5-2022

Clot Analogs for the Development of Improved Treatment Methods In Ischemic Stroke

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Clot Analogs for the Development of Improved Treatment Methods in Ischemic Stroke

An Honors Thesis submitted in partial fulfillment of the requirements of Honors Studies in Biomedical Engineering

By

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Spring 2022

Biomedical Engineering

College of Engineering

The University of Arkansas

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Abstract

According to the CDC, nearly 800,000 individuals experience stroke each year in the United States [1]. Greater than 70% of strokes are of ischemic etiology and involve the occlusion of key arteries in the cardiovascular system [2]. Tissue plasminogen activator (IV-tPA) is the current gold standard for thrombolytic approaches [3]; however, this therapeutic is only effective between 3 and 4.5 hours from the patient's last know well [4]. As a result, less than 5% of acute ischemic stroke patients receive IV-tPA. In order to increase the viability of AIS treatment as a whole, it is essential that thrombolytic techniques are combined with recanalization methods – so that the individuals unable to present to the emergency department during the treatment window still have options. The proposed device aims to decrease exposure to ischemia and increase the viability of reperfusion by combining precedent with novelty. The study seeks to show the potential for benchtop laboratory modeling of blood clots and plasmin using gelatin and bleach respectively.

Section I: Introduction

The modernization of medicine through the past 25 years has shown great potential in curative technologies. However, for a small group of illnesses, physiological complexity still eludes the rapid development of healthcare. Cerebrovascular accident (CVA), or stroke, is one such illness that presents substantial complexity in the development of treatment techniques. Stroke is a class of cerebrovascular dysfunction that debilitates blood vessels responsible for transportation of oxygen to the brain. According to the British Medicine Journal, one in six people will have a stroke in their lifetime, more than 13.7 million have a stroke each year, and 5.8 million a year die as a result [2]. The estimated cost of stroke between 2017 and 2018 – including medical fees, medications to treat stroke, and missed days of work – amounted to $53 billion [1]. Clinical presentations are categorized into three classes: acute ischemic stroke, hemorrhagic stroke, and transient ischemic attack. The most common of these is AIS.

AIS results from the occlusion of cerebral arteries. It is defined as the sudden occlusion of a blood vessel and a resultant loss in neurological function [4]. The two most common occlusion agents in AIS are the thrombus and the embolus. While the origin and the focality of these agents is slightly nuanced, both contribute to the development of AIS. Atherosclerosis, or the deposition of plaque on the inner walls of an artery, is the most frequent cause of thrombus formation [5]. In the case of AIS, prolonged aggregation of plaque creates unstable, vulnerable lesions in cerebral arteries that then fall victim to atherosclerotic plaque rupture – wherein the clotting cascade is activated and thrombi form [6]. Importantly, once the clotting cascade has activated, a glycoprotein called fibrinogen is activated. Fibrinogen is converted to fibrin which is a primary constituent of blood clots [7]. Alternatively, AIS development can begin prior to the perfusion of cerebral arteries.

Cardio-embolic thrombosis is one such clotting pathway that begins when a thrombus forms in a distal region of the circulatory system. This thrombus may then shed small fragments, called emboli, that are transported to the cerebral arteries [8].

Nearly 70% of global stroke cases are ischemic in nature, and incidence increases to 87% when considering the United States alone [2]. AIS has therefore remained at the forefront of stroke research efforts since its initial characterization. While physicians of the 20th century employed protocols for the management and rehabilitation of stroke patients, they were limited by the technology of their time [3]. As such, localized, intra-arterial treatment was highly experimental and was rarely performed secondary to a high risk and a marginal precedent. However, the development of diagnostic imaging and the increased precision of vascular access has allowed for the widespread use of new treatment paradigms in AIS: thrombectomy and thrombolysis [9], [10]. Mechanical thrombectomy seeks to remove clots entirely via catheterization [9]. Thrombolysis is used to dissolve clots that occlude an artery [10]. In both types of treatments, the fundamental outcome is reperfusion of ischemic tissue.

