TRANSCATHETER DELIVERY OF FIBRIN GEL FOR TREATMENT OF INTRACRANIAL ANEURYSMS: AN *IN VITRO* STUDY

By

Stephanie M. Harvey

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State University of New York at Buffalo, USA

To my loving fiancée, Jeff, who without his support I would not be who I've become today

And to my loving family, Mom, Dad, Tammy, Gordon, Joel and Kelly, and my wonderful friends; who have guided and encouraged me to strive for the best and have supported me in obtaining my goals

Thank you

Abstract

Liquid embolic is potentially advantageous endovascular treatment for use in intracranial aneurysms; however, concerns have been raised regarding the toxicity of the non-natural agents being used and the numerous risks to the patient, such as unwanted parent artery occlusion. Fibrin gel, the result of combining fibrinogen and thrombin, is a potential biologic embolic agent capable of occluding the aneurysm from blood flow and promoting healthy tissue in-growth. The purpose of this research was to test endovascular injection of fibrin gel, and determine its potential as an effective treatment of intracranial aneurysm.

Canine fibrinogen and bovine thrombin solutions were made prior to experiments. An *in vitro* system, consisting of a glass straight, sidewall aneurysm model in a flow loop, was used to simulate a flow environment. A pulsatile pump circulated room-temperature saline throughout the system. A coaxial delivery system was used in delivering the two components. Numerous experiments were performed with the focus primarily being on the delivery and testing forms of neck protection. Angiography was used in all experiments to visualize the gel formation.

With balloon protection, successful filling of the aneurysm cavity was achieved consistently and no visible gel formed within the main vessel or distally. We found that the gel was homogeneous, stable and not easily extracted at high flow rates. Experiments, in which no balloon protection was used, also produced positive results, however, critical regions of the aneurysm dome remained left unprotected by the fibrin gel and thus it was concluded that some form of neck protection is required for this treatment to be successful.

The results of this research illustrated that when delivered properly, fibrin gel potentially can be a viable and effective treatment for occluding intracranial aneurysms. Further research includes *in vivo* testing of and improving the radiopacity of the fibrin gel via the use of an appropriate contrast media.

