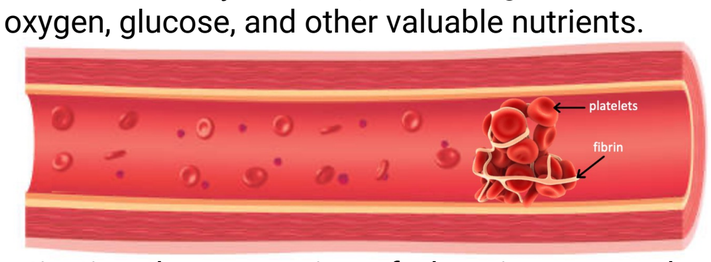
Design of a Novel, Dual-Functioning, Tissue Plasminogen Activator and Factor XI-Inhibiting Anticoagulant Therapeutic for Rapid Ischemic Stroke Treatment

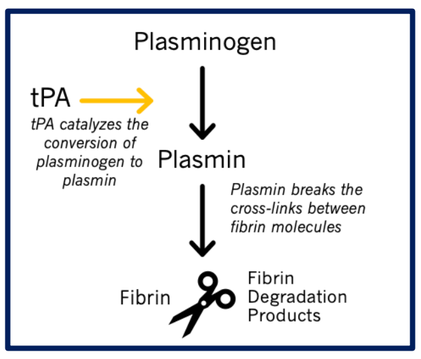
Introduction

- Stroke is the second leading cause of death worldwide, with 15 million people suffering from its effects each year. This impacted group has a 5.5 million mortality rate, with an additional 50% of survivors suffering from chronic disabilities (WHO & Donkor).
- Most strokes (87%) are classified as ischemic, where an artery narrows or becomes wholly blocked, restricting normal blood flow and depriving the brain of oxygen, glucose, and other valuable nutrients.



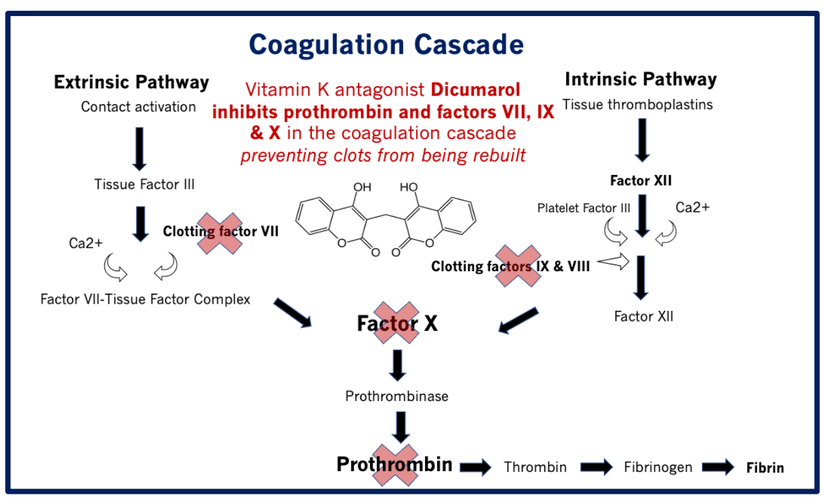
Currently, Tissue Plasminogen Activator (tPA) is the leading treatment for ischemic stroke. It is the only drug approved by the FDA for acute ischemic stroke, which functions by activating the conversion of plasminogen to plasmin, an enzyme responsible for the breakdown of clots.

- There remain significant challenges in emergency ischemic stroke treatment: the inability of bench-side to clinical trial candidates to deter the coagulation cascade (the process by which platelets and fibrin continue to accumulate at the clot site), avoid the risk of reperfusion injury, and ensure tPA is administered precisely
- A novel, dual-functioning clot removal and anticoagulant system has life-saving implications.



