Paper Critique

MRI-COMPATIBLE SKULL-EMBEDDED IMPLANT FOR DIRECT MEDICINE DELIVERY

EN.601.456 Computer Integrated Surgery II

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Table of Contents

My Project Summary

My work aims to address the problem of stagnancy in patient standard of care for Glioblastoma Multiforme (GBM) [1]. Currently, over 99% of promising therapies [2] are unable to reach the tumor and be effective due to the blood-brain barrier, which is a barrier that mediates communication between the peripheral and central nervous system. Therefore, the overall goal of my work is to develop a skull-embedded implant that enables chronic infusion of medicine directly into the brain to cross the blood-brain barrier, which could potentially benefit not only GBM patients, but patients with all kinds of neurological diseases that can be treated with direct drug delivery. My role in this project is to code for the pumps and develop the Bluetooth connectivity of the implant device for real-time interaction between the device, cellular devices, and the monitoring network.

Paper Selection

The paper selected for this review and critique is:

Oh S, Odland R, Wilson SR, et al. Improved distribution of small molecules and viral vectors in the murine brain using a hollow fiber catheter. J Neurosurg. 2007;107(3):568-577. doi:10.3171/JNS-07/09/0568 [3]

Although my role in this project does not directly involve the development of the catheter of the implant device, as a member of the team working on this project, I should have a good understanding on the fundamental mechanism of drug infusion to brain tumor tissue by the device, which is the primary motivation for the initiation of this project.

Since the catheter implemented in implant device is the foundational pathway via which drugs reach brain tumor tissue, having better knowledge base on the advantages and limitations of the catheter implemented will not only improve my understanding on the mechanism through which the product of the project can benefit GBM patients, the choice of catheter also imposes physical constraints to the delivery rate of drugs, which is relevant to the coding of the pumps, which I am responsible for in this project.

Summary of Problem & Key Result

In summary, the study conducted in this paper aims to design a new catheter to be used in convection-enhanced delivery (CED) to increase the flow rate and decrease the total infusion time for treatments of brain tumor. With this aim in mind, the authors designed and manufactured a novel catheter to be compared with a conventional needle used in standard clinical CED procedures. Below are the specifications of the designed hollow fiber catheter:

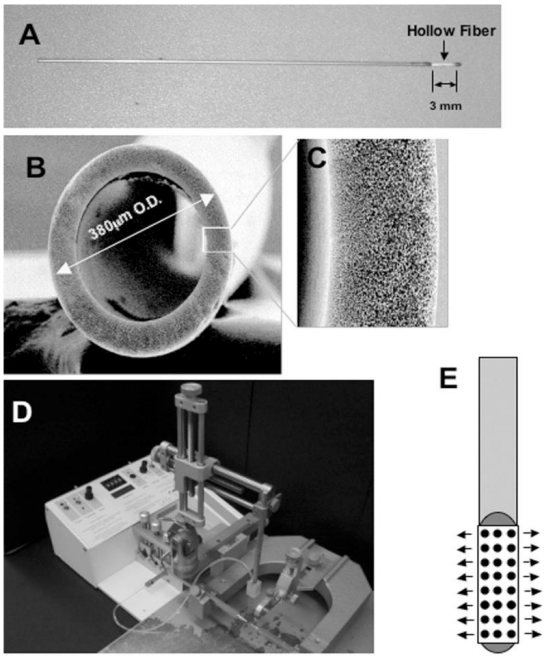

Figure 1 (adapted from the paper [3]). Five figures illustrating the designed hollow fiber catheter. A: a photo illustrating a 3-mm hollow fiber catheter attached to the end of a stainless-steel cylinder body. B: Magnified cross-sectional image featuring the hollow fiber catheter with an outer diameter of 380 μm, which is similar to that of a conventional 28-gauge needle (355 μm). Original magnification × 200. C: Magnified image depicting the area of the hollow fiber catheter outlined by the white box in panel B. Original magnification × 1000. D: Photograph showing the system used for stereotactic CED into the mouse brain. The needle or catheter was mounted directly onto the arm of the frame. The catheter(s) were connected to tubing attached to a syringe, which delivered infusate at a flow rate controlled by the micropump. E: Schematic depicting the hollow fiber catheter shown in panel A. Infusate is delivered along the shaft of the entire hollow fiber as depicted by the arrows.