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TABLE OF CONTENTS

CHAPTER I 1

I.1 INTRODUCTION.. 1

I.2 BACKGROUND 2

I.3 MOTIVATION / OBJECTIVE 4

CHAPTER II 6

II.1 PROPERTIES INFLUENCING FIBRIN GEL 6

II.2 CREATION OF FIBRIN GEL 8

II.3 DISCUSSION 8

CHAPTER III: ENDOVASCULAR DELIVERY TECHNIQUE 11

III.1 FLOW STUDY SYSTEM 11

III.2 INJECTION TECHNIQUE 11

III.3 RESULTS 13

III.4 DISCUSSION 17

III.5 CONCLUSION 19

CHAPTER IV: ADDITION OF STENT.. 20

IV.1 MOTIVATION 20

IV.2 METHODS 20

IV.3 RESULTS 21

IV.4 DISCUSSION 21

CHAPTER V: DIRECT FIBRIN INJECTION …………………………..………………..… 23

V.1 MOTIVATION …………………………………………………………..…… 23

V.2 METHODS ……………………………………………………………..…. 23

V.3 RESULTS ……………………………………………………............................ 23

V.4 DISCUSSION …………………………………………………………..….. 24

V.5 CONCLUSION ……………………………………………………………..… 25

CHAPTER VI: RADIOPACITY

VI.1 MOTIVATION ………………………………………………………...….. 26

VI.2 METHODS …………………………………………………………...... 26

VI.3 RESULTS …………………………………………………………...…. 27

VI.4 DISCUSSION …………………………………………………………...… 29

VI.5 CONCLUSION ……………………………………………………...……….. 30

CHAPTER VII: PRELIMINARY IN VIVO ………………………………………..……..…... 31

VII.1 MOTIVATION ……………………………………………………..….…. 31

VII.2 METHODS ………………………………………………………...…. 31

VII.2.A ANIMAL MODEL / ANEURYSM CREATION ………………………………...31

VII.2.B MATERIALS ………………………………………………………………...... 33

VII.2.C INJECTION TECHNIQUE ………………………………….................. 33

VII.3 RESULTS 35

VII.3.A RCCA: DISTAL ANEURYSM ………………………………………….. 35

VII.3.B RCCA: PROXIMAL ANEURYSM ……………………….................... 36

VII.3.C LCCA: DISTAL ANEURYSM ……………………………................... 36

VII.3.D LCCA: Proximal Aneurysm ……………………………………………… 37

VII.3.E Follow Up Angiography Post Fibrin Injection ……………….. 38

VII.4 Discussion ………………………………………………............................ 38

VII.5 Conclusion …………………………………………................................ 39

Chapter VIII: Conclusions / Future Recommendations 40

Chapter IX: Appendix ……………………………...............………..............….. 43

Chapter X: References ………………………………………………………..……... 44

LIST OF FIGURES

Figure 1: Specimen of cerebral aneurysms ………………………………………….…..... 1

Figure 2: Illustration of surgical clip across the neck of an aneurysm ……………..…. 1

Figure 3: Angiogram of platinum coil within an intracranial aneurysm ……………….. 2

Figure 4: Schematic drawing of the coagulation process ……………………………… 6

Figure 5: Diagram and photograph of *in vitro* setup ……………...…………………... 11

Figure 6: Picture of glass aneurysm model with steam shaped microcatheter placed in dome ……………………………………………………………………………...…. 12

Figure 7: Aneurysm model with inflated balloon ……………………………….…...... 12

Figure 8: Photograph of y-connector with 2 MTI syringes. The inner catheter was introduce into the microcatheter using the y-connector …………………………...... 13

Figure 9: Picture of aneurysm model post fibrin injection and after delivery system removal ……………………………………………………………………………..... 15

Figure 10: Digital subtraction images of fibrin gel with balloon protection ……….…... 15

Figure 11: Angiographic images after delivery of fibrin gel using partial filling technique and no balloon protection ………………………………………………………………. 16

Figure 12: Unsubtracted image of aneurysm model with stent …………………….….. 21

Figure 13: Digital subtraction image illustrating successful filling of the aneurysm dome after stent removal …………………………………………………….…………….….. 21

Figure 14: Photograph of 1ml MTI syringe …………………………………………… 23

Figure 15: Digital subtraction images of post direct fibrin injection ………………….. 24

Figure 16: Digital subtraction image of the addition of barium sulfate and Oxilan contrast media to the components …………………………………………………… 27

Figure 17: Images of coaxial delivery of the two components combined with Visipaque contrast media and image of direct fibrin injection combined with Visipaque contrast media ……………………………………………………………………………… 28

Figure 18: Schematic drawing of the four created aneurysms *in vivo* ………………... 32

Figure 19: Angiographic images illustrating the patency of all four aneurysms ……... 33

Figure 20: Subtracted image of distal aneurysm on right common carotid post fibrin injection ………………………………………………………………………… 35

Figure 21: Subtracted image of proximal aneurysm on right common carotid post fibrin injection ……………………………………………………………………….. 36

Figure 22: Subtracted image of distal aneurysm on left common carotid post fibrin injection ………………………………………………………………………… 37

Figure 23: Subtracted image of proximal aneurysm on left common carotid post fibrin injection ………………………………………………………………………… 37

Figure 24: Angiographic image showing occlusion of a distal branch of the external carotid artery ……………………………………………………………………… 38

CHAPTER I

I.1 Introduction

Intracranial aneurysms are a form of cerebrovascular disease characterized by the ballooning of an arterial wall (Figure 1). If left untreated, it may expand until it ruptures, leading to subarachnoid hemorrhage. Unfortunately, most intracranial aneurysms are asymptomatic, i.e., patients do not experience any symptoms and are discovered only accidentally during unrelated diagnostic procedures or when the aneurysm ruptures [1]. Thousands of people suffer from intracranial aneurysms and the majority of these patients suffer subarachnoid hemorrhage [3]. Nearly half of these cases will result in death and many more will result in severe neurological deficits despite the best medical and surgical intervention.