Intravenous tissue plasminogen activator (IV-tPA), or alteplase, was FDA approved in 1995 and remains the only thrombolytic agent with such approval for stroke treatment [3]. It is through the development of IV-tPA that a new “gold standard” has been established for AIS treatment [3]. As the methodologies involving alteplase have evolved, three main principles of AIS care have emerged: to “achieve timely recanalization of the occluded artery and reperfusion of the ischemic tissue,” to “optimize collateral flow,” and to “avoid secondary brain injury” [11]. Recanalization is the process of reinstating blood flow to the cerebral arteries that were originally occluded in the

ischemic insult. Reperfusion is the restoration of non-ischemic conditions via return of blood flow to the previously hypoperfused areas of the brain [11]. Reperfusion via recanalization is essential for successful stroke treatment [12]. In AIS, the ischemic penumbra is the primary target for thrombolytic treatment. The ischemic penumbra is a mass of cerebrovascular tissue that emanates from the central arterial occlusion and deteriorates with the passage of time [12]. Thus, timely thrombolytic treatment is one of the most important factors for improving outcomes in patients with stroke [11].

Presently, with alteplase as the "gold standard," the window for successful treatment is 3 to 4.5 hours [4]. The National Institute of Neurological Disorders and Stroke (NINDS) reported that stroke victims who are administered IV-tPA within a 3 hour window from their last known well are 30% more likely to "have minimal or no disability at 3 months than those who received placebo" [4]. With the complications inherent in stroke presentation, it is unrealistic to expect that alteplase alone is a universal solution to AIS, which is a reason why less than 5% of individuals with AIS receive this treatment [10]. Furthermore, the fixed pharmacokinetics of alteplase cannot be changed, meaning the percentage of alteplase clearance due to time since last-know well in stroke has not changed for the past 15 years [10], [13]. Additionally, a 2013 study reported that the rate of effective recanalization – such that ischemia is reversed – is only 25% ; and with this, there is still a 30% chance that existing thrombi will reocclude another site within the cerebral network to cause further complications [5]. Therefore, the limiting factor in AIS treatment is not the thrombolytic agent, but the concurrent therapies used to propagate safe recanalization for patients that present outside the critical zone of 3 to 4.5 hours. As a result, it is imperative to investigate complementary therapies to be used in tandem with thrombolytics.

Literature indicates that the optimal time frame for alteplase therapy is between 3 and 4.5 hours [4]. For individuals with AIS that present after 4.5 hours, fewer treatment options exist. Mechanical thrombectomy is a therapy with bright prospects as a complementary therapy to IV alteplase. Mobilization of emboli in the cerebral vessels poses a significant threat in all AIS patients; therefore, complete removal of the blood clot responsible for ischemia is one solution that has been investigated to promote reperfusion. The Solitaire FR Device is a mechanical thrombectomy tool developed by Medtronic. Instead of prioritizing clot dissolution for AIS treatment, this device was made with the express purpose of removing thrombi from cerebral arteries. The Solitaire FR Device does so via sequential deployment of a microcatheter and nitinol mesh stenting system. The device begins reperfusion upon contact with the occlusive thrombus. Afterwards, the nitinol traps the clot inside its meshing to allow for clot removal.