- Material: Porous polysulfone
- Nominal pore size: 45 μm
- Outer diameter: 380μm
- Length: 3mm

This research consists of four studies, all of which are used to compare the distribution and efficacy of drug and gene between using single-port clinical catheter and hollow fiber catheter. It is hypothesized that the newly-designed porous catheter improves the distribution of CED-mediated drug delivery in the central nervous system compared with single-lumen catheters by providing multiple pathways for infusate to travel around each cell in the brain, which can be anywhere from 10 to 100 μm in diameter.

The results of the experiment, put generally, proved the hypothesis that compared to single-lumen catheters which are used in existing treatments, using a porous catheter improves the distribution and efficacy of CED-mediated drug therapy and gene therapy.

Significance

There had been many attempts to improve infusate distribution via CED. One successful attempt is to administer hypertonic mannitol to lower free water content in the brain, thereby shrinking cells and widening interstitial pathways to dramatically increase the distribution of genetic vectors injected into the CNS. [4], [5] However, attempts to increase interstitial fluid velocity failed. Increasing the driving pressure will not increase velocity but serve only to deform the tissues, which then accelerates impedance mismatch due to compaction of tissue, and further decreases porosity and hydraulic conductance. If the free fluid accumulates at a rate faster than it can dissipate, it will either flow backward along the shaft of the needle or tear into the tissue by shear force.

Hollow fiber catheter which has been proved to improve the distribution of drug via CED in this paper provides the solution to the problem of impedance mismatch (a concept from electrical engineering) - the low capacity of convective fluid flow within the brain is unable to accommodate the high infusion rate of agents to brain tissue. In the particular case of CED method for GBM treatment, the interstitial pressure in brain tumor tissue is 25 times greater than that of normal brain tissue (1–2 mm Hg, which is relatively low). This may account for the uneven distribution and leakage of drug into the subarachnoid space that has been observed in brain tumor clinical trials.[6], [7], [8]

Hollow fiber catheter walls contain millions of nanoscale pores, which effectively reduce the impedance mismatch not by decreasing the impedance within the tissue, but by increasing impedance in the catheter. This is achieved by the approximation of the porosity of brain tissue in the walls of the catheter, which is known as “pore connectedness.” [9]

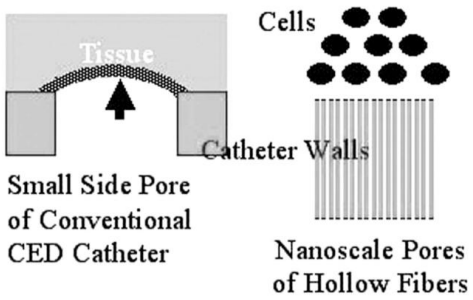

Figure 2 (adapted from the paper [3]). Schematic demonstrating tissue—pore interaction for the conventional catheter and hollow fiber catheter. The tissue deforming force is a product of infusion pressure and cross-sectional area of the tissue—pore interface. A 1-mm pore of a conventional CED catheter generates five million times more tissue deforming force than the nanoscale pores of hollow fiber catheters.

Increased impedance to flow through the hollow fiber catheter wall is compensated for by an increased surface area and a reduced potential for generation of the tissue-deforming force. The surface area of a 3-mm hollow fiber is more than 25 times the surface area of the open tip of a

28-gauge needle. Given that force is a product of pressure and area, the open tip of a 28-gauge needle will generate 874,000 times more deforming force than the pores of the hollow fiber catheter.

Another advantage of the hollow fiber catheter is the uniform drug delivery along the length of the catheter. Compared with that in multiple-pore catheters, transmural outflow of drug is reasonably homogeneous from the proximal to distal end. [10] Such linear homogeneity allows the use of long catheters up to several centimeters in length, which further increases the rate of delivery in the case that delivery to a large volume of brain tissue is required. With increased surface area of the hollow fiber, relatively large volumetric flow is possible at nominal flow velocities.