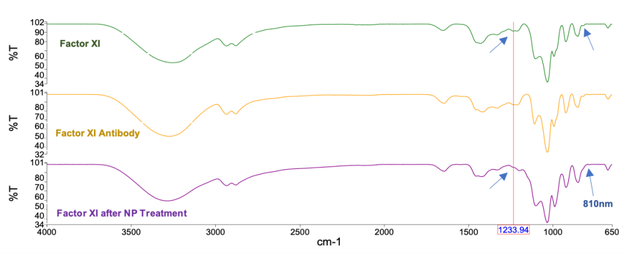
Antagonist to Deter the Coagulation Cascade

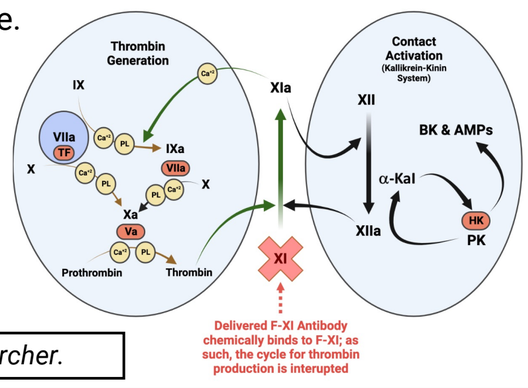
- The coagulation cascade is the process through which a blood clot is actively built. There are two classifications: hemostasis and pathologic thrombosis.
- The two major pathways of the cascade are the intrinsic and extrinsic pathways, which combine to form the common pathway.



There has been no attempt to break apart the clot while deterring the coagulation cascade. Thus, a therapeutic that simultaneously cleaves the formation of fibrin and deters the accumulation of platelets and fibrin would relieve a significant challenge in the bench-side to the clinical trial process.

- In the first iteration of the anticoagulant component, Vitamin K antagonist Dicumarol, a coumarin-like compound found in sweet clover, was identified as a candidate that successfully inhibits fibrin formation.
- As an additional component of the research, it was found that a Vitamin K antagonist like Dicumarol is not suitable in some cases, as it must be used over a prolonged period for effectiveness and poses an additional risk of bleeding. As such, an F-XI antibody that does not pose such risk was selected and subsequentially verified as a viable alternative.





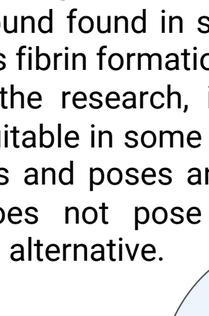
All data and images were created by the student researcher.

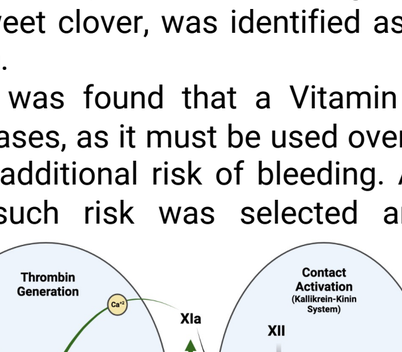
Fabrication of the Therapeutic Structure

The engineering of the novel, dual functioning, tPA and Dicumarol-loaded therapeutic was broken down into a series of steps, initializing with the fabrication of the interior Dicumarol-Fe_3O_4 nanoparticle system.

1 *Synthesis of Interior Anticoagulant-Fe_3O_4 Nanoparticles*

Carboxylic-acid coated Fe_3O_4 nanoparticles provide several advantages for use as the particles in the center of the microbubble drug delivery system. These NPS were fabricated in a repeated fashion by adding layers of EDC, chitosan, and the anticoagulant, respectively verified by ATR-FTIR analysis (right).





2 *Fabrication of SiO_2-tPA Component*

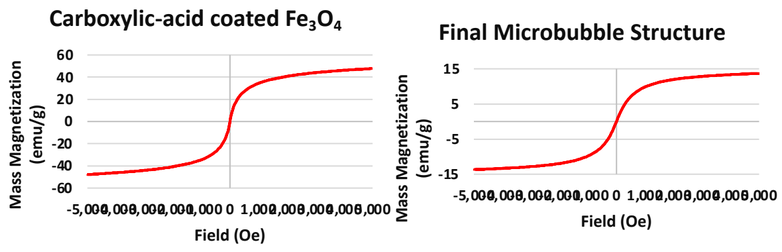
SiO_2-tPA consists of the secondary layer encapsulating the drug-carrying Fe_3O_4 nanoparticles, offering a medium for continued tPA stability and appropriate release at the clot site. SiO_2 was prepared by mixing ethanol, deionized water, TEOS, and NH_4OH under mechanical stirring, validated by ATR-FTIR spectroscopy.

