Focused Ultrasound Assisted Delivery of Thiolated Nanoparticles in Tumor Microenvironments
(Technical Project)

Comparative Analysis of Sociocultural Status of the United States During Major FDA Regulation of Animal Testing
(STS Project)

A Thesis Prospectus
In STS 4500
Presented to
The Faculty of the
School of Engineering and Applied Science
University of Virginia
In Partial Fulfillment of the Requirements for the Degree
Bachelor of Science in Biomedical Engineering

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October 27, 2023

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On my honor as a University student, I have neither given nor received unauthorized aid on this assignment as defined by the Honor Guidelines for Thesis-Related Assignments.

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Introduction

Brain cancers such as glioblastomas are the most common central nervous system tumors and are extremely aggressive with rapid development (McFaline-Figueroa & Lee, 2018). Glioblastomas are characterized partly by low levels of immune cells (leukocytes), which limits the body's ability to fight them (Singh et al., 2021). Symptoms of brain tumors include mental impairment, seizures, and headaches, all of which can have a traumatic impact on one's life (Demeule et al., 2004; McFaline-Figueroa & Lee, 2018). Currently, patients have a mean survival of approximately 15 months after diagnosis, and procedures intending to treat these tumors have low success rates (Demeule et al., 2004). For children, brain cancer is the second leading cause of cancer mortality; for those under 34, it is fourth (Demeule et al., 2004). New innovative techniques are needed to help those with brain tumors, such as glioblastomas, survive and recover because the current techniques are not effective enough as evidenced by the poor outlook.

Many current research projects involve overcoming the blood brain barrier (BBB). The BBB is mainly composed of endothelial cells (Takeshita & Ransohoff, 2012). These endothelial cells limit what can enter the brain from the bloodstream thus shielding the brain from toxins (Ding et al., 2016). Focused ultrasound is a novel technology that has been used to open the BBB to allow for easier delivery of drugs (Gasca-Salas et al., 2021; Izadifar et al., 2020). This technology has the potential to be paired with a variety of other delivery techniques.

The proposed technical project aims to enhance the precision of drug delivery directly to the tumor site, minimizing potential side effects, while also circumventing the challenges presented by the BBB. These innovations could potentially extend the median survival rate of patients and have greater outcomes than current treatments, improving the quality of life for

those afflicted with glioblastomas. This will be done by generating a nanoparticle that is better able to bind to the tumor site through the addition of a small chemical group called a thiol. This thiol group can then interact with the increased level of thiol groups in the tumor microenvironment (Goerdeler et al., 2023).

Pre-clinical testing for novel methods such as this one generally use an animal model and up until recently were required to do so. Animal testing involves the use of various animal types as test subjects for new treatments and causes the death of countless animals as a result (Hajar, 2011). Animal testing is a topic heavily debated from both cultural and political fronts with many different justifications behind each viewpoint such as animal rights and the need to ensure human safety. The proposed STS project will explore the animal testing methods used to perform this and similar studies. This project seeks to gain an understanding of what social and political factors have led to changes in regulations regarding this testing. This will be studied through a literature review with a focus on times when major changes have occurred in the regulation.

Technical Topic

Microvascular endothelial cells are the main type of cell found in the blood-brain barrier (BBB), with pericytes and astrocytes surrounding them (Takeshita & Ransohoff, 2012). The endothelial cells act as a barrier and a mediator between the blood and the brain by forming tight junctions limiting what can enter the brain from the bloodstream, and by repulsing charged compounds (Ding et al., 2016). The BBB protects the brain from toxic substances and can limit the movement of inflammatory cells into the brain parenchyma (Takeshita & Ransohoff, 2012). The BBB can be bypassed by combining focused ultrasound therapy with microbubble injection (Gasca-Salas et al., 2021; Izadifar et al., 2020). This therapeutic method temporarily opens specific areas of the BBB, allowing more effective drug delivery (Gasca-Salas et al., 2021;

Izadifar et al., 2020). Additionally, it has been found that tumor microenvironments show an increased level of exofacial thiols relative to benign cells (Slezak et al., 2022). To take advantage of this unique property of tumor microenvironments, we propose to develop a thiolated nanoparticle design that leverages the increased levels of free thiols in tumor microenvironments as a means to target and deliver therapeutics specifically to cancerous cells.