Background

The objective of Convection-Enhanced Delivery (CED)

CED is a delivery method for the delivery of drugs into the brain that circumvents the obstacles posed by the blood-brain barrier and dilution of infusates in the bloodstream. [11], [12], [13], [4], [14] It works by establishing a pressure gradient between cells via the bulk flow of infusates in the catheter, such that infusates will continuously diffuse away from the catheter, resulting in the widespread distribution of infusates in large areas of brain tissue. [15], [16]

Current development of CED

CED is optimal when the diameter of the catheter is small and the flow rate is low (<= 0.5 µl/minute). [5], [17] As of now, CED is capable of uniformly distributing infusate to cover distances of 1-2.9 cm from the site of catheter placement in cat and nonhuman primate brain.[15], [18] For CED-mediated infusion of adeno-associated virus, a 35.5-µl volume delivery covers nearly 75% of the putamen in rhesus monkeys. [11] The distribution of infused liposomes or viral vectors can be increased further when mannitol is coadministered to increase the size of the interstitial space and to promote diffusion. [4], [5]

Problem of the CED method for GBM treatment

A significant drawback of the CED method is its low infusion rate, such that it can take hours to days to deliver therapeutic doses. This low infusion rate required for CED is due to impedance mismatch discussed in the Significance section. In addition, attempting to infuse large volumes of drug over a short time period creates a deforming force on tissue, eventually narrowing the interstitial space and promoting a shear plane or tissue tearing. [5], [8], [17]

Recombinant adenoviral vectors

The RAdLUC and RAdGFP used in this study are first-generation replication-defective recombinant adenovirus type 5 vectors expressing the transgene under the transcriptional control of the human cytomegalovirus intermediate early promoter within the E1 region.

Firefly luciferase

Firefly luciferase is the light-emitting enzyme responsible for the bioluminescence of fireflies. It catalyzes the oxidation of firefly luciferin, which requires oxygen and ATP. To quantify the expression of firefly luciferase, an assay is developed to lengthen the duration of which light is produced from the action of firefly luciferase.

GFP

GFP stands for Green fluorescent protein, which exhibits bright green fluorescence when exposed to light in the blue to ultraviolet range. Therefore, GFP expressions can be visualized using fluorescence microscopy.

Experiments

The authors have conducted the following four studies to compare the distribution and efficacy of infusate delivery between using a 3-mm-long hollow fiber catheter, the newly designed catheter with millions of nanoscale pores to increase surface area and bulk flow, and a 28-gauge needle, a model of a single-lumen catheter of similar size, which is a standard single-port clinical catheter for CED. [4], [8], [19]

Study 1: Dye infusion into Agarose Gel (in vitro)

A total of 2 µl Evans blue dye was infused into a 4% agarose gel by using a microinjection pump via the two aforementioned catheters, with a flow rate of 0.1 µl/minute.

Study 2: Dye infusion into mice brain (in vivo)

Three mice were treated using the hollow fiber catheter, and three other mice using the 28-gauge needle. 2 µl of Evans blue dye was infused into the right striatum of each mice over 20 minutes at the flow rate of 0.1 µl/minute. Mice were killed and the brains were sectioned within 1 hour of dye infusion. An automatic picture area measurement program (ImageJ) was used to measure the area of dye distribution in each section, which was measured in every fifth 10-µm-thick section covering the entire labeled area of the brain to determine the total volume of dye distribution.

Study 3: Adenoviral-mediated Gene Transfer (Gene transfer and expression)

Three mice were injected with the adenoviral vector RAdLUC, which encodes the expression of firefly luciferase, into the striatum using the hollow fiber catheter, and three other mice using the 28-gauge needle. A total of 1.4 × 107 particles of RAdLUC are delivered in 2 µl of saline over 20 minutes at a flow rate of 0.1 µl/minute. The expression of luciferase was measured at 24, 48, and 72 hours after injection using in vivo bioluminescent imaging.

The experiment was repeated using an identical dose of virus and identical infusion parameters, except for using four mice per group. Mice were killed 48 hours after injection of RAdLUC. The

brains were removed, homogenized, and immediately assayed for luciferase expression using an in vitro activity assay.

Study 4: Adenoviral Vector Infusion (Distribution of gene transfer)

Firefly luciferase is a useful reporter gene to determine total gene transfer and expression but does not address the distribution of gene transfer into the brain parenchyma. Therefore, three mice were injected with the adenoviral vector RAdGFP into the striatum using the hollow fiber catheter, and three other mice using the 28-gauge needle. A total of 1.4 × 107 particles of RAdGFP are delivered in 2 µl of saline over 20 minutes at a flow rate of 0.1 µl/minute. Mice were killed two days after the administration of RAdGFP, whose brains were analyzed using fluorescence microscopy to visualize GFP expression. To quantify the area of gene delivery, measurements of GFP-positive areas were made rostral to caudal relative to the injection site, covering the majority of transduced tissue.

