Efficacy of nanoparticle-encapsulated BCNU delivery in a pCPP:SA scaffold for treatment of Glioblastoma Multiforme

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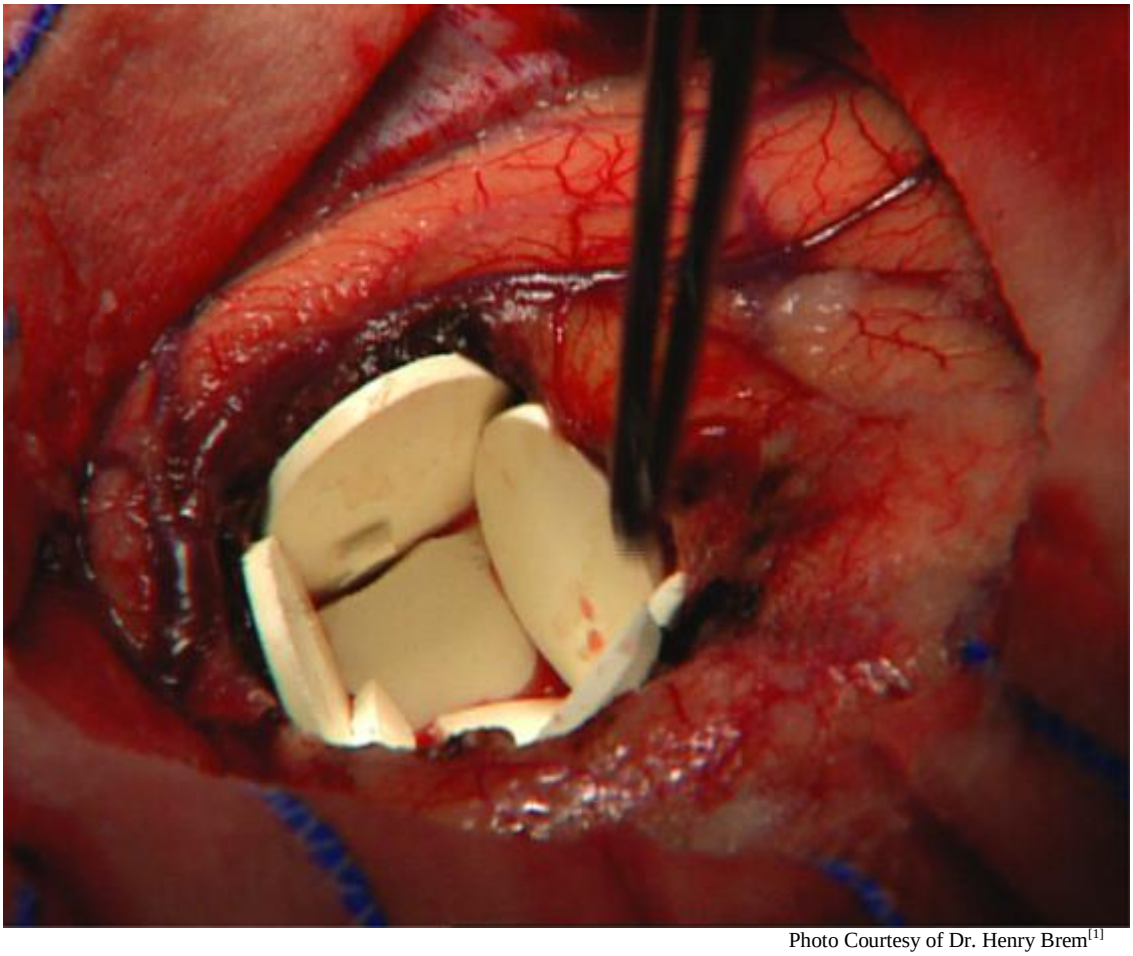

Photo Courtesy of Dr. Henry Brem[1]

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I. Executive Summary

Glioblastoma multiforme is a high-grade malignant glioma, caused by rapidly proliferating, metastasizing astrocytes in the brain[2,3,4,5]. This glioma has an average survival time of 12 months post-diagnosis and results in approximately 13,000 deaths annually[6,7]. The primary treatment protocol for this glioma involves delivering carmustine (BCNU) drug using the commercially available Gliadel® wafer following surgical tumor resection and radiation treatment[2]. Drug penetration using the wafer, however, only extends to a maximum distance of 1.00 cm away from the resected tumor. Ideally, to prevent the recurrence of malignant gliomas, threshold levels of BCNU (0.0252 mol/m^3) should be present up to 2.50 cm from the outer boundary of the remaining tumor margin[8]. The purpose of our study is to model a new drug delivery method for treatment of glioblastoma multiforme (GBM).

Our new approach is to incorporate BCNU into nanoparticles and then uniformly distribute these BCNU-loaded nanoparticles into a scaffold made of the same material used for Gliadel® wafers. Hence we examined the release of BCNU from polylactic acid (PLA) nanoparticles originally contained in a pCPP:SA scaffold (1,3-bis(p-carboxyphenoxy)propane (CPP) and sebacic acid (SA) in a 20 to 80 molar ratio) similar to the wafer material of Gliadel®. Further diffusion of the drug into the tumor and normal brain tissue regions is expected because the BCNU is protected from enzymatic degradation while within the nanoparticles.

In COMSOL, the scaffold, tumor tissue, and healthy brain tissue were modeled as a compound hemispherical region in a 2D, axisymmetric geometry with two transient diffusion species. The 1 mm thick scaffold contained uniformly distributed nanoparticles that were released by diffusion through the scaffold. BCNU was released from the nanoparticles over time before diffusing further on its own. A BCNU release profile obtained from the specific nanoparticle-BCNU formulation by Yan et al[9]. was used to make a BCNU generation term as a function of nanoparticle concentration and time. A 36 hour delay was also included in the flux function to represent an outer shell around the nanoparticle that would prevent BCNU release until 36 hours after implantation.

After running the COMSOL simulation for ten days, the results for the encapsulated BCNU nanoparticle model indicate that the furthest distance at which a therapeutically effective concentration of BCNU is present is 2.967 cm from the center of the resected tumor, which is 0.467 cm from the edge of the resected tumor. The results for the non-encapsulated BCNU show the effective distance is 3.227 cm from the center of the resected tumor, which is 0.727 cm from the edge of the resected tumor. As such, the scaffold/nano-particle drug delivery approach appears to be significantly less effective in delivering threshold concentration levels of BCNU to further distances from the resection site in comparison to the wafer method. While our method does not increase the effective distance, the encapsulation protects the drug from degradation thereby significantly increasing its post-implantation half-life.

Increasing the distance of therapeutic BCNU delivery to the target regions would significantly improve the number of glioma cells eradicated by the treatment and prolong the life of those who are diagnosed with glioblastoma multiforme. However, our results indicate that nanoparticles, at least in our formulation, were not useful to such ends. Further study is required in order to confirm such findings for other nanoparticle formulations.

II. Introduction

Glioblastoma multiforme is a high-grade malignant glioma, caused by rapidly proliferating, metastasizing astrocytes in the brain[2,3,4,5]. Almost 52% of all brain tumor cases are glioblastoma multiforme[4]. This glioma has an average survival time of 12 months post-diagnosis and results in approximately 13,000 deaths annually[6,7]. Glioblastoma multiforme is extremely difficult to treat because the tumors are heterogeneous and the tumor cells penetrate the adjacent regions of the brain and therefore cannot be entirely removed during surgical removal. Current treatment approaches include: radiation therapy, chemotherapy, gene therapy, surgery or a combination of two or more[10]. The treatment chosen is highly predicated on the characteristics of the tumor, how quickly it proliferates, the symptoms it induces, and its location in the brain[10]. Furthermore, most glioma cells are partly resistant to radiation and chemotherapy[4,11]. A significant hindrance to the delivery of chemotherapeutic agents to areas in the central nervous system is the existence of the blood brain barrier, new methods of intracranial drug delivery are being investigated to release potent concentrations of drug to tumor cells, while evading the undesirable systemic effects[11]. There is a pressing need to develop more effective methods to improve treatment approaches and to produce higher life expectancies.