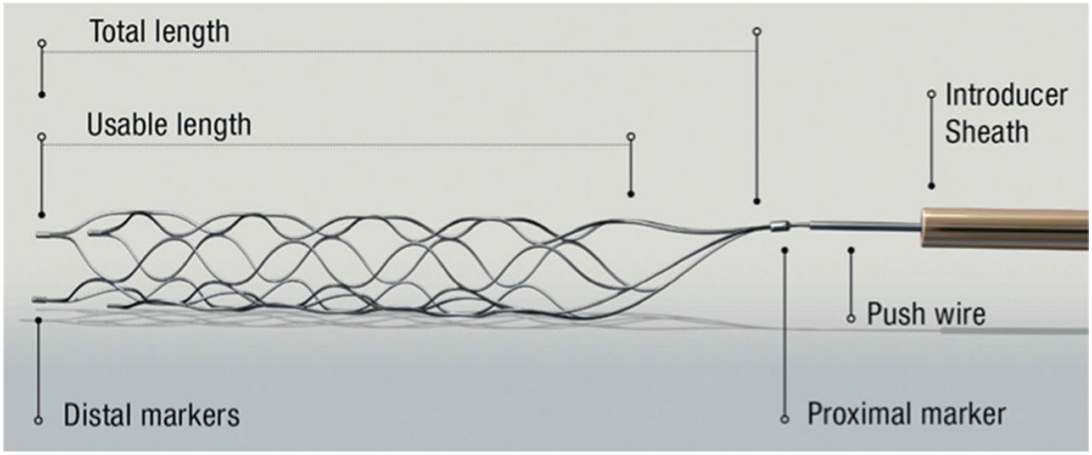

Figure 1: illustrating the Medtronic Solitaire FR Device for thrombectomy [12]

A 2011 study with the Solitaire FR thrombectomy device was 89% successful in recanalization with 9% associated operative complications [14]. Moreover, a 2015 study of the Solitaire FR

thrombectomy device illustrated reduced “severity of post-stroke disability and increased… rate of functional independence” when treated within 8 hours of last known well [9].

Combination therapy of thrombolytic agents and mechanical thrombectomy proves advantageous for non-ideal presentations of AIS. Whereas less than 5% of individuals with AIS receive intravenous alteplase [10], thrombectomy can be performed with greater liberty. Consequently, further investigation into this treatment paradigm is warranted for the improvement of stroke therapies. Although the time since last known well cannot always be controlled, there exists plenty of opportunity to improve quality and variety of care for those suffering from AIS.

To address the need for greater quality of care in individuals with AIS, the Cardiovascular Biomechanics laboratory (CB Lab) has proposed a novel stroke treatment device. In theory, this device will offer better prospects to individuals that present to the emergency department after the alteplase threshold of 3 to 4.5 hours. Though similar to the Solitaire FR Device, the implementation of new techniques provides ample opportunity for the improvement for this new device. However, before these techniques may be implemented on a large scale, they must first be validated individually. Hence, a benchtop model of the stroke-clot pathway was created. This model was created in order to minimize expenses and to maximize feature development. The focus of this thesis rests in the advancement of an *in-vitro* model for simulating a novel device created by the CB Lab. Ultimately, the benchtop simulation will substantiate the efficacy of each feature proposed for the stroke treatment device and will provide an outline for implementation *in-vivo*.

Section II: Materials and Methods

Section 2.1: Gelatin and Bleach

The aforementioned benchtop simulation was composed of two analogs. Fibrin was represented through the use of gelatin and plasmin was represented through the use of bleach. Knox unflavored gelatin powder was used to simulate blood clots and Clorox bleach was used to simulate a plasmin analog.

Table 1: the benchtop model for device simulation

In-vivo	***In-vitro***
Fibrin	Gelatin
Plasmin	Bleach

Section 2.2: Micropore Membranes

The micropore membranes were donated from Dr. Ranil Wickramasinghe's laboratory. They are designed to function in plasmin delivery. In the *in-vitro* studies conducted for this project, the micropore membranes delivered bleach to gelatin. **Figure 2** displays the micropore membranes at 10,000x magnification.