Results and Discussion

Results from Study 1: Dye infusion into Agarose Gel (in vitro)

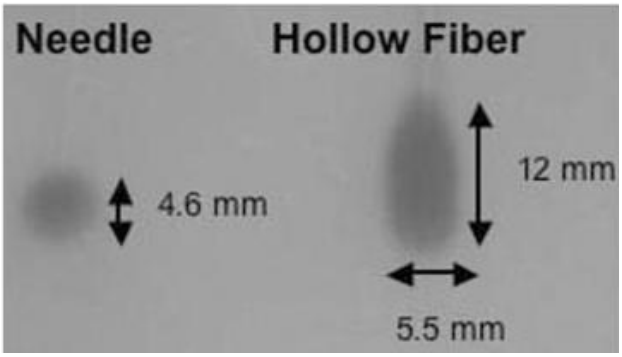

Figure 3 (adapted from the paper [3]). An image showing the distribution of 2 µl of dye infused into an agarose gel over 20 minutes using a 28-gauge needle or a 3-mm hollow fiber.

The volume of dye distribution was calculated according to the equation for the volume of an ellipsoid: $V = 4/3\pi abc$, where a, b, and c are the lengths of the three semi-axes. The infused volume using the hollow fiber catheter was calculated to be 189.97 mm^3 as opposed to 50.94 mm^3 using the conventional needle, which is 3.7 times greater.

Results from Study 2: Dye infusion into mice brain (in vivo)

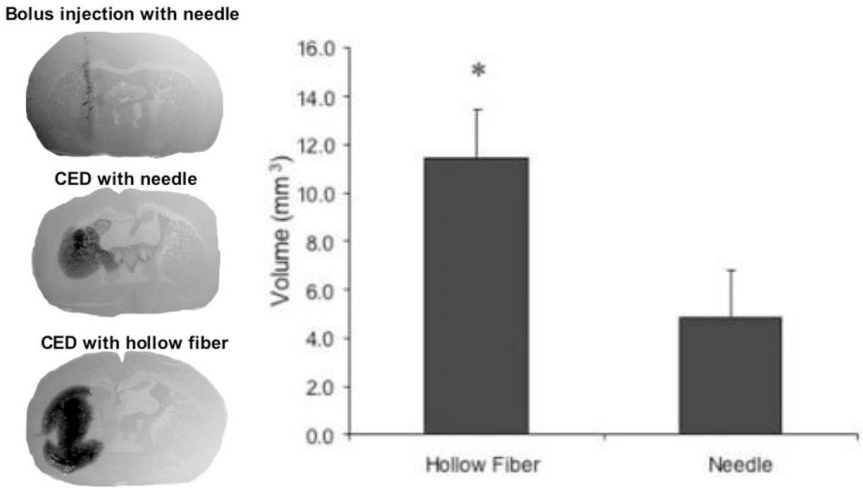

Figure 4 (adapted from the paper [3]). Left: Images of tissue sections depicting the distribution of 2 µl of dye infused into the mouse brain via stereotactic targeting to the striatum using a bolus injection as control (10 seconds), or CED (20-minute injection) with a 28-gauge needle, or a hollow fiber catheter. Right: Bar graph demonstrating the volume of dye distribution in the murine brain infused with 2 µl of dye over 20 minutes using a hollow fiber catheter or 28-gauge needle (three mice/group, five sections per animal). Use of the hollow fiber catheter significantly increased the volume of the brain labeled with dye. *$p \leq 0.05$, t-test.

There is a noticeable increase in dye distribution in coronal sections for CED via a hollow fiber catheter compared to a conventional needle (Figure 4, left). The distribution of Evans blue dye in the brain was 2.7 times larger for delivery using a hollow fiber catheter relative to a conventional needle (Figure 4, right).

Results from Study 3: Adenoviral-mediated Gene Transfer (Gene transfer and expression)