Figure 1: Specimen of cerebral aneurysms

Current treatments of intracranial aneurysms fall in two classes: surgical clipping and endovascular intervention. Surgical clipping is a highly invasive procedure that involves opening the skull and placing a metal clip across the neck of the aneurysm (Figure 2). Although this treatment is effective in occluding aneurysms from blood flow, many risks are associated with clipping. In general, aneurysms are difficult to access and more complications are associated with surgical clipping once the aneurysm has ruptured, such as anesthesia complications. In addition, surgical clipping is associated with higher rates of mortality. Thus,

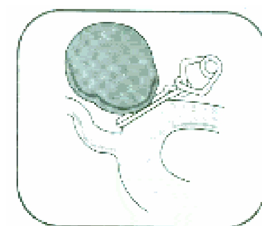

Figure 2: Illustration of surgical clip across the neck of an aneurysm

endovascular procedures are becoming more preferred as treatments for aneurysm patients over surgical clipping whenever possible.

I.2 Background

Endovascular treatments are becoming more and more commonly used since they are less invasive, enable the clinicians to gain access to more aneurysms and subject the patient to less risk. This form of treatment involves delivery of balloons, coils, stents, liquid embolics or a combination of them [1-4, 26] via micro-catheters through the arteries to the site of the aneurysm to achieve occlusion. Among these, coiling is the most commonly used endovascular treatment and is FDA approved (Figure 3). It involves placing platinum coils into the aneurysm sac to promote thrombosis of the aneurysm, which eventually leads to complete occlusion from the blood flow. Coiling has been found to be highly effective for small aneurysms with small neck sizes. However, for large or giant aneurysms, major problems occur with coiling such as compression of the surrounding brain tissue due to the large mass of coils, coil migration into the parent artery, which could cause stroke and incomplete occlusion or coil compaction, which may lead to regrowth of the aneurysm [5, 27-30].

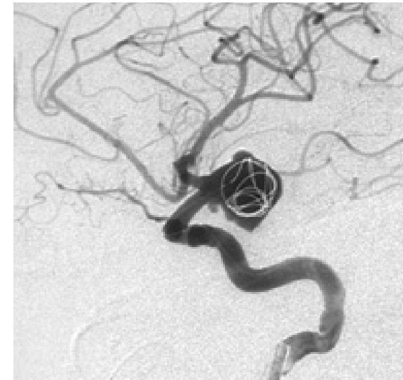

Figure 3: Angiogram of platinum coil within an intracranial aneurysm

Liquid embolic is potentially advantageous endovascular treatment, as long as the liquid solidifies quickly, integrates well with the aneurysm sac and does not block the main vessel. Several research facilities and biotechnology companies are testing liquid embolic agents such as synthetic polymers for use in intracranial aneurysms [7-12, 19,

20]. Giant aneurysms unable to be coiled due to their large neck size and aneurysms with complex geometry benefit from the use of liquid embolics, as the embolic agent conforms to the aneurysm shape. Concerns have been raised, however, regarding the toxicity of these non-natural agents being used [8, 9]. These materials are also associated with many problems such as incomplete occlusion of the aneurysm sac, unwanted parent artery occlusion, catheter-polymer adhesion and distal embolization [9-12, 19].

To combat the negative effects of synthetic embolic agents, an alternative is to use biologically active liquid embolics. Fibrin gel, the result of combining fibrinogen and thrombin, is a potential embolic agent, capable of occluding the aneurysm from blood flow and promoting healthy tissue in-growth. Direct thrombin injections have previously been studied for treatment of peripheral and pseudoaneurysms; however, risks of distal emboli and parent vessel occlusion were reported [34-36]. Endovascular delivery of fibrin gel into the aneurysm sac would imitate the final stages of blood coagulation, resulting in the formation of a stable fibrin clot, independent of the patient's own coagulation process [20]. Other areas of medicine are currently using fibrin gels or fibrin based matrices for numerous applications such as wound healing, sealing of tissues to facilitate hemostasis and drug delivery [21-25]. Although current applications of fibrin gels are in other areas of medicine, numerous research studies on animal models and few clinical cases on peripheral aneurysms have been evaluating the benefits of using fibrin gel [13-18].

Previous studies [13-18] have demonstrated the long-term beneficial effects of fibrin gels but have used delivery methods that are impracticable for use in intracranial aneurysms. The majority of studies used percutaneous intrasaccular injection of the gel,

which involves direct puncture of the aneurysm sac with a needle [13,14,16,18] and few studies used endovascular delivery [15,17]. The fibrin gel that these studies used was commercially available Beriplast or Tissucol. However, both fibrin sealants are similar in that the main components are plasma proteins (fibrinogen), aprotinin, thrombin and calcium chloride.