All treatment modes intend to initially maximize the number of tumor cells eradicated. Generally surgery is first done to remove the bulk of the initial tumor cells. The remaining carcinogenic cells are then removed using radiation and chemotherapy[4,11]. Unfortunately, high-grade gliomas like glioblastoma multiforme have a high propensity for re-growth, and available treatments can only attempt to delay their recurrence as long as possible[11]. In one chemotherapeutic approach to treat recurrent gliomas, surgeons perform a craniotomy to surgically resect most of the tumor leaving a 2.00 mm margin of cancerous tissue. Up to eight Gliadel® wafers are then placed in the resection cavity for the directed and controlled release of BCNU, a drug used to kill the remaining glioma cells[2,12]. Each Gliadel® wafer (diameter: 14.5 mm, thickness: 1.00 mm) is composed of a 20:80 molar ratio poly[bis(p-carboxyphenoxy) propane: sebacic acid] (p(CPP:SA, 20:80)) and contains 7.7 mg of homogenously distributed BCNU. BCNU is a chemotherapeutic drug that has proved effecting in killing glioma cells. The wafers degrade over two to three weeks, allowing the drug to diffuse into the proximate tissue[2,12].

The goal of all treatments for glioblastoma multiforme is to eradicate all glioma cells up to 2.5 cm from the outer tumor boundary. Gliadel® wafer treatment has been found to extend only 1.00 cm from the outer tumor boundary. Thus, we chose to study the efficiency of a new treatment approach in which BCNU is encapsulated in nanoparticles to extend the drug's half-life post-implantation and allow functional BCNU to reach further into the target region. The scaffold/nano-particle method presented examines the release of BCNU drug from polylactic acid (PLA) nanoparticles contained in a pCPP:SA scaffold using a 2D axisymmetric, transient diffusion COMSOL model. We expect this model will ensure further diffusion of the BCNU into the tumor and normal brain tissue regions by postponing degradation of the drug.

II.A Design Objectives

Using COMSOL, we will model the concentration profile of PLA nanoparticles diffusing out of a pCPP:SA scaffold and of BCNU released from these nanoparticles in order to:

I. Determine how the concentration of the carmustine (BCNU) in the tumor region changes over time (concentration profile)
II. Determine the difference in the effective radius of the BCNU in the brain by comparing nano-particle encapsulated BCNU release from scaffold to free BCNU release from scaffold (like Gliadel®).

II.B Problem Schematic

A 2D axisymmetric, transient diffusion COMSOL model will be used, in which we expected further diffusion of the drug into the tumor and normal brain tissue regions due to protection of BCNU from degradation while contained in the nanoparticles. The scaffold, tumor tissue, and healthy brain tissue were modeled as a compound hemispherical region with a 1 mm thick scaffold composed of 1,3-bis(p-carboxyphenoxy)propane (CPP) and sebacic acid (SA) in a 20 to 80 molar ratio (p(CPP:SA, 20:80)), a 2mm thick tumor region, and semi-infinitely thick (2.5cm) healthy tissue region. Initially, the pCPP:SA scaffold contains uniformly distributed nano-particles to be released by diffusion. The PLA nanoparticles contain BCNU, which is released via surface erosion over time by a function extrapolated from release profile data obtained by Yan et al (see Appendix A). A 36 hour delay was also included in flux function to represent an outer layer around the nanoparticle that would prevent BCNU release until 36 hours post-implantation.

We present our treatment as an analysis of 2-species:

I. Nanoparticle concentration
II. BCNU concentration

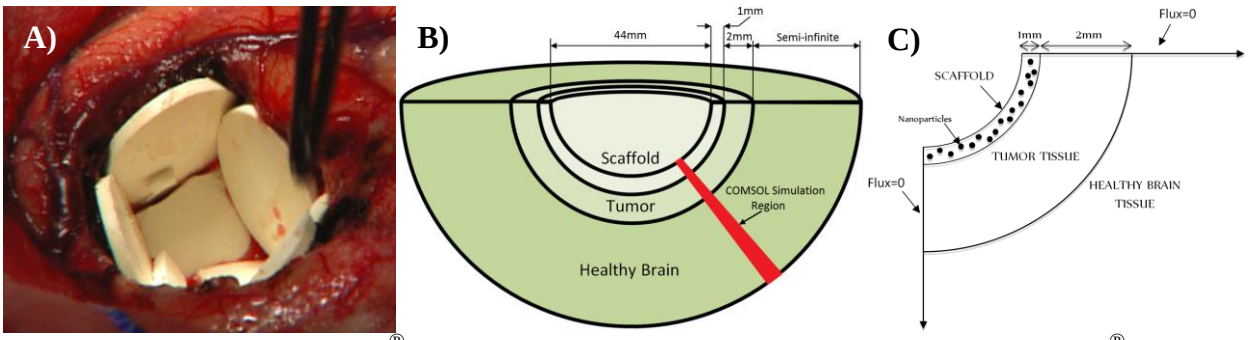

Figure 1. Schematics. A) Gliadel® Implantation. After surgical tumor resection, up to 8 Gliadel® wafers are added to release BCNU into the surrounding tissue. **B) pCPP:SA Scaffold Schematic.** The tumor to be resected is 50.0 mm in diameter and the margins after surgery are 2.0 mm. The required scaffold is 1mm thick with an outer diameter of 46.0 mm. **C) Simplified Schematic.** PLA Nanoparticles (54nm diameter) impregnated with BCNU diffusing through the pCPP:SA scaffold and tumor tissue.

III. Results and Discussion

III.A Concentration Profiles

To determine the effective release of drug into the tumor region, simulations of PLA nanoparticles and BCNU diffusion (Figures 6 and 7) were run for 10 days. To uncover how the drug and nanoparticles have moved through the target regions by the end of the simulation, surface concentration plots were generated in COMSOL and are presented below. These plots show the concentration profiles of the species at the final time of 10 days.

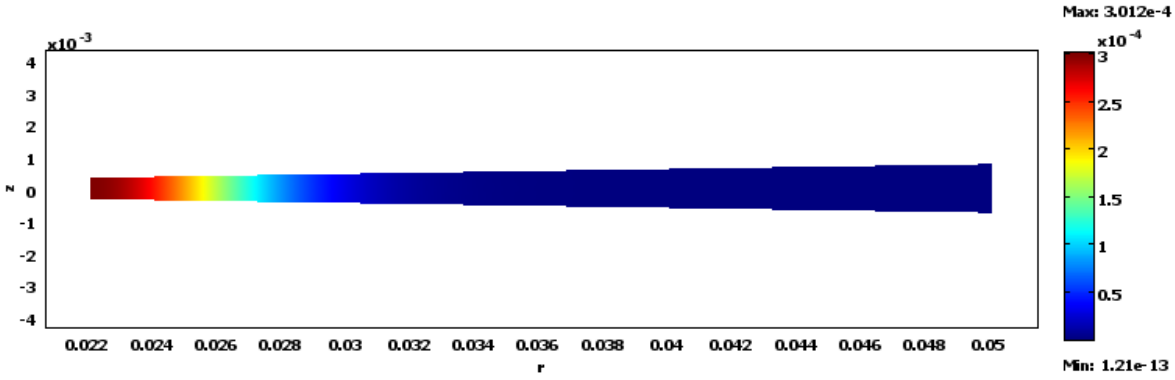

Figure 6. Surface Plot of PLA nanoparticle concentration at 10 days (mol/m^3). The diffusion of PLA nanoparticles (54nm) carrying BCNU drug though the scaffold, tumor region, and healthy brain tissue after 10 days.

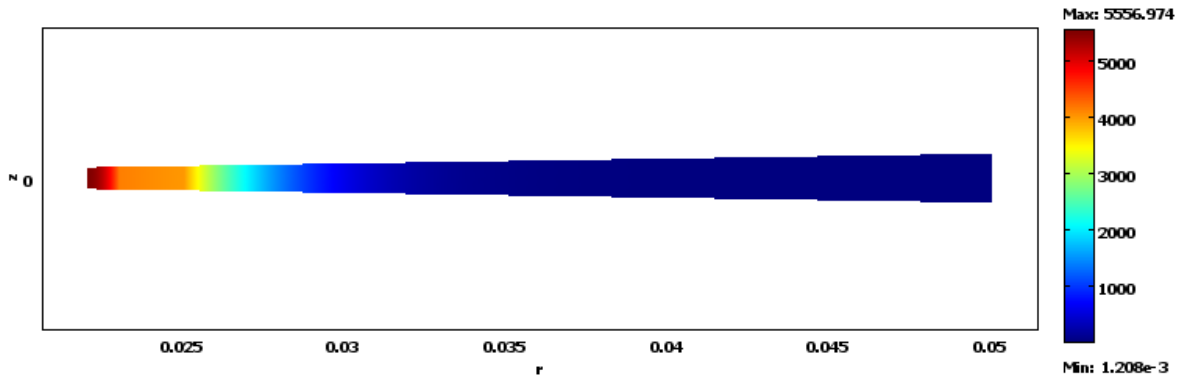

Figure 7. Surface Plot of BCNU Concentration at 10 days (mg/m^3). The diffusion of BCNU drug through the scaffold, tumor region, and healthy brain tissue after 10 days.