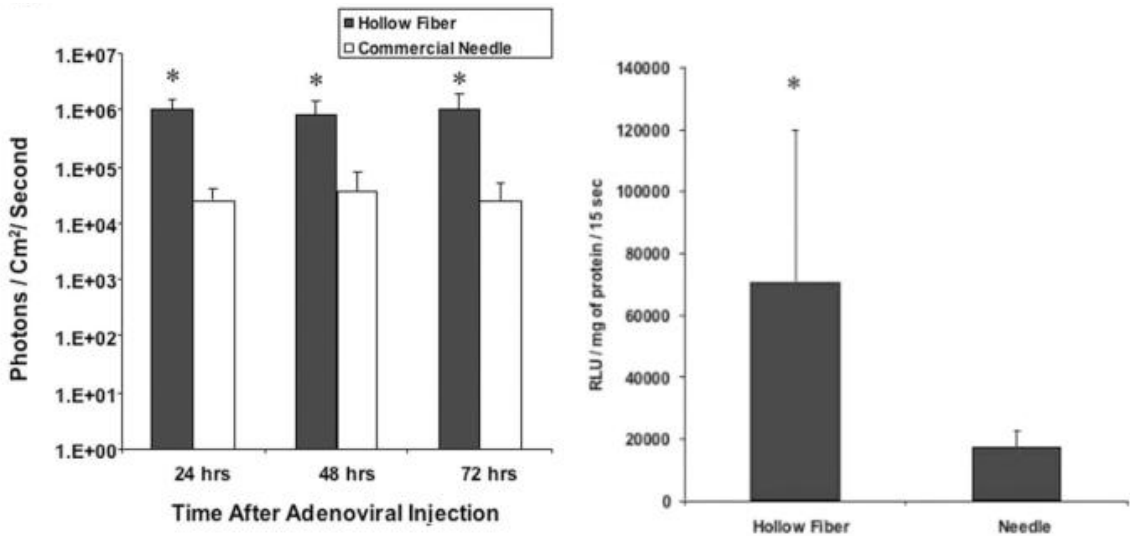

Figure 5 (adapted from the paper [3]). Left: Bar graph demonstrating firefly luciferase expression in animals. *p ≤ 0.05, Student t-test. Right: Bar graph revealing luciferase expression 48 hours after injection with RAdLUC. Only the murine brains were homogenized and assayed for luciferase activity 48 hours after gene delivery. *p ≤ 0.05, Student t-test. RLU = relative light units.

Luciferase in vivo imaging showed that the delivery of RAdLUC using the hollow fiber catheter increased gene expression by 10 times compared to using the conventional needle, and this significant difference remained constant from 24 to 72 hours after the injection of the virus (Figure 5, left).

From the in vivo assay, it was found that the whole-brain luciferase activity measured in vitro was four times greater when a hollow fiber catheter was used for infusion into the mouse striatum (Figure 5, right), indicating that hollow fiber catheter delivery increased gene transfer fourfold relative to needle transfer.

Results from Study 4: Adenoviral Vector Infusion (Distribution of gene transfer)

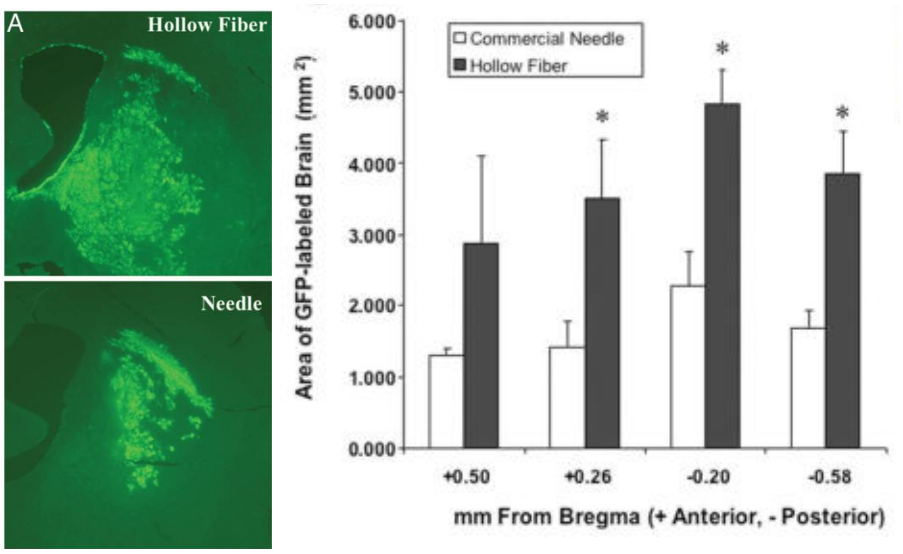

Figure 6 (adapted from the paper [3]). Left: Fluorescence microscopy images demonstrating the distribution of GFP-transduced cells in representative adult mice injected with RAdGFP delivered using a hollow fiber catheter or needle (three animals/group, flow rate of 0.1 µl/minute). Note the increase in gene transfer in representative sections when the catheter was used. Original magnification × 2. Right: Bar graph revealing the area of GFP-transduced brain tissue in four sections from three animals at the indicated distance (mm) from the bregma. Use of the hollow fiber catheter led to a significant increase in the labeled area throughout a large region of the brain. *p ≤ 0.05, Student t-test.