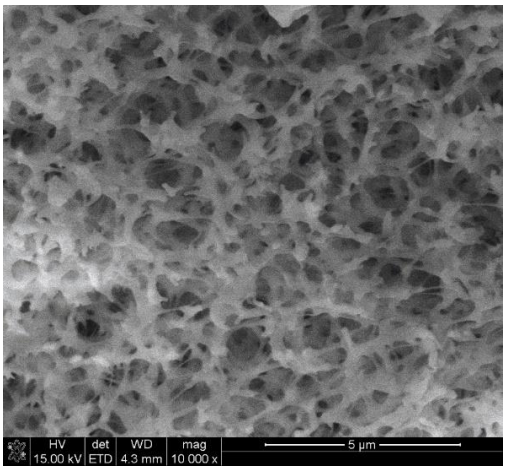

Figure 2: scanning electron microscopy image of micropore membranes

Section 2.3: Gelatin Casting Procedure

Knox unflavored gelatin was used for all qualitative and quantitative data collection. Liquid gelatin was first poured in a silicon cube mold then placed in a refrigerator to set. In order to normalize the preparation of gelatin, the following procedure was created and followed:

<u>Gelatin preparation:</u>

1. Boil 236.5mL water in an electric kettle
2. Add 13 g of gelatin to the boiling water
3. Stir until completely dissolved
4. Slowly add 236.5mL of cold water; continue stirring during this process
5. Add approximately 87.8mL of gelatin mixture to each cube mold
6. Refrigerate for 4 hours or until firm

Figure 3: gelatin casted with micropore membranes and syringes

Section 2.4: Dissolution of Gelatin

The dissolution of gelatin was performed in a number of different ways. Primary trials involved quantifying the percentage mass loss after leaving gelatin in varying concentrations of bleach and water dilutions. The first ratio was 100:1 water to bleach. Subsequent trials were 5:1, 1:1, and 0:1, or 100% bleach. Each clot analog was placed in bleach for 30 minutes. The dissolution of these clot analogs was measured quantitively via percentage mass change. Bleach and gelatin trials were also performed with the addition of manual, periodic stirring. As shown in **Figure 4**, the beaker on the left-hand side contains gelatin in a bleach bath at time t = 0 minutes. The right-hand picture illustrates gelatin in a bleach bath at time t = 20 minutes.

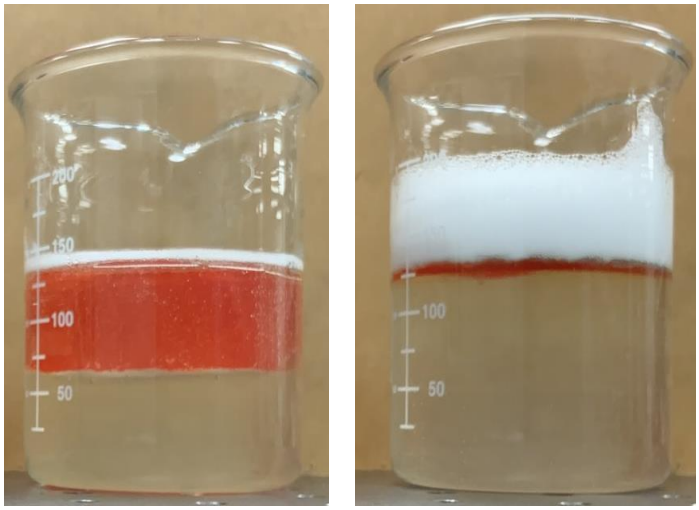

Figure 4: an example of gelatin dissolution with bleach

Dissolution of gelatin was also monitored via timelapse recordings. This was especially necessary for the implementation of the micropore membrane as dissolution was much subtler when delivered through the membrane.

Section 2.5: Device Design Features

Following the characterization of gelatin dissolution, the next step was the implementation of device design features. The micropore membrane, TPU filament, and nitinol wire were all used in order to simulate the conditions of the device. Bleach was delivered through the micropore membrane and inside the gelatin.