As can be seen in Figure 6, the nanoparticle penetrates into the healthy tissue region by the end with a concentration greater than $5x10^{-3}$mol/m^3 about 5mm into this region (r=0.03m). As can be seen in Figure 7, the final BCNU concentration profile shows that drug concentration is approximately 4000 mg/m^3 the within the tumor region ($0.023 \leq r \leq 0.025$ m) which is less than the threshold level of 5392.8 mg/m^3. It certainly can be seen that by the final time point of our

simulation, drug degradation has taken the BCNU concentration within the tissues down to below threshold. However, prior to the final time, BCNU concentrations above threshold are obtained as can be seen below.

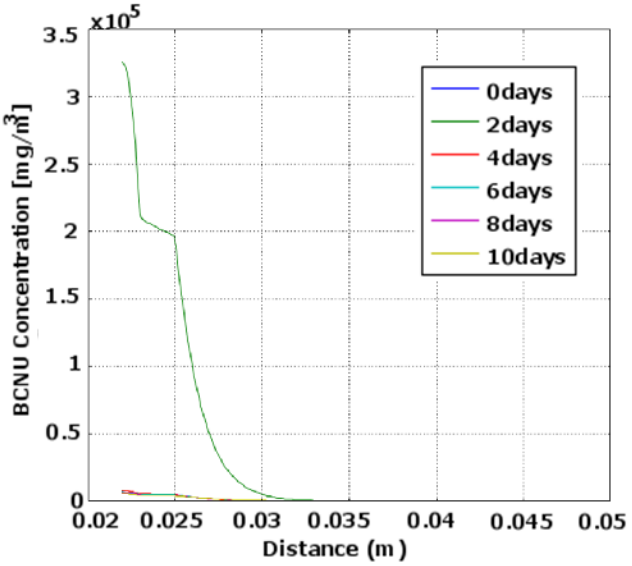

Figure 8. BCNU Concentration Profiles with Nanoparticles at 0, 2, 4, 6, 8, and 10 days. After a 36-hour delay the nanoparticles will release BCNU into the scaffold, tumor region, and healthy tissue region. The drastic release profile shown at 2 days comes from the initial bulk BCNU discharge from the nanoparticles. Shortly after two days, significantly less BCNU is released from the nanoparticle.

After one and a half days post-implantation, the nanoparticles begin to release BCNU; this is visible in Figure 8 at the 2 day time point. As the nanoparticles begin to diffuse through the domain, we see that the reach of the BCNU appears to be dependent on the nanoparticle release profile (see Figure 15, Appendix A). Figure 8 also points to the conclusion that maximum BCNU concentrations occur around the end of the quick release period.

In order to determine the maximum concentration at any distance throughout the simulation, plotting of the concentration gradients every hour is essential. With the profiles from all times in the simulation overlaid on one plot as in Figure 9, effective distance is ascertained by determining where the maximum concentration corresponds to the threshold BCNU level required for sufficient glioma cell death.

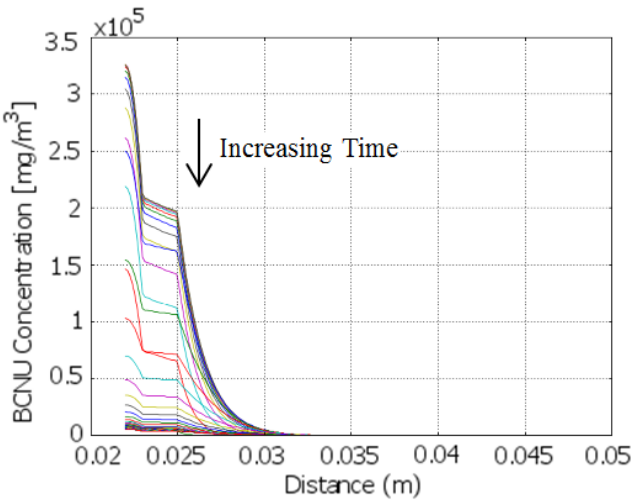

Figure 9. BCNU Concentration Profiles with Nanoparticles at every hour. The concentration profiles of BCNU at every successive hour during the simulation over a period of 10 days had lower maximum concentrations.

As evident above, the BCNU concentration profile decreases with time. At all times, the slope of the concentration gradients is lowest in the tumor region. This occurs because the tumor region has a diffusivity that is an order of magnitude higher than that of the scaffold and healthy tissue regions. The tumor and healthy tissue regions also show exponential decay in BCNU concentration due to the degradation of drug. This decay is not as visible in the tumor region where high diffusivity causes a low concentration gradient. From Figure 9, we ascertain that the effective distance of this model is 0.00467m from the scaffold, 0.02967m from the center of the resected tumor.

The reason for the limited reach of BCNU into the healthy region can be attributed to its dependence on nanoparticle diffusion. Nanoparticle concentration profiles provide insight into this dependence since the nanoparticles must travel into the region before most of the BCNU can be released.

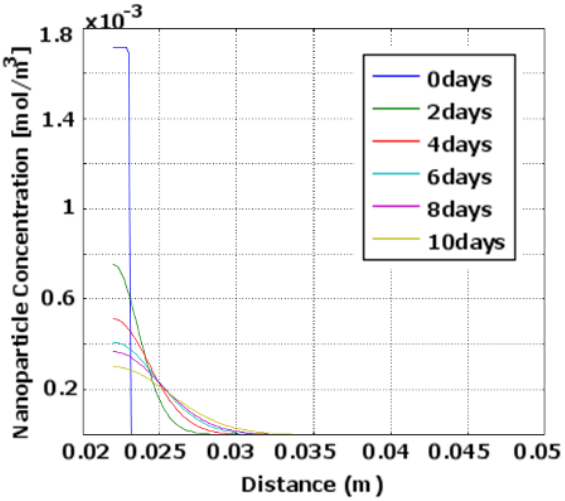

Figure 10. Nanoparticle Concentration Profiles at 0, 2, 4, 6, 8, and 10 days. Gradient-dependent nanoparticle release from the scaffold every two days for a period of 10 days. The concentration gradient decreases as fewer nanoparticles are released from the scaffold over time.

Initially, the concentration of the nanoparticle in the scaffold/tumor boundary is at its highest level as indicated by the dark blue curve. The initial concentration of nanoparticles within the scaffold region is at its highest, around 1.7 x 10^{-3} mol/m^3, but decreases rapidly as seen in the day 2 curve. After the initial implantation day, the nanoparticle concentration drops by about 10^{-3} mol/m^3. As time increases, the rate of nanoparticle diffusion into the tumor region and healthy tissue region decreases causing a progressive decrease in concentration gradient as shown by the slopes of the concentration profiles.

To determine how our model may compare to the commercially available Gliadel®, we developed a Gliadel-like model in which BCNU was released directly from the pCPP:SA scaffold without the use of nanoparticles. Therefore, the BCNU released and the effective distance traveled can be obtained by comparing the BCNU concentration profiles with and without the use of nanoparticles.

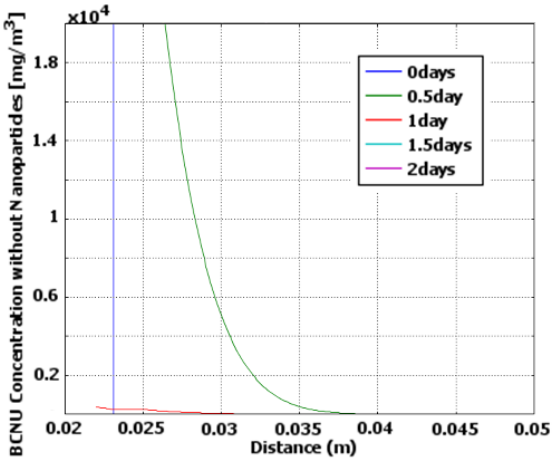

Figure 11. BCNU Concentration Profiles without Nanoparticles at 0, 0.5, 1, 1.5, and 2 days. The majority of the BCNU held within the scaffold is released and diffuses through the tumor and healthy tissues regions in less than 1 day.

Unlike the release in the nanoparticle case, the non-encapsulated BCNU is able to traverse the domain in a much smaller time period (Figure 8). This indicates that the nanoparticles are able to hold the BCNU for significantly longer periods of time as it moves through the tissue. The model lacking the nanoparticles allows for greater distance of BCNU diffusion into the healthy tissue region, with BCNU extending almost 0.005m farther than in the nanoparticle model. Figure 11 shows that most of the BCNU is consumed by 2 days post-implantation. Comparing Figure 8 and Figure 11, we see an order of magnitude difference between the concentration remaining at the 2 day mark for the no-nanoparticle model and at the 10 day mark for the nanoparticle model. We can presume the more sustained presence of BCNU is due to the nanoparticles protecting the drug from degradation. This drastic difference would not be possible without the use of nanoparticles.