Despite the infeasible delivery method, the previous studies have demonstrated the benefits to using fibrin gel and its ability to occlude the aneurysm and promote healthy tissue in-growth. Clinical cases that occluded arterial, iliac or pseudoaneurysms with fibrin gel, achieved successful results with few complications [13, 15, 17]. Studies that performed histological analysis [14, 16, 18], found that after approximately three days, the fibrin gel began to be infiltrated by a leukocyte reaction, the initial stage of scar tissue formation. Within the first week post fibrin injection, there was a small remnant of fibrin clot remaining and granulation tissue was observed in the aneurysm wall and space previously occupied by the fibrin gel. After two weeks, granulation tissue was still present in the aneurysms as well as advanced tissue organization within the inner portions of the sac. Endothelial cells were found present along the orifice of the aneurysm and after three weeks, the aneurysms were filled with reactive tissue including capillary proliferation [14].

I.3 Motivation / Objective

Due to the histological benefits of previous studies, it becomes plausible that fibrin gel would be an alternative form of treatment for intracranial aneurysms. The natural healing mechanism of the fibrin would result in shrinkage of the aneurysm sac, thus eliminate any mass effect on the surrounding tissue. In addition, due to the biologic nature of the

embolic, additives such as growth factors, can be added to the gel to further promote healing and revascularization. The purpose of this study was to determine the feasibility of using a coaxial endovascular delivery system to inject the fibrin gel directly into the aneurysm sac. Upon appropriate delivery techniques, the use of fibrin gel has the potential to become a safe and effective liquid embolic agent, used for occluding intracranial aneurysms. In addition, factors influencing the structure and gelation of fibrin gel, such as pH and calcium chloride concentration, were researched in order to produce a stable gel for our studies. Radiopaque materials were also tested in order to make the gel visible with fluoroscopy.

CHAPTER II

II.1 Properties Influencing Fibrin Gel

Fibrin gel is a biologically active embolic that mimics the final stages of the coagulation process. Once the coagulation system becomes activated, thrombin releases two monomers, fibrinopeptide A and fibrinopeptide B, which convert the fibrinogen molecules into fibrin monomers [32]. These monomers then form a three dimensional network of fibrils that becomes the clot, the final stage of the coagulation process (Figure 4). In creating the fibrin gel *in vitro*, many factors, such as pH and thrombin concentration, effect the gelation time and overall structure and stability of the gel mass.

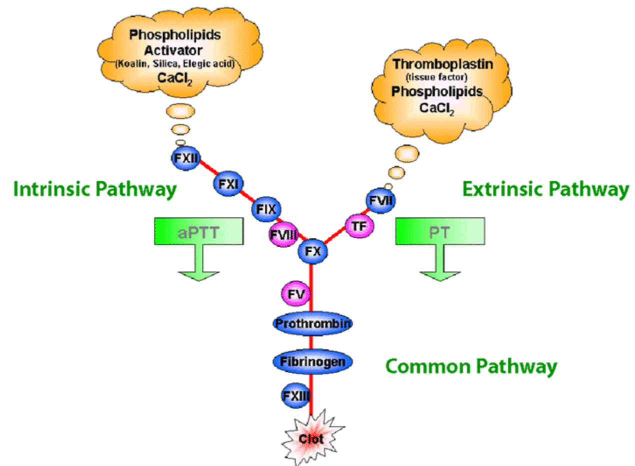

Figure 4: Schematic drawing of the coagulation process

Protein concentration of the fibrinogen and thrombin are the main factors associated with the creation of fibrin gel. Fibrinogen concentration affects the rate at which the fibrinogen becomes activated by the thrombin. The higher the concentration of fibrinogen, the rate at which fibrinopeptide A is released increases, thus the conversion of fibrinogen to fibrin increases [32]. In addition, the adhesive strength of the fibrin gel is directly correlated with the fibrinogen concentration; the higher the concentration of fibrinogen, the higher the adhesive strength [37]. Thrombin concentration also influences the mechanical strength but plays a more important role in the gelation time [21]. The

coagulation rate of the fibrin increases as the concentration of thrombin increases [21, 38].