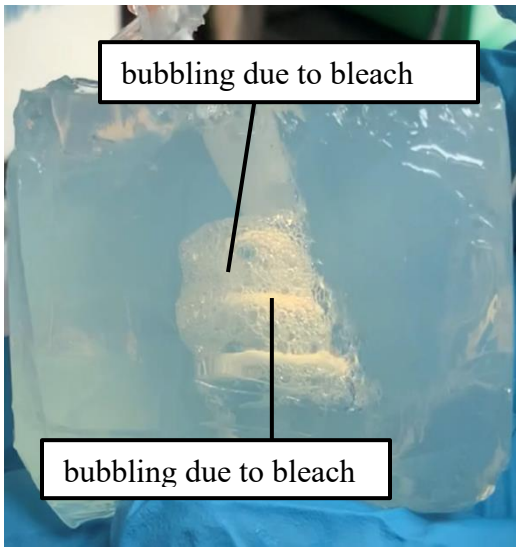

Figure 5: bleach delivery to gelatin via micropore membrane

Section III: Results & Discussion

The purpose of this study was to better understand the nature of gelatin as an embolus analog. First, a replicable procedure was created within the laboratory in order to ensure that each batch of gelatin would be uniform. Next, gelatin dissolution trials were performed with gelatin and a bath of bleach. After 7 dissolution trials we found the optimal bleach concentration to be 100% bleach. 16 new gelatin cubes were submerged in a bleach bath, without stirring, and the results are displayed in **Figure 6**. The first four samples, 1-4, are control samples; however, the remainder of the gelatin cubes illustrate an average mass percent decrease of 48%.

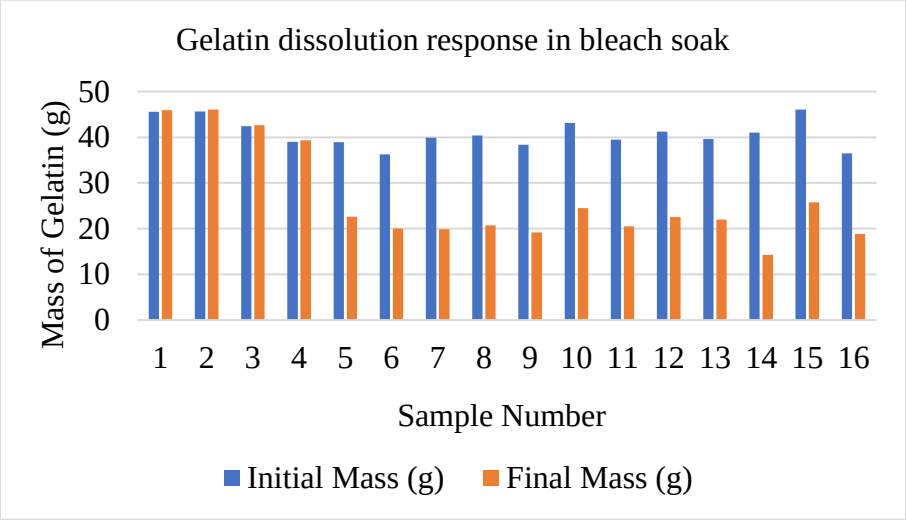

Figure 6: the dissolution of gelatin in a stationary bleach bath; samples 1-4 were water controls

After discovering the average 48% dissolution of undiluted bleach on the gelatin, the next experiment implemented stirring of the gelatin-bleach suspension. In all 6 trials using this methodology, the gelatin dissolved completely. The bleach dissolution trials provided evidence that the combination of a thrombolytic agent – bleach in this case – with mechanical disturbance – such as periodic stirring –increased the rate of dissolution in the crude bleach bath prototype.

The next step in developing the device involved the insertion of micropore membranes into the casted bleach. This design feature was added over the span of multiple weeks with the ultimate goal of coiling the micropore membrane inside the gelatin and doing so in a way that allowed for the perfusion of bleach from an external syringe. **Figure 5** shows a successful, though solely qualitative trial where bleach was delivered to the gelatin and the area around the micropore membrane began foaming.

