Technical Project: eLiposomes as a Targeted Thrombolytic Drug Delivery Vehicle During Sonothrombolysis

STS Project: Actor-Network Theory and FDA Approval in Recent Years

A Thesis Prospectus

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By
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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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General Research Problem: Blood Clot Treatment

What can be done to improve patient outcomes when treating severe blood clots?

The leading causes of death in the United States are cardiovascular diseases, the most deadly of which is ischemic heart disease followed by ischemic stroke (Stone et al., 2017). Ischemic events are blockages in someone's blood vessels that prevent the delivery of blood and oxygen to a specific organ, leading to cell death, tissue damage, and eventual organ failure. A major cause of ischemic events are blood clots, which can be difficult to treat successfully in time-sensitive cases depending on location, size, and time to treatment. Modern treatment plans include surgical intervention to remove the blood clot or the use of drugs such as anticoagulants and thrombolytics to break down the clot. Anticoagulants may fail to clear the clot effectively, while thrombolytics or surgical intervention are both tied to an elevated risk of bleeding and large chunks of clot debris (Goel & Jiang., 2020). In cases of life-threatening, time-sensitive occlusions like ischemic heart disease, pulmonary embolisms, and ischemic strokes there remains a need for a treatment that is rapid, safe, and reliably successful.

In order to attempt to develop such a treatment as well as consider a possible path to clinical usage, I aim to explore the technical and sociotechnical components of each problem respectively through two projects. For my technical project I will attempt to use targeted, ultrasound-sensitive, drug carrying nanoparticles as a new thrombolytic agent for therapeutic use in combination with ultrasound sonication. For my STS project, I aim to use Actor-Network theory to examine the network that a novel therapy navigates before gaining Food and Drug Administration (FDA) approval and how that network has itself been changed over time.

Technical Project: eLiposomes as a Targeted Thrombolytic Drug Delivery Vehicle During Sonothrombolysis

Can the application of eLiposomes to sonothrombolysis lead to improved thrombolysis rates whilst minimizing thrombolytic dosage?

The technical project consists of uniting two novel techniques in ultrasound therapeutics: sonothrombolysis and emulsion liposomes. Sonothrombolysis is the incorporation of therapeutic ultrasound as the sole therapy or in combination with other therapies to treat a blood clot blockage in blood vessels (Goel & Jiang, 2020). Emulsion liposomes (eLiposomes) are manufactured vesicles that contain a perfluorocarbon nanodroplet emulsion on the inside, and are sensitized to ultrasound by vaporizing the nanodroplets into expanding gas bubbles, bursting the vesicle membrane and releasing therapeutic drugs (Javadi, 2013).

Thrombolytics are medications used to dissolve blood clots within the circulatory system by promoting the breakdown of fibrin, a protein that forms the mesh-like structure of a blood clot. Thrombolytics primarily target the fibrin, which holds the clot together, and facilitate the body's natural clot-dissolving processes by converting plasminogen, a protein within the fibrin matrix, into plasmin, an enzyme that breaks down fibrin and leads to the dissolution of the clot. Thrombolytics are used in medical emergencies when rapid clot dissolution is essential, such as in the treatment of heart attacks, strokes, or massive pulmonary embolisms where they help restore blood flow to vital organs and prevent further tissue damage. Despite all these incredible

capabilities, in the United States, only 2% of ischemic stroke patients are administered thrombolytics (Eissa et al., 2012). This is because usage of thrombolytics is strictly vetted due to a slightly elevated risk of life-threatening bleeding in the brain and other organs as an unintended side effect at the current standard dose (Bivard et al., 2013).