In the model without nanoparticles the BCNU concentration profiles (Figure 12) decrease with time, similarly to that in the simulation where nanoparticles were used.

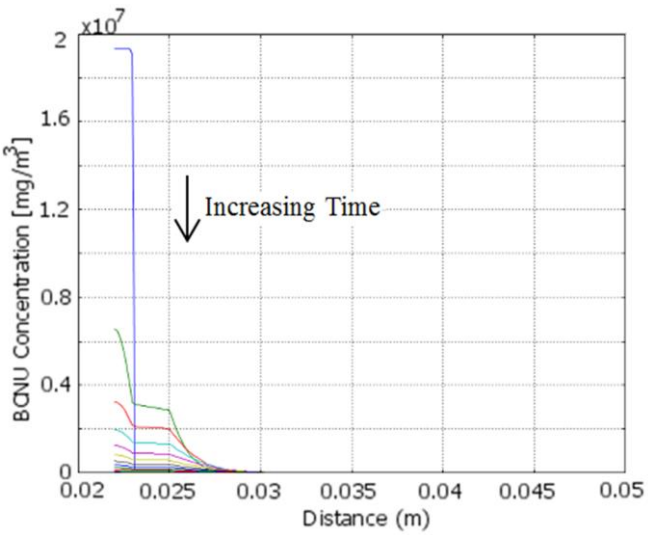

Figure 12. BCNU Concentration Profiles without Nanoparticles at every hour. The concentration profiles of BCNU at every successive hour during the simulation over a period of 10 days had lower maximum concentrations.

For all time points, the slope of the concentration gradients is also lowest in the tumor region because of the high diffusivity in this medium. After the initial day, both the tumor and healthy tissue regions express a sharp exponential decay in BCNU concentration which is attributed to the degradation of drug. In comparison to the steepness of the decay in the healthy tissue region, the slope of the concentration gradient in the tissue region is flatter because the high diffusivity produces a lower concentration gradient. Using an expanded version of this plot, the effective distance of BCNU release without nanoparticles was determined to be 0.00727m from the tumor region (0.03227m from the center of the resected tumor), which is almost twice as far as our nanoparticle-encapsulated model.

III.B. Mesh Convergence

In order to ensure that our mesh converged and to eliminate the potential discretization error sometimes caused by coarse meshes, the solutions of several different meshes 3 days into the simulation were analyzed. We analyzed their convergence by obtaining the average concentration within the tumor region and the flux at a point along the tumor-healthy tissue interface. Using these values, the relative percent change in the average concentration within the tumor region and the relative percent change flux at a point along the tumor-healthy interface were calculated to demonstrate mesh convergence (Figure 12).

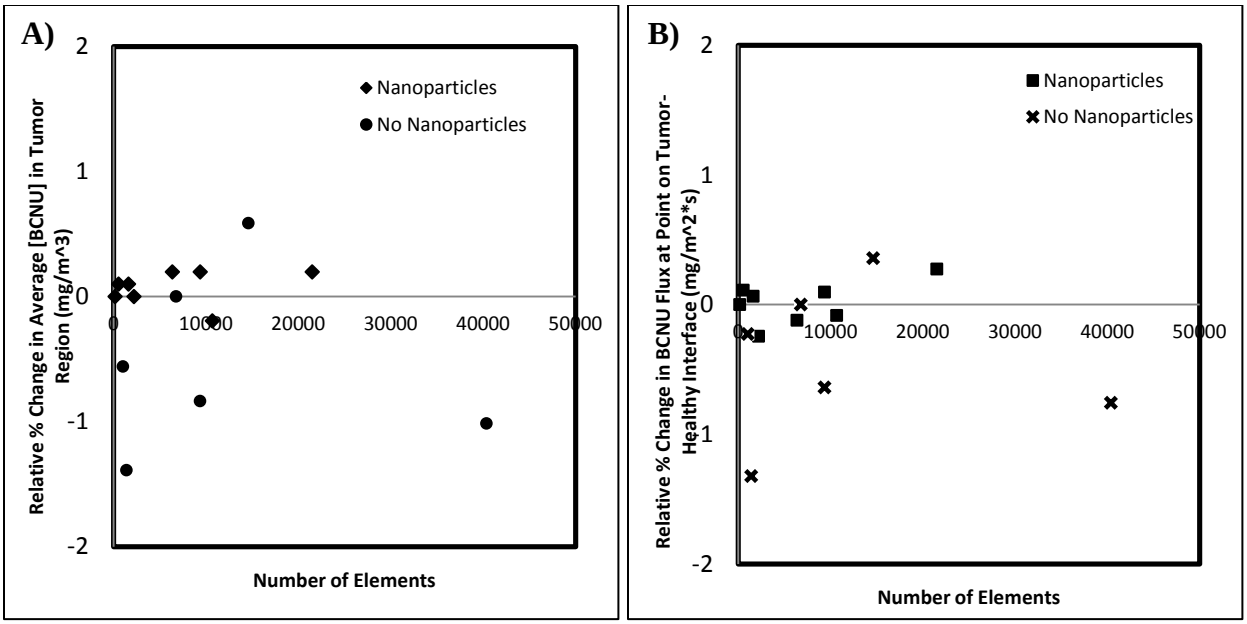

Figure 12. Mesh Convergence. A) Mesh Convergence of Average BCNU Concentration in the Tumor Region with and without Nanoparticles. Even at the lowest number of elements, the relative percent change in average BCNU concentration is less than 1% for the nanoparticle case and less than 1.5% for the no nanoparticle case. **B) Mesh Convergence of BCNU Flux at a Point on the Tumor-Healthy Tissue Interface.** Even at the lowest number of elements, the relative percent change in BCNU flux is less than 1% for the nanoparticle case and less than 1.5% for the no nanoparticle case

It was found that even at the lowest number of elements, the relative percent change in average BCNU concentration was less than 1% and 1.5% in the nanoparticle case and no nanoparticle case respectively while the relative percent change in flux was less than 1% and 1.5% as well for the nanoparticle case and no nanoparticle case respectively. As such, we could have chosen to use meshes of fewer than 1000 elements for both models. However, as it did not take very long to solve when the number of elements was increased, we chose to err on the side of caution and went with the meshes shown in Figure 13.

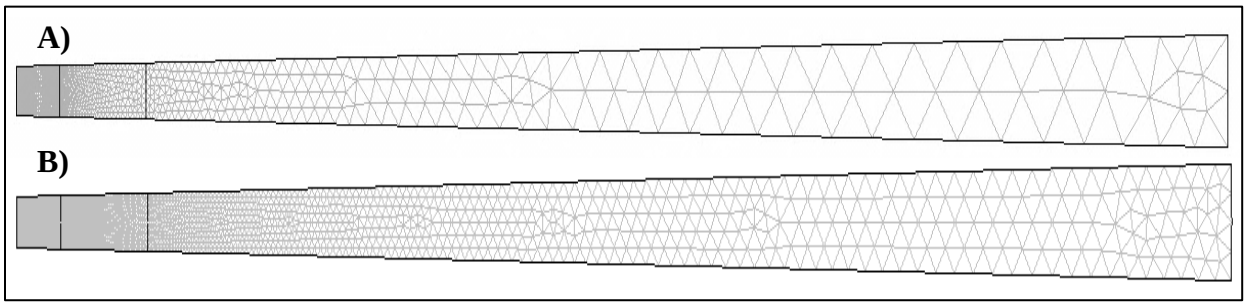

Figure 13. The Final Meshes. A) Final Mesh for Nanoparticle Model with 2220 elements. **B) Final Mesh for No Nanoparticle Model** with 6818 elements

All meshes produced a solution with the same general shape and we found that the mesh converged by 198 elements for the nanoparticle model and by 1050 elements for the no nanoparticle model. Therefore, a mesh of 2220 elements and 6818 elements were chosen for the nanoparticle model and no nanoparticle model, respectively.

III.C Accuracy Check

An analytical solution characterizing BCNU diffusion from pCPP:SA scaffolds shows that the effective distance of the drug is 1.018 cm from the polymer site (Appendix C3). Past research conducted on BCNU diffusion from pCPP:SA scaffolds has also demonstrated that the effective distance of the drug is about 1 cm from the polymer site[13]. However, our COMSOL model of this same condition gave a predicted result of .727 cm from the polymer site, as seen in Figure 12, which is about 30% smaller than the analytical solution and the results found in the literature.

