	0.042	0.004	4.724E-07	4.274E-08
10	0.055	0.011	6.220E-07	1.192E-07
20	0.067	0.006	7.502E-07	6.974E-08
30	0.079	0.009	8.920E-07	1.024E-07
40	0.091	0.012	1.026E-06	1.383E-07

Trial 2 of absorbance and concentration of lipoprotein after five 10 minute intervals to determine release rate from hydrogel.

Time passed (mins)	Absorbance @ 227 nm	Absorbance @ 270 nm	LDL concentration @ 227 nm (mg/uL)	LDL concentration @ 270 nm (mg/uL)
0	0.022	0.004	2.486E-07	2.796E-12
10	0.018	0.007	2.025E-07	2.277E-12
20	0.014	0.001	1.575E-07	1.771E-12
30	0.030	0.012	3.374E-07	3.795E-12
40	0.081	0.044	9.111E-07	1.025E-11
50	0.104	0.067	1.170E-06	1.316E-11
60	0.136	0.093	1.530E-06	1.721E-11
70	0.118	0.078	1.327E-06	1.493E-11