To circumvent the risk of bleeding from using thrombolytic drugs, sonothrombolytic therapies have been researched as an alternative approach to clot destruction using cavitation agents like microbubbles or nanodroplets. Sonothrombolysis uses cavitation agents in combination with focused ultrasound to mechanically break up the fibrin matrix of the clot by causing cavitations in bubbles with the ultrasound field, rapidly growing and shrinking in response to the shifts between low and high pressure sound waves, leading to abrasions at the clot surface. Microbubbles and nanodroplets are typically made of a perfluorocarbon gas or liquid, a nonpolar and biologically inert gas that does not easily dissolve in the blood and is eventually exhaled from the lungs. While successful at dissolving blood clots, the usage of them can damage blood vessel tissue due to intense ultrasound pressures leading to violent collapses and jetting of gas bubbles (Li et al, 2019). Thrombolytics are occasionally used in combination with sonothrombolysis for an even more successful dual therapy, but the thrombolytics are still released freely into the blood vessel and will ultimately be found throughout the body rather than just at the blood clot site, maintaining a risk for side effects even at reduced dosages (Goel & Jiang, 2020). My goal is selectively release thrombolytics with focused ultrasound at the site of the blood clot to minimize the spread of thrombolytics to the rest of the body.

I aim to accomplish this by improving upon the “Ultra” method described by Javadi et al. to internalize a nanodroplet in a liposome vesicle initially carrying the thrombolytic (2013). I will also attach the cyclic RGD protein sequence to the vesicle membrane as it specifically binds to platelet integrin αIIbβ3, a protein that is only significantly expressed on platelet cells that are forming blood clots with red blood cells, allowing for clot specific binding by the liposome (Zhang et al., 2018). An ultrasound field focused at the clot will vaporize the nanodroplet inside the liposome into a microbubble several times its initial volume, selectively releasing the thrombolytic by breaking open the liposome only at the blood clot site. Additionally, the microbubble will cavitate with the ultrasound field to mechanically breakdown the clot whilst the thrombolytic also dissolves the clot (Dixon et al., 2019).

l will first characterize the ultrasound sensitivity of the liposome by sonicating it with ultrasound with parameters (1MHz, 1MPa, 7.5% duty cycle) used in other studies related to nanodroplet sonication (Goel et al., 2021) and evaluating release with a calcein fluorescence assay. I will then evaluate the efficiency of my cavitation agent compared to other used cavitation agents and freely circulating thrombolytics in a flow loop. My hope is to demonstrate comparable or superior thrombolysis rates compared to nanodroplets and the standard dosage of unencapsulated thrombolytics, whilst using a fraction of the standard dosage.

STS Project: Actor-Network Theory and FDA Approval in Recent Years

How have human and non-human actors been shaped in the cases of FDA approval, and have they shaped the process of FDA approval?

Assuming my technical project is successful in its goals, I would then be interested in analyzing the FDA approval process for clinical translation. The STS project is an exploration using the lens of Actor-Network Theory (ANT) of the human and non-human actors that novel therapeutic treatments must interact with before gaining FDA approval for use with patients in the United States and how it has changed in the 21st century. The FDA approval process relies upon multi-phase clinical testing of a new drug or therapy in human volunteers and patients after the presentation of convincing pre-clinical evidence of a drug's efficacy in treating a disease in animal models. However, to examine the system that this process operates within in purely social terms would fail to capture the interplay of human actors and non-human elements that determine the success or failure of approval. To that end, I want to apply ANT to analyze the mutual shaping and dynamics between actors and actants in the process of some cases of FDA approval in order to develop a better understanding of the priorities of the approval process and also to determine whether they have changed over time.

Actor-Network Theory is a methodological approach devised by Bruno Latour to analyze sociotechnical systems involved in producing facts about the world, such as scientific research or disease diagnosis, and examining how common notions of truth, "black boxes", are constructed and eventually uncritically accepted. It does so by considering all acting components (actants) in a system, whether they are human or non-human, and it strives to have an emphasis on symmetry in the importance of shaping between human and non-human actants in the construction of facts (Walsham, 1997). It does not suggest that scientific facts are purely socially constructed nor does it suggest that scientific facts are objectively true, it merely tries to examine what factors lead to the acceptance of something as fact within a system.