Initially, we will generate a protocol for the utilization of Ellman's reagent in mouse brain endothelial cells (bEnd.3), murine glioma cells (GL261) (both *in vitro)*, and murine tissue (*ex vivo)* to color free-thiol groups for colorimetric quantification (*DTNB (Ellman's Reagent) (5,5-Dithio-Bis-(2-Nitrobenzoic Acid)*, n.d.). Measurements will be made using a spectrophotometer at 412 nm to confirm if the cancerous GL2621 cell line has greater numbers of thiols than the bEnd.3 cell line which would indicate that the thiolated nanoparticle should exhibit increased binding efficacy.

The next major step will be to synthesize thiolated and non-thiolated nanoparticles. These nanoparticles should have a zeta potential of +/- 2 mV and a diameter of 40-60 nm as quantified using dynamic light scattering with a Zetasizer. These values have shown success with similar plasmid delivery sizes and in similar cell lines in the past. The values are backed up by literature sources which showed that nanoparticles smaller than 200nm are ideal, as any higher will activate the lymphatic system and become removed from circulation, and that nanoparticles between 30 and 60 nm show the best binding ability (Hoshyar et al., 2016; Rizvi & Saleh, 2018). The values shown for zeta potential are verified by the literature which suggests that between -10 and 10 mV are best for binding (Clogston & Patri, 2011).

The nanoparticle binding efficiency will be measured via a fluorescent tag or High-performance liquid chromatography (HPLC) and compared to the non-thiolated nanoparticle

control. Based on the assay results, the number of thiol groups on the nanoparticle's surface will be optimized, as well as the size and main body composition by changing the parameters of the nanoparticle synthesis protocol.

The next step will require the utilization of focused ultrasound and microbubbles for nanoparticle binding to the tumor microenvironment. First focused ultrasound (FUS) parameters such as frequency, pressure, and exposure time will be optimized for the cell lines. A successfully optimized protocol will allow for the enhanced permeation of the thiolated nanoparticles through the BBB in a mouse model. The optimized FUS protocols will be combined with microbubbles and nanoparticle injection in an *in vivo* model. The binding will be analyzed in the animal model by using flow cytometry to quantify the difference in binding between the thiolated and unthiolated nanoparticles.

The accomplishment of these tasks will augment our understanding and capability to innovatively and effectively address the challenges posed by glioblastomas and similar brain cancers. Our proposed approach promises to enhance the precision and efficiency of drug delivery directly to the tumor site, minimizing systemic exposure and potential side effects, while also circumventing the challenges presented by the BBB. The synergy of these innovations could potentially greatly extend the median survival rate of patients and have greater outcomes than current treatments, improving the quality of life for those afflicted with these devastating diseases.

STS Topic

The history of animal testing is one intertwined with public outrage as a catalyst for change in many ways. A 2022 law, the FDA Modernization Act 2.0, removed the requirement for animal testing before drug sales (Hernandez, 2023). This law was supported by democrats

and republicans as well as by organizations concerned with animal welfare (Hernandez, 2023). This new law eliminates a requirement that stood since 1938 that required animal testing for drugs prior to marketing (Hajar, 2011). With the elimination of the animal testing requirement, animal testing as a whole may no longer be practiced (Hernandez, 2023). Inspired by these changes the question I hope to answer is: How have social and political attitudes toward animal testing welfare and consumer medical safety standards of the US changed since the inception of animal drug testing requirements in 1938 to when it ceased to be required in 2022?

In 1938 the Federal Food, Drug, and Cosmetic Act (FDCA) was passed and gave the Food and Drug Administration (FDA) the authorization to oversee the production, marketing, and distribution of food, drugs, and medical devices (Lam & Patel, 2023). This law was passed due to poisoning caused by an antibiotic, elixir sulfanilamide, which was not properly tested and thus contained the toxin diethylene glycol leading to the deaths of around one hundred people (Hajar, 2011; Lam & Patel, 2023). Prior to this law the FDA had compiled a list of drugs that had been approved but had either no benefit or induced harm calling these the "The American Chamber of Horrors" (Commissioner, 2019). These drugs started a push for new regulation but the elixir sulfanilamide poisoning caused widespread public outcry and fear which caused President Franklin Delano Roosevelt to sign the FDCA into law (Commissioner, 2019; Hajar, 2011). While this is the most popular cause, it has also been theorized that the law was passed due to influence from the pharmaceutical industry as larger companies are better able to afford to follow FDA regulation thus forcing out competition of smaller companies (Carpenter & Sin, 2007). These different factors together created an environment that allowed the passage of the law.