3 *DLS Particle Sizing*

DLS particle sizing of the progressive coating steps, beginning with the original COOH-Fe_3O_4 NPs highlights growth from ~23nm to 41nm.

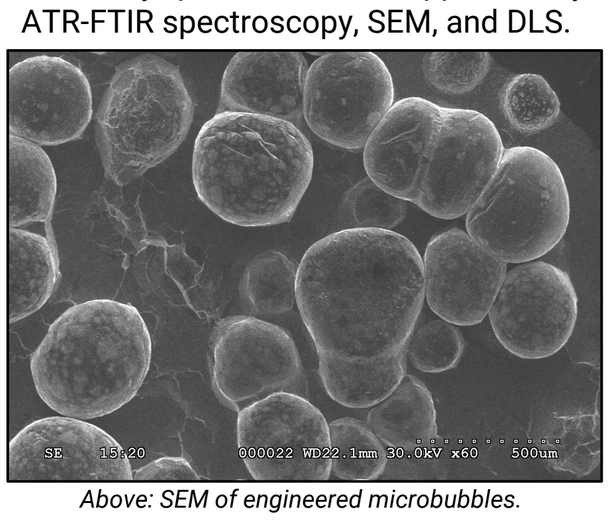
Delivery and Functionality of Microbubbles

4 *Mass Magnetization Study*

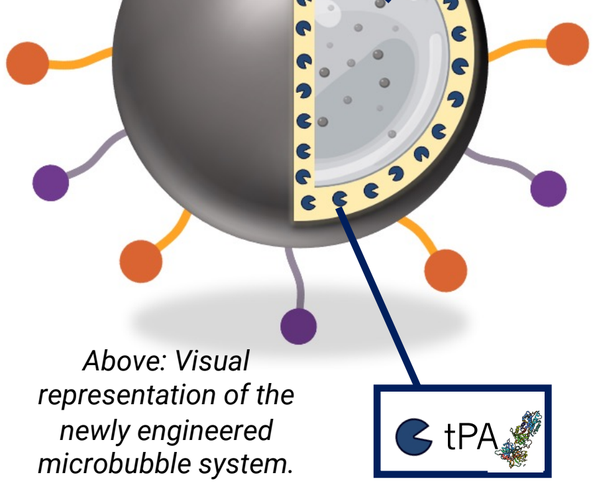


The magnetic susceptibility of the newly purchased COOH-coated Fe_3O_4 compared to that of the completed microbubble structure demonstrates that the final delivery system remains magnetic, with a +/- 15 emu/g mass magnetization.

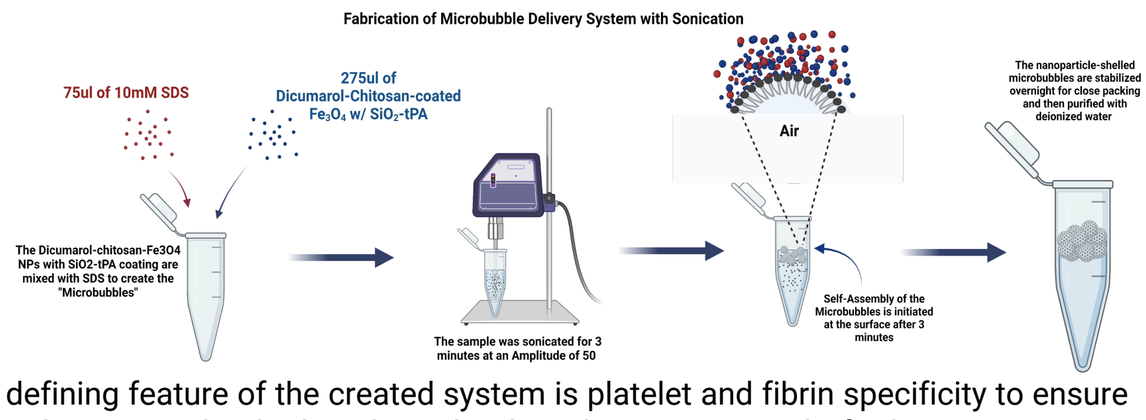
Microbubble shells were created via sonication of these components in SDS/shape with an average diameter of ~200um (process summarized below). Each successive synthetic step in the assembly process was supported by ATR-FTIR spectroscopy, SEM, and DLS.