The FDA approval is a very complicated, expensive, and long process that outputs black boxes about the efficacy and safety of the drugs and therapies that it approves. It consists of pre-clinical testing, then three phases of patient testing in human volunteers to demonstrate that a drug is non-toxic, analyze the cost-benefit of side effects compared to therapeutic success, and to stay vigilant for unforeseen side effects that occur in the mid to long term (Bonet Olivencia & Sasangohar, 2021). Work has previously been done on applying concepts from ANT on the FDA approval process to analyze the dynamics between the pharmaceutical industry, doctors, patients, and the FDA in the construction of the black box of approval, with points about the limitations of the approval process such as the overrepresentation of adult men in clinical trials and the moral hazard of having pharmaceutical companies running their own trials that they are allowed to select data from (Busfield, 2006). However, this analysis has primarily centered a sociological over a sociotechnical perspective, with most of the emphasis being on relationships between human actors, particularly between the FDA and pharmaceutical companies. Being very broad in scope, it additionally does not examine a particular case of drug approval to delineate exact actants, but instead refers generally to the overall process. While unrelated to FDA approval, ANT has also been used to examine the construction of health-related knowledge on a sociotechnical level that is more symmetric between human and non-human actants, such as the

effects of cholesterol on heart disease or patient adherence to breast cancer treatment (Cabling et al., 2022; Garrety, 1997).

While an important overview of the tensions between pharmaceutical regulation and industry exists using some ANT concepts, there remains a novelty in examining particular cases of FDA drug approval over time through a sociotechnical lens that emphasizes the importance of symmetry in ANT. In addition to highlighting the role of non-human actants to have a more comprehensive understanding of the system, by looking at cases of FDA approvals at different points in time insight can be gained into how the approval system itself has been shaped by its components over time, with potential extrapolations of how the system will continue to evolve in the future. To accomplish this, I have limited my scope to analyzing the FDA approvals of three drugs across time: Doxil, Ponatinib, and Aduhelm, each approved respectively in 1995, 2012, and 2021 (Barenholz, 2012; Cortes et al., 2013; Dyer, 2022). Doxil was chosen for analysis due to its similar targeted liposomal delivery system of a pre-existing toxic drug like my technical project. The latter two drugs have been involved in serious scandals relating to the FDA, and I aim to use ANT to compare and contrast the components of each drug approval to determine whether significant changes have occurred in the approval process over time that may have contributed to the undetected side effects of Ponatinib and the rushed approval of Aduhelm.

To do this, I will investigate primary documents of each phase of testing during clinical trials to determine the non-human attributes of the tests, such as differences in the number of participants, the age range of participants, whether the approval process was fast-tracked or priority reviewed, whether there was the presence of a control group where applicable, whether there were different doses of the drug evaluated in humans to determine the minimum effective dose, and any other important components of the test that may affect how comprehensive it was evaluated. I will also look for regulatory changes or new laws that affect regulation such as the Food and Drug Administration Amendments Act of 2007 to see if there were any stated changes to the requirements for pre-clinical data or clinical trials and whether there were any changes in the relationships between human actors. If any news sources report notable shifts in relationships between actants from the contemporary standard procedure in the cases of the two scandals, I will also note those. The goal is to be able to make sufficiently comprehensive graphs of the combined human and non-human actants for each drug approval to determine qualitatively if there have been large shifts in the system of FDA drug approval over time, and if so, whether they follow a trend.

Conclusion

By developing a deeper understanding of the FDA approval process, I hope to more thoughtfully consider what it means for a product to gain FDA approval and the feasibility of my technical project coming into clinical use with or without a sponsor, assuming its success. By working on developing my technical product, I hope to gain deeper insight into the dynamics of thrombolysis, and as to whether pairing cavitation agents and thrombolytics into one particle would improve the rates of thrombolysis whilst allowing for a notable reduction in the risk of hemorrhaging. Overall, I hope to combine a new insight into the FDA approval process with a

novel treatment method to help the millions of people suffering from blood clot related illnesses every year to survive and continue to lead fruitful and happy lives.

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