The FDA Modernization Act 2.0 signed into law in 2022 opened the door to alternatives to animal testing as this testing ceased to be required (Hernandez, 2023). This law was proposed by Rand Paul, a Republican senator, supported by non-partisan organizations such as PETA, and signed into law by Joe Biden, a Democratic president (Hernandez, 2023). Concerns about the reliance of the FDA on animal testing have been presented in Congress since 1998, which had led to limitations on animal cosmetic testing and now drug testing (Adashi et al., 2023). These concerns largely stem from a place of ethics and animal rights but are also augmented by studies that have shown limited translation of animal model results to human testing (Robinson et al., 2019). Taken together, these factors represent a very different environment than that present when the 1938 law was passed.

From the last two paragraphs, it is clear that the 1938 and the 2022 laws differ in the context surrounding their implementation. The 1938 law was inspired by public fear and outcry for their own safety coupled with the interests of large corporations. The 2022 law was again inspired by public outcry, although for animal rights rather than personal safety, but was this time paired with scientific questions about the efficacy of animal testing. These variations provide justification for why it is worthwhile to answer the research question.

To answer this question I will gather review articles discussing animal and cosmetic testing, look into the text of the two major regulator laws mentioned, look into review articles regarding animal cruelty at each time point, and attempt to explore news sources from both time points relating to reactions to the laws. These sources will mainly be gathered with a focus on both the date of publication and the publication source to help determine their ability to accurately paint the culture and politics of the time periods in question. These sources and the information gathered through them will be analyzed through the lens of Winners's "Do Artifacts

Have Politics?" in order to treat the practice of animal testing as an artifact that has inherent politics (Winner, 1980). This research and the variations discovered can provide insight into different paths toward legislation in this field and can help add context to future changes.

Conclusion

The technical work aims to create a thiolated nanoparticle that enhances transfection and binding efficiency in the tumor microenvironment. This nanoparticle has broad applications in cancer treatment, with a focus on brain cancer using focused ultrasound. The STS research project investigates the historical and social factors influencing the requirement and elimination of animal drug testing in medical marketing and development. These insights benefit both animal testing advocates and animal rights proponents. Animal testing advocates might explore alternative methods, while animal rights supporters gain historical context for their advocacy. Together these projects tackle the complexity of cancer drug development. The technical aspect seeks an innovative cancer treatment approach. Simultaneously, the STS project delves into the historical and ethical facets of treatment development, shedding light on changing perspectives over time.

Word count: 2025

Citations

Adashi, E. Y., O'Mahony, D. P., & Cohen, I. G. (2023). The FDA Modernization Act 2.0: Drug Testing in Animals is Rendered Optional. *The American Journal of Medicine*, *136*(9), 853–854. https://doi.org/10.1016/j.amjmed.2023.03.033

Carpenter, D., & Sin, G. (2007). Policy Tragedy and the Emergence of Regulation: The Food, Drug, and Cosmetic Act of 1938. *Studies in American Political Development*, *21*(2), 149–180. https://doi.org/10.1017/S0898588X0700020X

Clogston, J. D., & Patri, A. K. (2011). Zeta potential measurement. *Methods in Molecular Biology (Clifton, N.J.)*, *697*, 63–70. https://doi.org/10.1007/978-1-60327-198-1_6

Commissioner, Office of the. (2019). Part II: 1938, Food, Drug, Cosmetic Act. *FDA*. https://www.fda.gov/about-fda/changes-science-law-and-regulatory-authorities/part-ii-1938-food-drug-cosmetic-act

Demeule, M., Régina, A., Annabi, B., Bertrand, Y., Bojanowski, M. W., & Béliveau, R. (2004). Brain Endothelial Cells as Pharmacological Targets in Brain Tumors. *Molecular Neurobiology*, *30*(2), 157–184. https://doi.org/10.1385/MN:30:2:157

Ding, Q., Lu, P., Xia, Y., Ding, S., Fan, Y., Li, X., Han, P., Liu, J., Tian, D., & Liu, M. (2016). CXCL9: Evidence and contradictions for its role in tumor progression. *Cancer Medicine*, *5*(11), 3246–3259. https://doi.org/10.1002/cam4.934

DTNB (Ellman's Reagent) (5,5-dithio-bis-(2-nitrobenzoic acid). (n.d.). Retrieved October 24, 2023, from https://www.thermofisher.com/order/catalog/product/22582