The final conception of this project was a prototype that involved a nitinol analog. Nitinol is used in a host of biomedical applications for its shape memory **Figure 7** shows the syringe tip for bleach delivery, the micropore membrane for bleach dispersion, and the TPU filament as a nitinol surrogate.

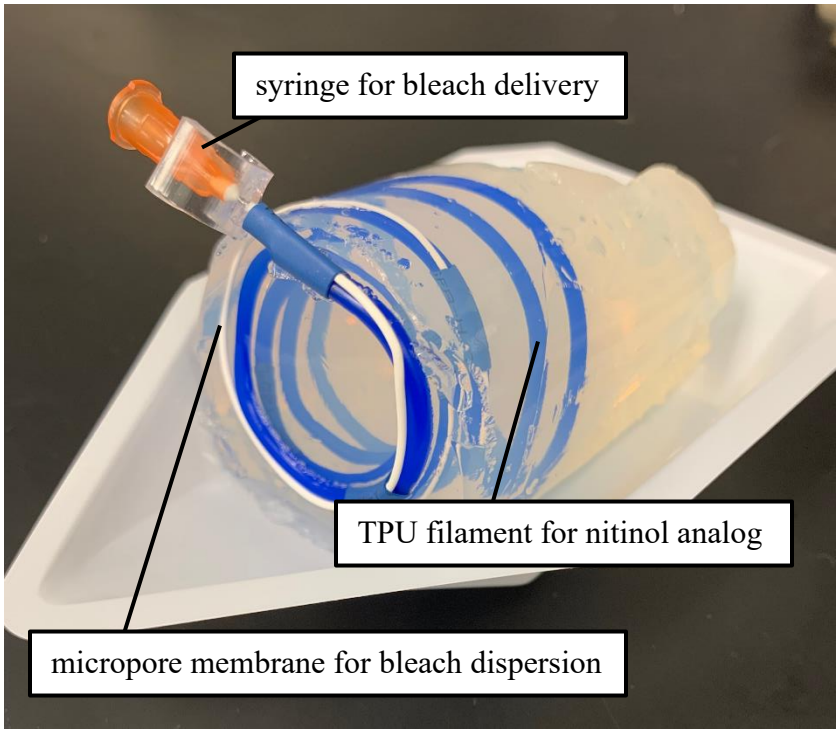

Figure 7: the final prototype achieved through this project

Section IV: Conclusion

AIS is a pathology with abundant opportunity for improvement. The current "gold standard," IV-tPA, falters because of the difficulty in reconciling time of presentation with other contraindications. This study allowed for the exploration of a novel stroke device using a gelatin-bleach simulation for the characterization of device-based modifications. Further dissolution experiments will be conducted with the goal of increasing likeness to the novel device. Moreover, the validation of device-based modifications in this low-risk benchtop model will allow for testing with natural emboli with a greater understanding of how each component of the novel treatment system will integrate with the others.

Section V: Future work

The qualitative nature of this study is a limitation that requires further work and quantitative data collection. The gelatin-bleach simulation has allowed for the investigation of gelatin dissolution properties, the efficacy of micropore membrane inside gelatin molds, and the difference in dissolution in bleach bath versus micropore membrane. Future work should consider the implementation of TPU or nitinol in place of copper wire inside the micropore membranes. Furthermore, once the components of the novel device have been validated, a full experiment with all device features could be conducted in the gelatin-bleach system. Once a prototype is achieved for this device, the final goal lies in human trials. The device proposed by the CB Lab has the potential to increase the variety and reduce time-sensitivity of medical care in AIS

Section VI: Acknowledgements

I would like to sincerely thank Dr. Morten Jensen, Dr. Ranil Wickramasinghe, Kaitlyn Elmer, Sam Stephens, and Ben Yip for their assistance and their guidance in this project.

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