The discrepancy between our model and the analytical solution may be due to the fact that the latter models one-dimensional diffusion of the drug, whereas the former models two-dimensional diffusion. The discrepancy between our model and past studies may be due to several additional factors. First, the penetration distance listed in the literature could have been defined as the distance at which no more BCNU is present, whereas we defined this distance be the one at which the BCNU concentration is minimally effective. This definition would make their penetration distance larger. Second, we assumed the degradation rate of the BCNU to be a first order reaction. However, if the degradation were actually a zero order reaction, the minimally effective BCNU concentration could be able to go further out into the brain tissue.

The lack of agreement between the experimental and theoretical penetration distances raises questions about the accuracy of our model. However, because the implementations for our models with and without the nanoparticle are otherwise identical, we are still able to compare the two COMSOL models to each other.

III.D Sensitivity Analysis

Sensitivity of our model to various input parameters was assessed based on the result of parameter modification on the effective radius of BCNU outside of the tumor region. Results, which are shown in Figure 14, show low sensitivity to changes in realistic diffusivity values, to the span of quick release of BCNU from nanoparticles, and to the delay time of release from our nanoparticles. They do however show relatively high sensitivity to the rate of BCNU degradation and initial BCNU concentration.

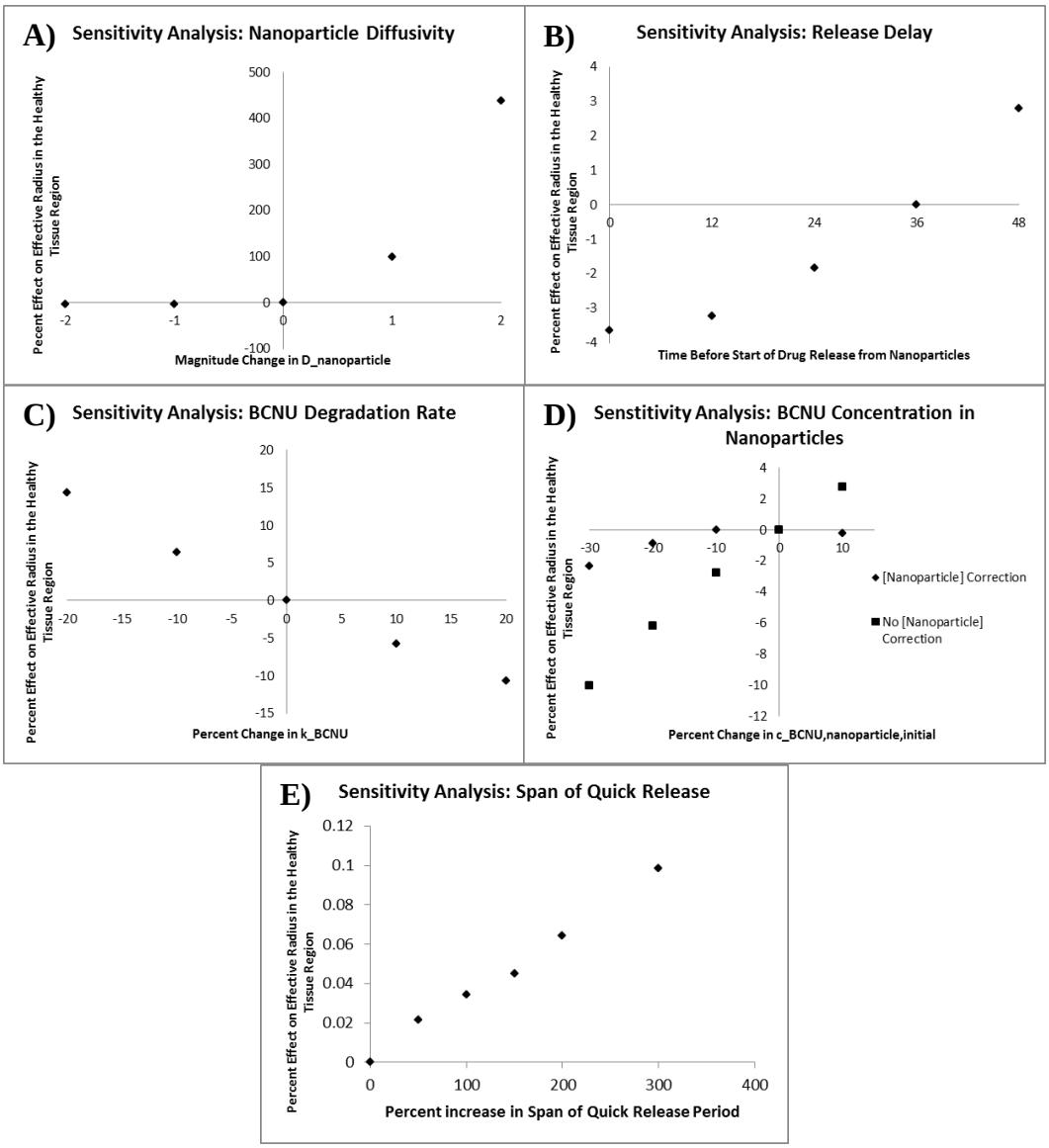

Figure 14. Sensitivity Analysis. A) Nanoparticle Diffusivity was adjusted by orders of magnitude and the effective radii of the formulations were compared. **B) Time of Release Delay** was adjusted from 0 hours to 48 hours and the effective radii of the formulations were compared with the 36hour delay as the baseline. **C) BCNU degradation rate** was adjusted by various percentages and the effective radii of the formulations were compared. **D) BCNU concentration in nanoparticles** was adjusted by various percentages and the effective radii of the formulations were compared. For each adjustment, there was a trial where the mass of drug administered was allowed to change with the initial concentration and one where the nanoparticle concentration was also adjusted in order to keep the total mass of drug administered the same. **E) Span of Quick Release** was adjusted and lengthened by various percentages in order to increase the percent of BCNU introduced to the nanoparticles that would come out during the period when the nanoparticles released 1.72% of their contents per hour.

III.D.i Diffusivity

Due to the high uncertainty of our Stokes-Einstein estimated diffusivity values, sensitivity of the model to nanoparticle diffusivity was assessed based on magnitudinal changes in diffusivity values. Results, seen in Figure 14A, show a very large increase in effective range when the magnitude of diffusivity was increased. However, due to the fact that the viscosities of brain tissue and scaffold are more likely to be higher than that of water, it is much more likely

that the actual diffusivities of the nanoparticles would be lower than our inputs and not higher. It should also be noted that the ~450% difference obtained and reported for the +2 magnitude value is likely different from the true increase of a +2 magnitude alteration on nanoparticle diffusivity. In this case, our semi-infinite geometry assumption failed, as the entire region had a maximum BCNU concentration greater than the effective dose. It is possible that extending the simulation region could both increase or decrease this value, but as stated, such a diffusivity would be unlikely to be realized.

Compared to the effect of increased diffusivities, the effect of decreased diffusivities was a relatively small. Both 1 and 2 magnitude decreases led to only about -3.5% change in the effective radius. The similarity of -2, -1, and 0 magnitude differences confirms a lack of effectiveness of our nanoparticle delivery method. In the -1 and -2 magnitude diffusivity case, the nanoparticles barely diffuse out of the scaffold before releasing BCNU so if the effective radius of these cases are only a little bit different, then our nanoparticle method may not be very effective.

III.D.ii Release Delay

The time when drug began to be released from the nanoparticles was adjusted by 12 hour increments, and as can be seen in Figure 14B effective distance increases with the time of delay. Compared to the 0 hour case, where drug diffusion is almost completely unprotected from degradation in the tissues, our 36 hour delay model only had a 3.6% higher effective radius. This fact supports previous findings that a nanoparticle formulation is not helpful. The positive slope of the plot and 48 hour delay's 3% increase on effective radius point towards increasing the delay in drug release as an effective way to improve the delivery mechanism.

III.D.iii Degradation Rate

The data in Figure 14C shows degradation rate to have had a near linear effect on the effective radius. Though not a 1:1 ratio, it is apparent that, for example, if our rate coefficient is 20% too high, then the actual radius of effectiveness for a nanoparticle delivery method could be 15% greater.

III.D.iv BCNU Concentration

When effective of drug concentration was modified, the resulting effective radii were seen to increase with increasing initial BCNU concentration (see the No [Nanoparticle Correction results in Figure 14D). This was to be expected, however the ratio of concentration increase to effective radius increase is again less than 1:1. Also, due to concerns about the side-effects of high BCNU doses, actually increasing the administration of drug is likely a bad idea, so we tested the sensitivity of the effective radius to $[BCNU]_{nanoparticle}$ increases that coincided with proportionally decreased nanoparticle concentrations (Figure 14D). In such alterations, the effect of altering the initial drug concentration relatively disappears.