Gasca-Salas, C., Fernández-Rodríguez, B., Pineda-Pardo, J. A., Rodríguez-Rojas, R., Obeso, I., Hernández-Fernández, F., del Álamo, M., Mata, D., Guida, P., Ordás-Bandera, C., Montero-Roblas, J. I., Martínez-Fernández, R., Foffani, G., Rachmilevitch, I., & Obeso,

J. A. (2021). Blood-brain barrier opening with focused ultrasound in Parkinson's disease dementia. *Nature Communications*, *12*(1), Article 1. https://doi.org/10.1038/s41467-021-21022-9

Goerdeler, F., Reuber, E. E., Lühle, J., Leichnitz, S., Freitag, A., Nedielkov, R., Groza, R., Ewers, H., Möller, H. M., Seeberger, P. H., & Moscovitz, O. (2023). Thiol-Mediated Uptake of a Cysteine-Containing Nanobody for Anticancer Drug Delivery. *ACS Central Science*, *9*(6), 1111–1118. https://doi.org/10.1021/acscentsci.3c00177

Hajar, R. (2011). Animal Testing and Medicine. *Heart Views : The Official Journal of the Gulf Heart Association*, *12*(1), 42. https://doi.org/10.4103/1995-705X.81548

Hernandez, J. (2023, January 12). The FDA no longer requires all drugs to be tested on animals before human trials. *NPR*. https://www.npr.org/2023/01/12/1148529799/fda-animal-testing-pharmaceuticals-drug-development

Hoshyar, N., Gray, S., Han, H., & Bao, G. (2016). The effect of nanoparticle size on in vivo pharmacokinetics and cellular interaction. *Nanomedicine*, *11*(6), 673–692. https://doi.org/10.2217/nnm.16.5

Izadifar, Z., Izadifar, Z., Chapman, D., & Babyn, P. (2020). An Introduction to High Intensity Focused Ultrasound: Systematic Review on Principles, Devices, and Clinical Applications. *Journal of Clinical Medicine*, *9*(2), 460. https://doi.org/10.3390/jcm9020460

Lam, C., & Patel, P. (2023). Food, Drug, and Cosmetic Act. In *StatPearls*. StatPearls Publishing. http://www.ncbi.nlm.nih.gov/books/NBK585046/

McFaline-Figueroa, J. R., & Lee, E. Q. (2018). Brain Tumors. *The American Journal of Medicine*, *131*(8), 874–882. https://doi.org/10.1016/j.amjmed.2017.12.039

Rizvi, S. A. A., & Saleh, A. M. (2018). Applications of nanoparticle systems in drug delivery technology. *Saudi Pharmaceutical Journal : SPJ*, *26*(1), 64–70. https://doi.org/10.1016/j.jsps.2017.10.012

Robinson, N. B., Krieger, K., Khan, F. M., Huffman, W., Chang, M., Naik, A., Yongle, R., Hameed, I., Krieger, K., Girardi, L. N., & Gaudino, M. (2019). The current state of animal models in research: A review. *International Journal of Surgery (London, England)*, *72*, 9–13. https://doi.org/10.1016/j.ijsu.2019.10.015

Singh, K., Hotchkiss, K. M., Patel, K. K., Wilkinson, D. S., Mohan, A. A., Cook, S. L., & Sampson, J. H. (2021). Enhancing T Cell Chemotaxis and Infiltration in Glioblastoma. *Cancers*, *13*(21), 5367. https://doi.org/10.3390/cancers13215367

Slezak, A. J., Mansurov, A., Raczy, M. M., Chang, K., Alpar, A. T., Lauterbach, A. L., Wallace, R. P., Weathered, R. K., Medellin, J. E. G., Battistella, C., Gray, L. T., Marchell, T. M., Gomes, S., Swartz, M. A., & Hubbell, J. A. (2022). Tumor Cell-Surface Binding of Immune Stimulating Polymeric Glyco-Adjuvant via Cysteine-Reactive Pyridyl Disulfide Promotes Antitumor Immunity. *ACS Central Science*, *8*(10), 1435–1446. https://doi.org/10.1021/acscentsci.2c00704

Takeshita, Y., & Ransohoff, R. M. (2012). Inflammatory cell trafficking across the blood-brain barrier (BBB): Chemokine regulation and in vitro models. *Immunological Reviews*, *248*(1), 228–239. https://doi.org/10.1111/j.1600-065X.2012.01127.x

Winner, L. (1980). Do Artifacts Have Politics? *Daedalus*, *109*(1), 121–136.