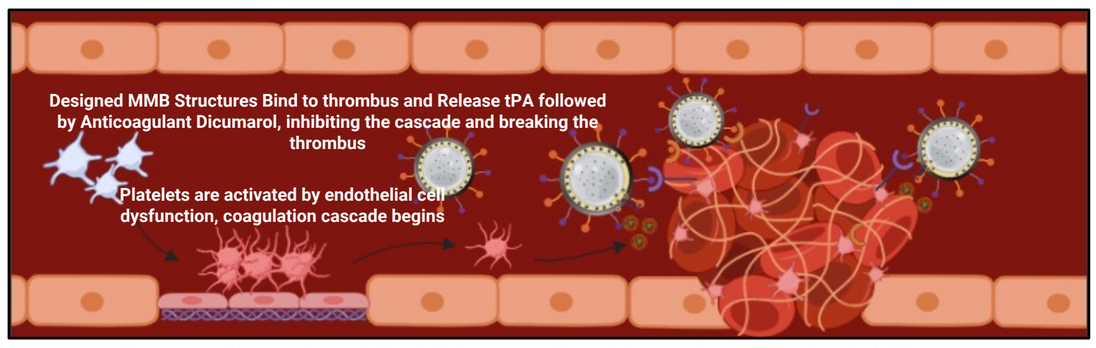
Above: SEM of engineered microbubbles.



Above: Visual representation of the newly engineered microbubble system.



A defining feature of the created system is platelet and fibrin specificity to ensure the therapeutic binds directly at the thrombus site instead of releasing in a non-localized manner. Integrin αIIbβ3 is expressed at high levels in platelets and their progenitor cells, playing a central role in hemostasis and arterial thrombosis. For specific binding to platelet αIIbβ3, a platelet binding peptide and a fibrin binding peptide were conjugated. The peptides were then attached to the chitosan-coated exterior of the previously fabricated microbubble, completing the synthesis of the dual-targeting system by combining the nanoparticles containing Dicumarol Fe_3O_4 nanoparticles and SiO_2-tPA surface.



Above: An illustration of the full functionality of the engineered microbubble system.

Clot Emulation & Verification

A vertical channel gel system composed of fibrinogen, thrombin, and agarose was developed to validate the effectiveness of fibrin lysis based on the engineered structure. Liquid gels containing fibrinogen, thrombin, and agarose were placed in each channel and refrigerated for cooling. A timed, *in-vitro* study was completed using five orientations to determine the efficacy of the engineered system. 15 ul of each treatment were added to the top of each vertical channel gel for 10 gels with 2

of each type. The gels were then incubated at 37°C and observed over 12 hours, with the change in fibrin area observed. The microbubble containing the peptide (blue) resulted in a ~20% of the fibrin dissolved, which is near twice as high as free tPA (orange).

The student researcher's blood was used to gauge the efficacy of the engineered system. BleedStop is distributed along the edges of the well-absorbed blood to form a solidified "halo" shape leaving the center clear and empty. 15µL of each of the fibrinolytic drugs to be tested were added with a multichannel pipette. The degradation of the halo clots was measured straight after with a plate reader, The dissolution of the halo-shaped human blood clots was compared to that of free tPA versus the MMB-SiO_2-tPA.