It is observed that brains infused using a hollow fiber catheter displayed a much larger area of transduced cells (Figure 6), and hence a greater distribution of gene transfer compared with that using a conventional needle.

In summary, results of both the in vitro gel model and in vivo mouse model indicated that the hollow fiber catheter significantly increased the distribution of Evans blue dye compared with a needle. In addition, results of both the in vivo imaging study and in vitro assay revealed a significant increase in total luciferase expression as well as the distribution of GFP when a hollow fiber catheter was used compared with a conventional needle. These results indicate that compared with single-lumen catheters, porous catheters may improve the distribution of CED-mediated drug delivery, as well as gene distribution and expression for gene therapy in the CNS.

Assessment

I think that the authors of this paper effectively addressed the problem put forward at the beginning of the paper, which is to evaluate the distribution and efficacy of drug therapy and gene therapy using a hollow fiber catheter in CED as compared to a conventional needle. However, there are aspects that the paper could improve on. Below are the good and bad points about the paper:

“Good”

- Very rich background information about CED is provided, which outlines the problem that this study is addressing very clearly.
- The design of the hollow fiber catheter is described in detail in the paper, such that it could be reproduced by other researchers to conduct other studies to propagate their findings.
- Photographs and the plots provided summarized the key results effectively, which makes the results of this paper accessible and understandable.

“Bad”

- There is not enough background information provided for the materials and methods used in the study, e.g. there is no explanation of what GFP stands for or how firefly luciferase enables the visualization of gene expression.
- The paragraphs in the Materials and Methods section can be more organized by introducing materials and methods according to the sequence in which the four studies are introduced. The sequence of the subtitles listed below makes the section especially hard to follow:
    - Mice and Experimental Groups
    - Anesthesia, Surgery, and Infusate Delivery
    - Recombinant Adenoviral Vectors
    - Dye Infusion Into Agarose Gel and Distribution Calculations
    - Perfusion and Tissue Processing
    - Measurement of Evans Blue and GFP Distribution in the Murine Brain
    - Firefly Luciferase Assays
- There is a typo for the flow rate in the catheter that is optimal for current CED methods, which is crucial for understanding the existing problem that this paper attempts to solve. In the first paragraph of the Discussion section, “This delivery method permits optimal distribution of infusate when a small-diameter catheter is used and when the flow rate is very low (that is, ≥ 0.5 µl/minute)”, the flow rate should have been <= 0.5 µl/minute instead of >= 0.5 µl/minute.

The research

In my opinion, the four studies conducted very comprehensively evaluated the use of a hollow fiber catheter compared to a conventional needle on the distribution and efficacy of the drug therapy and gene therapy in the central nervous system. The choice of infusate (Evans blue dye, RAdLUC encoding the firefly luciferase expression, and RAdGFP), and the choice of model (in vitro and rodents) for such comparisons are also appropriate considering that this research is the first to involve hollow fiber catheters in CED. For future directions, the following can be considered:

- Repeat the four studies using primate brains.
- Repeat the four studies on brain tumor tissues.
- Repeat the four studies to compare the distribution and efficacy of the drug therapy and gene therapy in the central nervous system between using a hollow fiber catheter and other existing methods that also increase the drug delivery rate in CED, such as co-administering mannitol with the use of a conventional needle which increases the size of the interstitial space and to promote diffusion.

Conclusion and Personal Relevance

In conclusion, this paper produced a successful prototype of the hollow fiber catheter to be used in CED to improve the distribution and efficacy of drugs therapy and gene therapy.

The catheter used in the current design of the implant device is not a hollow fiber catheter as described in this paper, but a single-lumen catheter that is commonly used in standard clinical CED for treatments for GBM. After discussing with the lab manager, I found out that it is because the current prototype of the implant device focuses on the functionality of the device. After this prototype passes tests to ensure that its functionality achieves our expectation, there is potential to replace the catheter with the one described in this paper so as to increase the upper limit of the drug delivery rate.

After completing a review for this paper, I have gained much more insights into the principles behind CED, and hence a greater motivation to participate in this project. I also came to realize the limitations of single-lumen catheters, for instance, the potential to deform brain tissues when the flow rate is too high. There is currently no upper bound set on the pump rate in the code implemented, and I will discuss with our project supervisor the possibility to implement such an upper bound.

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