III.D.v Span of Quick Release from Nanoparticles

When the amount of time that nanoparticles released their contents quickly (1.72%/hr) was adjusted and lengthened by various percentages in order to increase the percent of BCNU introduced to the tissue quickly, the effective range of the nanoparticle formulation was seen to

increase in a near linear fashion. This was to be expected as increasing the amount of drug introduced quickly from 21.5% in the original case to 86% in the 300% time increase case would put more drug into the tissue. However, even in the 300% increase case where 86% of the BCNU incorporated into each nanoparticle came out quickly after the 36hour delay, the effective range in the healthy tissue region only increased by about 10%. Thus the lack of penetration of our nanoparticle model cannot be solely due to lack of complete release. Perhaps particles with an even faster rate of BCNU release would be required for a nanoparticle formulation to be helpful.

IV. Conclusions and Design Recommendations

IV.A Conclusions

After completing our model without nanoparticles in a way that should be comparable to a normal Gliadel® delivery, it was found that our numbers did not compare favorably. The BCNU concentrations remained higher for the nanoparticle encapsulated case than the non-encapsulated case. There was an order of magnitude difference in BCNU concentration between the two by the two day mark. On the other hand, and more importantly, the nanoparticle model only had an effective penetration radius of 0.00467m while the model without nanoparticle encapsulation had an effective penetration radius of 0.00727m. Thus, the non-encapsulated method would kill more metastasizing glioma cells and better prevent tumor recurrence.

It is possible that the lack of effectiveness for our nanoparticle system was an imbalance between the release rate from the nanoparticles and the degradation rate of the BCNU. The release rate from nanoparticles might not have been high enough to compete with the degradation rate to bring BCNU concentration levels up to threshold.

IV.B Design Recommendations

Considering the reasons as to why the addition of the nanoparticle does not demonstrate any improvements over simple diffusion, one design recommendation for this drug delivery model is to change the n. It is possible that selecting a material that allows for a faster release of the BCNU will allow more of the drug to be in the tissue at a given time, so that it is not overpowered by the rate of drug degradation.

An alternative design recommendation that we suggest is to change the composition of the nanoparticle. The results of our sensitivity analysis showed that the effective radius was particularly sensitive to nanoparticle diffusivity and initial concentration of BCNU loaded into the nanoparticles. However, the only way to increase nanoparticle diffusivity is to reduce particle size. But at 54nm in diameter, the nanoparticles that we are using are already very small, near realistic manufacturing limits, and increasing the initial concentration of drug risks damaging healthy brain tissue. So we recommend changing the structure of the nanoparticle, possibly with the inclusion of probes that target molecular signatures specific to tumor cells, to have selective uptake of the nanoparticle by the tumor cells, where the nanoparticles will then unload the drug[14]. This method of drug delivery would allow the nanoparticles to move further out into the tissue, since they would not degrade until taken up by tumor cells, and would allow us to still use a smaller initial concentration of BCNU, since the drug would be released directly within the tumor cells.

Our sensitivity analysis also showed that the effective radius is sensitive to the drug degradation rate. So another additional design recommendation is that instead of using BCNU, we use a drug like Paclitaxel. Not only does Paclitaxel have a much slower degradation rate, but it also is larger than BCNU, so it is much less subject to loss via transcapillary exchange[15]. These advantages would possibly allow for deeper penetration into the brain tissue, as well as an effective concentration that could be sustained for a longer period of time.

For continued work with this nanoparticle drug-delivery model, we also recommend changing the diffusion-only assumption. Originally, we did not include convection in this model, thinking that convection in the brain would be minimal. However, upon further research, we learned that there is a convective condition in the brain following surgery. Transient edema, or

the accumulation of fluid, is associated with surgical trauma and, furthermore, there is an interstitial fluid flow that results from pressure gradients that are established in the presence of a tumor[16]. So including convection in subsequent models could increase the effective distance of the BCNU in our model without nanoparticles, closer to that which is observed in the literature.

IV.C Realistic Constraints

If future work with this model of nanoparticle-encapsulated BCNU drug delivery shows that the nanoparticle contributes to increasing the effective distance of the drug, then there are still potential constraints in design. We have not yet investigated the effects of the minimally effective BCNU concentration on healthy brain cells. The established minimum concentration is the minimum needed to kill tumor cells, but it is also possible that healthy brain cells will also be destroyed. In this case, we will need to optimize the model so that we maximize the amount of tumor tissue destroyed, and minimize the amount of healthy tissue that is damaged.

But assuming that health safety is not compromised, another potential constraint is the Food and Drug Administration approval process. Before pharmaceutical drugs and devices are allowed to be introduced for sale to the public, the FDA must approve or give them clearance. FDA standards mandate that this product must undergo stringent testing through at least three phases of trials before getting clearance or approval. These trials are typically costly and time-consuming, on average costing around $154 million dollars, and taking 8 years from the time clinical trials begin until approval is received.

Appendix A. Mathematical Statement of Problem

General Governing Equation (cylindrical coordinates):

$$\frac{\partial c_A}{dt} + (v_r \frac{\partial c_A}{\partial r} + v_\theta \frac{\partial c_A}{\partial \theta} + v_z \frac{\partial c_A}{\partial z}) = D_{AB}(\frac{1}{r}\frac{\partial}{\partial r}(r\frac{\partial c_A}{\partial r}) + \frac{1}{r^2}\frac{\partial^2 c_A}{\partial \theta^2} + \frac{\partial^2 c_A}{dz^2}) + R_A$$

For all constants, see Table 1

Assumptions:

1. No bulk flow
2. Symmetric about z-axis (no change in θ direction)

I. Nanoparticle through the scaffold, tumor, and normal brain tissue

$$\frac{\partial c_{nanoparticle}}{\partial t} = D_{nanoparticle,tissue}\left[\frac{1}{r}\frac{\partial}{\partial r}\left(r\frac{\partial c_{nanoparticle}}{\partial r}\right) + \frac{\partial^2 c_{nanoparticle}}{\partial z^2}\right]$$

Where:

- $D_{nanoparticle,tissue}\ is\ D_{nanoparticle,scaffold}, D_{nanoparticle,tumor}, or\ D_{nanoparticle,normal}$
  All were approximated with the Stokes-Einstein Equation, $D = \frac{\kappa_b T}{6 \cdot \pi \cdot r \cdot \mu_{water}}$

Boundary conditions:

1. $z = 0,\ \frac{\partial c_{nanoparticle}}{\partial z} = 0$
2. $r = 0,\ \frac{\partial c_{nanoparticle}}{\partial r} = 0$
3. $at\ t = 0,\ c_{nanoparticle,scaffold} = 1.2168x10^{-4}\frac{mol}{m^3}$

II. BCNU through the scaffold, tumor and normal brain tissue

$$\frac{\partial c_{BCNU}}{\partial t} = D_{BCNU,tissue}\left[\frac{1}{r}\frac{\partial}{\partial r}\left(r\frac{\partial c_{BCNU}}{\partial r}\right) + \frac{\partial^2 c_{BCNU}}{\partial z^2}\right] + R$$

Where:

- $R = [generation] - [elimination] = F_{nano} - R_A$
  - $F_{nano,PLA} = flux_{percent\ mass} * M_{initial} * c_{nanoparticle} * N_A$
  - $R_A = -k_{BCNU}c_{BCNU}$
  - $c_{nanoparticle}$ at any time and position will be obtained from pt I.
  - $flux_{percent\ mass}$ as a function of time out of the nanoparticles obtained from release profile from literature that was a ratio of mass remaining to initial mass. Therefore it is multiplied by the initial mass of BCNU in the nanoparticle to get the total flux out per nanoparticle.
  - Assume R_A term drops out in the scaffold (No enzymatic breakdown).
- $D_{nanoparticle,tissue}\ is\ D_{nanoparticle,scaffold}, D_{nanoparticle,tumor}, or\ D_{nanoparticle,normal}$

Boundary conditions:

1. $\sqrt{r^2 + z^2} = 50mm, \frac{dc_{BCNU}}{dn} = 0$
2. $r = 0, \frac{dc_{BCNU}}{dr} = 0$
3. $z = 0, \frac{dc_{BCNU}}{dz} = 0$
4. $t = 0, c_{BCNU} = 0$ (All BCNU is initially encapsulated in nanoparticles)

Table 1. Parameter values used in nanoparticle-encapsulated BCNU simulation.

Parameter		Baseline value	Reference
$D_{BCNU, scaffold}$	Diffusivity	$2.0x10^{-10}$ m^2s^{-1}	3
$D_{BCNU, tumor}$	Diffusivity	$6.75x10^{-9}$ m^2s^{-1}	3
$D_{BCNU, brain}$	Diffusivity	$2.5x10^{-10}$ m^2s^{-1}	3
k_{BCNU}	Reaction rate	1.31×10^{-4} s^{-1}	3
$D_{nanoparticle, scaffold}$	Diffusivity	$8.41x10^{-12}$ m^2s^{-1}	Stokes-Einstein
$D_{nanoparticle, tumor}$	Diffusivity	$8.41x10^{-12}$ m^2s^{-1}	Stokes-Einstein
$D_{nanoparticle, brain}$	Diffusivity	$8.41x10^{-12}$ m^2s^{-1}	Stokes-Einstein
$c_{nanoparticle, scaffold, initial}$	Concentration	$1.558x10^{-3}$ mol m^{-3}	Designed parameter
$c_{BCNU, nanoparticle}$	Concentration	$2.5x10^{8}$ mg m^{-3}	Designed parameter
μ_{water}	Viscosity	10^{-3} Pa·s	Known
$F_{nano, PLA}$	BCNU flux from PLA nanoparticle	$f_{percent\ mass} \cdot M_{initial} \cdot c_n \cdot N_A$	Calculation
c_n	Nanoparticle concentration	COMSOL Output	Calculation
N_A	Avagadro's number	$6.022x10^{23}$ mol^{-1}	Known
SA	Nanoparticle surface area		Designed parameter
$M_{initial}$	Mass of BCNU in a single nanoparticle	$2.06134x10^{-14}$ mg	Designed parameter
R_a	Drug metabolism	$-k_{BCNU} * c_{BCNU}$	3
$F_{percent\ mass}$	flux out of nanoparticle expressed as percent of total mass out per second	Varies with Time	10
$C_{BCNU, scaffold}$	Concentration	$1.92296x10^{7}$ mg m^{-3}	

Figure 15A shows the percent of BCNU released from the 54nm PLA nanoparticles over time as measured by Yan et al[9]. As can be seen, there is an initial period of quick release followed by slow release. The $f_{percent\ mass}$, flux out of the nanoparticle as needed for our mathematical solution, must be expressed as a percent of total mass out per second. Thus, the absolute percentages released were turned into percentages released per time by taking the slopes of regression lines for both release rates as seen in Figure 15B. We converted the slopes of this model (1.72%/hr for the quick release and 0.0499%/hr for slow release) to percentages per second (0.000477778%/sec for the quick release and 1.38611%/sec for the slow release). We incorporated in a 36 hour period with zero release to add in a theoretical shell that could postpone drug release, and the resulting flux function became that shown in Figure 15C.

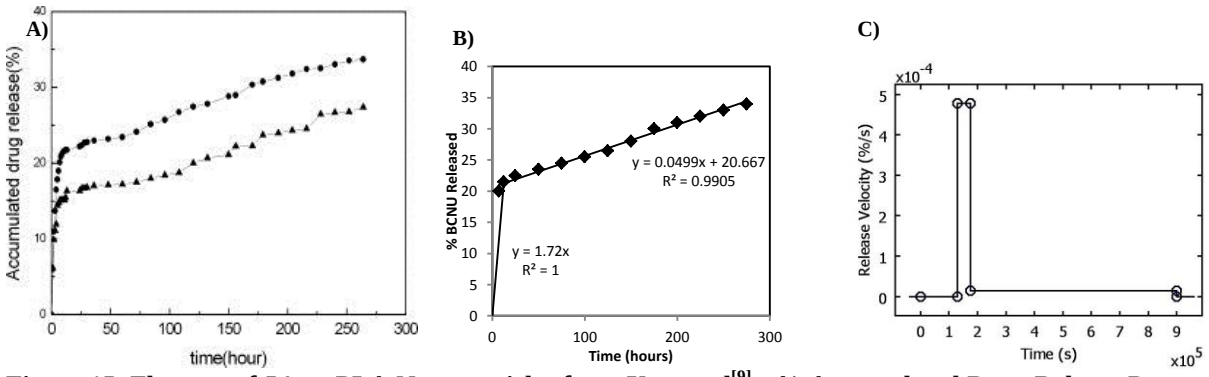

Figure 15. Flux out of 54nm PLA Nanoparticles from Yan et al[9]. A) Accumulated Drug Release Percentage over Time of two different nanoparticle formulations. The circles represent the 54nm particles used in our model. **B) Percent BCNU Released from Nanoparticle over Time** with regressions lines used for establishment of flux per time function**. C) Plot of the Flux Function inputted into COMSOL** A stepwise function was generated from the graph shown in B)

Appendix B. Solution Strategy

To examine the release of BCNU from the pCPP:SA scaffold with and without the use of nanoparticles, COMSOL Multiphysics 3.5A was used to solve a 2D-axisymmetric transient diffusion problem with reaction.

Linear System Solver: The direct (UMFPACK) solver was used to solve the equations and did so with coupling of nanoparticle concentration with BCNU calculations at each time step.

Time Stepping: The nanoparticle model was run over a period of 10 days, with solutions saved at every hour. The initial and maximum time step, set to AUTO, were determined by COMSOL.

Relative Tolerance: 0.01

Absolute Tolerance: 0.0010

Mesh: We used free meshes with higher numbers of elements in the scaffold and tumor regions where concentration gradient would be the largest. To accomplish this, triangular advancing front meshing was used and a maximum element size in the scaffold region was set along with a maximum growth rate in the tumor and healthy tissue regions. The maximum element size was set to 0.00004m and the growth rate in the tumor and healthy regions was set to 1.05 for the nanoparticle case. The maximum element size was set to 0.000025m and the growth rate in the tumor and healthy regions was set to 1.02 for the no nanoparticle case.

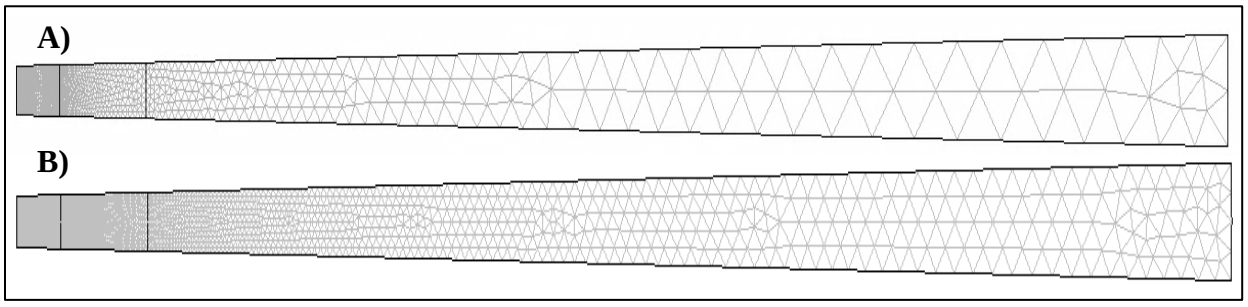

Figure 16. The Final Meshes. A) Final Mesh for Nanoparticle Model with 2220 elements. **B) Final Mesh for No Nanoparticle Model** with 6818 elements

The above meshes were garnered after tracking average BCNU concentration in the tumor region and flux across the tumor/healthy tissue interface for a number of different maximum element sizes and comparing the relative percent changes in tracked values. Data for mesh convergence analysis on average BCNU concentration in the tumor region can be seen in Table 2, Table 3, and Figure 12.

Table 2. Mesh Convergence Data for Model with Nanoparticles.

Max. Element Size	Number of Elements	Avg [BCNU] (mg/m^3)	Relative % Change
1.50E-03	198	4.67E+03	0
1.50E-04	560	4.68E+03	0.097560976
5.00E-05	1628	4.68E+03	0.097560976

4.00E-05	2220	4.67E+03	0
2.00E-05	6386	4.68E+03	0.195121951
2.00E-05	9394	4.68E+03	0.195121951
1.50E-05	10712	4.66E+03	-0.195121951
1.00E-05	21539	4.68E+03	0.195121951

Table 3. Mesh Convergence Data for Model without Nanoparticles.

Max. Element Size	Number of Elements	Avg [BCNU] (mg/m^3)	Relative % Change
1.00E-05	14640	1.63E-02	0.586273488
1.50E-04	1050	1.62E-02	-0.561173523
1.00E-04	1424	1.60E-02	-1.390902651
2.50E-05	6818	1.62E-02	0
2.00E-05	9394	1.61E-02	-0.838927774
8.00E-06	40417	1.61E-02	-1.016085853

Appendix C. Additional Visuals

Appendix C1: Design Parameter Formulation

In order to keep in accordance with Gliadel® where a maximum of 8 wafers can be implanted for a total mass of BCNU of 61.5mg, we needed to ensure that our input values would correspond to the same total mass. Therefore, after choosing a concentration of BCNU within the nanoparticles, only a specific concentration of nanoparticle would lead to a total mass of 61.5mg. To find this concentration, we started with the total mass and concentration within the nanoparticles:

$C_{BCNU,\ total}$ = 61.5 mg
$C_{BCNU,\ nanoparticle}$ = 2.5 x $10^8\ \frac{mg}{m^3}$

Then we determined the necessary volume of all nanoparticles for such a concentration to lead to a total mass of 61.5mg.

$$V_{Nano,Total} = 61.5\ mg\ \left(\frac{1\ m^3}{2.5\ x\ 10^8\ mg}\right) = 2.46\ x\ 10^{-7}\ m^3$$

This value, coupled with the volume of a single 54nm diameter nanoparticle was used to figure to figure out the total number of nanoparticles.

$$V_{Nano} = \frac{4}{3}\pi r^3 = \frac{4}{3}\pi(27\ x\ 10^{-9}\ m)^3 = 8.245\ x\ 10^{-23}\ m^3$$

$$\frac{V_{Nano,Total}}{V_{Nano}} = 2.983626\ x\ 10^{15}\ Nanoparticles$$

Dividing this by the volume of our scaffold would yield the nanoparticle concentration.

$$V_{Scaffold} = \frac{1}{2}\left(\frac{4}{3}\pi {r_{Outer}}^3\right) - \frac{1}{2}\left(\frac{4}{3}\pi {r_{Inner}}^3\right)$$

$$= \frac{1}{2}\left(\frac{4}{3}\pi(0.023\ m)^3\right) - \frac{1}{2}\left(\frac{4}{3}\pi(0.022\ m)^3\right)$$

$$= 3.18x\ 10^{-6}\ m^3$$

$$c_{Nanoparticle} = \frac{Number\ of\ Nanoparticles}{V_{Scaffold}}$$

$$= \frac{2.983626\ x\ 10^{15}\ Nanoparticles}{3.18x\ 10^{-6}\ m^3}$$

$$= 9.3825\ x\ 10^{20}\ \frac{Nanoparticles}{m^3}$$

This concentration was then converted to moles/m^3 so that the number inputted into COMSOL could be smaller and more manageable.

$$\frac{\boldsymbol{Moles\ of\ Nanoparticles}}{\boldsymbol{m^3}} = (9.3825\ x\ 10^{20}\ \frac{Nanoparticles}{m^3})(\frac{1\ mol}{6.022\ x\ 10^{23}\ \ Nanoparticles})$$

$$= \mathbf{1.558\ x\ 10^{-3}}\ \frac{\boldsymbol{Moles\ of\ Nanoparticles}}{\boldsymbol{m^3}}$$

For our reaction term, $M_{initial}$, the mass initially contained in each nanoparticle, was obtained by multiplying the volume occupied by each nanoparticle by the concentration of BCNU within each.

$$\frac{\boldsymbol{Mass\ BCNU}}{\boldsymbol{Nanoparticle}} = \mathbf{2.06134\ x\ 10^{-14}\ mg}$$

Finally, we also needed to get a concentration of BCNU for our no nanoparticle case. This was accomplished by dividing the total BCNU concentration (61.5mg) by the volume of the scaffold.

$$c_{No\ Nanoparticle} = \frac{C_{BCNU,Total}}{V_{Scaffold}} = \frac{61.5\ mg}{3.18\ x\ 10^{-6} m^3}$$

$$= 1.93396\ x\ 10^7\ \frac{mg}{m^3}\left(\frac{1\ g}{1000\ mg}\right)\left(\frac{1\ mol}{214\ g}\right)$$

$$= 90.37\ \frac{mol}{m^3}$$

Appendix C2: Analytical Solution of Model Without Nanoparticle

Using the following equation that models simple diffusion, we determined the analytical solution of BCNU diffusion through the scaffold, tumor and healthy brain regions. The following equation is used for each of the three regions.

$$\frac{c_a}{c_{a0}} = e^{-\sqrt{\frac{k}{D}}x}$$

c_a = BCNU concentration
c_{a0} = initial BCNU concentration
k = reaction rate of BCNU
D = diffusivity of BCNU through region
x = distance BCNU has diffused

In the scaffold region, the parameters are as follows:

$c_{a0} = 1.92296 \times 10^7$ mg/m^3
$k = 1.31 \times 10^{-4}$ 1/s
$D = 2.0 \times 10^{-10}$ m^2/s
$x = 1.0 \times 10^{-3}$ m (thickness of the scaffold region)

This gives us a concentration c_a at the scaffold/tumor boundary of $c_{a1} = 8560255.653$ mg/m^3

Then we use this concentration as the initial BCNU concentration for the tumor region, where the parameters are as follows:

c_{a1} = 8560255.653 mg/m^3
k = 1.31 x 10^{-4} 1/s
D = 6.75 x 10^{-10} m^2/s
x = 2.0 x 10^{-3} m (thickness of the tumor region)

This gives us a concentration c_a at the tumor/health brain boundary of c_{a2} = 6478630.291 mg/m^3

We then use this concentration as the initial BCNU concentration for the healthy brain region, where we wish to solve for the distance at which the concentration is the minimally effective concentration of BCNU. The parameters for the equation are as follows:

c_{a2} = 8560255.653 mg/m^3
c_a = 5392.8 mg/m^3
k = 1.31 x 10^{-4} 1/s
D = 2.5 x 10^{-10} m^2/s

This gives us an effective distance of 1.018 cm from the outer tumor edge.

Appendix C3: Original Design

Originally, our design involved a solid, hemispherical PLA scaffold containing pCPP:SA nanoparticles and or modeling was a 3-scale problem. The first was diffusion of nanoparticle through the scaffold and brain tissue. The second was diffusion of BCNU though the scaffold and brain tissues. This level involved a generation term for BCNU that was similar to the one for our current model. It contained a degradation term and a function to account for flux out of nanoparticles. However, unlike the current model, COMSOL was used to determine this function as opposed to data from previous studies such as Yan et al[9]. To get the flux function, Nanoparticles were modeled in COMSOL with uniform initial concentration and a simplification of [BCNU] outside the nanoparticles being set to zero. Then the flux at the outer surface over time was used for the generation term.

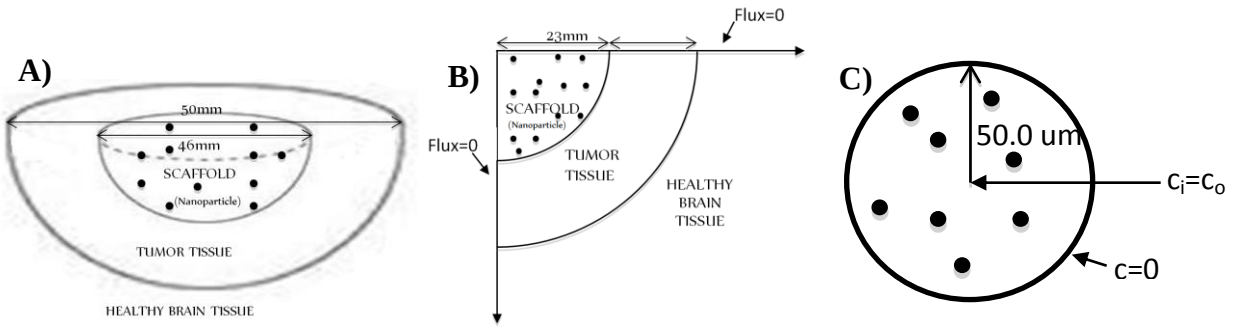

Figure 17. A&B) Old PLA Scaffold Schematic and Simplified Schematics for Macroscale Level of Diffusion through Scaffold and Brain Tissues. The scaffold was a solid hemisphere made out of PLA and the nanoparticles were 50µm in diameter and made of pCPP:SA. All other dimensions were unchanged. **C) Schematic for BCNU**

diffusion within nanoparticle. Diffusion of BCNU, originally at constant concentration, out of nanoparticles was modeled by setting the outer concentration to zero

It was found that near complete diffusion of BCNU out of the nanoparticles took less than 2 minutes which did not give the nanoparticles time to diffuse out of the scaffold before drug release. In light of this finding, we decided to change the nanoparticle material to PLA, which could be modified to have delayed release using shells of various materials. We found a paper, Yan et al., describing release from 54nm PLA nanoparticles[9], and we decided to use a shell model for our scaffold in order to place the nanoparticles closer to the brain initially. Then we added a time delay to the flux from the nanoparticle in order to represent the delay conferred by a shell before arriving at the current study.

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