Calcium chloride ($CaCl_2$) is an important factor necessary in creating a fibrin gel with quick gelation time and high strength. Wang et al [31] demonstrated through quantitative analysis that concentrations of $CaCl_2$ equal to 20mM yielded the quickest gelation time and the highest shear strength.

The pH of the solution also greatly affects the structure of the fibrin gel. Gels formed at a more acidic pH are generally rigid, porous and opaque, whereas gels formed at a more basic pH are transparent, dense and more malleable [39]. In addition to the structure of the fibrin gel, pH also contributes to what types of biologic processes will occur. A study by Nehls and Herrmann [39] found that for gels formed at pH of 6.5-7.2, cells migrated to the gel more rapidly compared to that of gels formed at pH of 7.6-8.0, were the slowest cell migration was observed. For gels formed at pH of 7.2-7.6, the most noticeable decrease in cell migration occurred.

Additional components that benefit the fibrin gel when used *in vivo* include aprotinin, factor XIII and fibronectin. Aprotinin, normally derived from bovine lung, is a naturally occurring protease inhibitor, meaning it delays degradation of the stable fibrin clot when *in vivo* [21, 38, 40]. Factor XIII is an important player in the final stages of blood coagulation [41]. When activated by thrombin and Ca^{2+}, Factor XIIIa forms covalent cross-links between the fibrin monomers, which further stabilize the clot, increase its tensile strength, increases the stiffness for wound closure and provides additional resistance to the fibrinolytic enzymes [21, 41]. Fibronectin is important for

promoting granulation tissue ingrowth and the overall formation of scar tissue, which is the net goal of the use of fibrin gel for treatment of cerebral aneurysms [42, 43].

II.2 Creation of Fibrin Gel

Freeze-dried canine fibrinogen (Sigma Aldrich) was mixed with tris-buffered saline solution (TBS) with a pH of 7.4-7.6. Fibrinogen solutions were made with an average concentration of 15 mg of fibrinogen per 1 ml of TBS. Thrombin from bovine plasma (Sigma Aldrich) was mixed with saline. Once the thrombin was in solution, it was then combined with TBS (pH 7.4-7.6) and calcium chloride, at a concentration of 20mM/ml [31]. The thrombin concentration used was either 80 U/ml or 160 U/ml. Equal volumes of each solution were combined to create the fibrin and by using higher concentrations of each, allowed for rapid coagulation of the gel.

II.3 Discussion

The protein concentrations of the fibrinogen and thrombin were varied in these preliminary studies. It was imperative to have quick coagulation times, and as previously reported, higher protein levels result in faster coagulation [21, 38]. Thus through the series of experiments, the protein concentrations were increased to increase the rate of coagulation. Initial fibrinogen solutions contained a protein concentration of approximately 4-5 mg/ml and the thrombin concentration started at 10 U/ml. Fibrinogen concentrations increased to 10mg/ml and finally to an average of 15mg/ml. Although higher concentrations of fibrinogen would allow for quicker coagulation, mixing higher amounts into solutions was difficult and not easily reproduced. Thrombin concentrations increased in levels varying from 10-160 U/ml. More consistent filling of the aneurysm

was found when using higher concentrations of thrombin and the average thrombin concentration was held constant at 80 or160 U/ml. There was no particular reason behind which concentration of thrombin was used and towards the end of the experiments the concentration of 160 U/ml was consistently used. Although the concentration of thrombin could be easily increased, this was avoided to reduce complications that are associated with thrombin. *In vivo*, if thrombin were to leak from the aneurysm, there is a risk of emboli forming, leading to vessel occlusion. The lower the concentration of thrombin, the easier the system can dilute any leakage. This is not to say thrombin at levels of 160 U/ml, the patient is at no risk, but the risks are significantly decreased. In addition, the levels of thrombin that were used produced successful results; therefore there wasn't the need to increase the concentration of thrombin.

Levels of calcium chloride ($CaCl_2$) were held constant at 20mM. Due to the results from previous studies [31, 32], a concentration of 20mM was shown to provide optimum results for coagulation rates and strength of the gel, and therefore there was no need to alter the level of $CaCl_2$. The pH levels were altered, however. Initially tris-buffered saline (TBS) with pH of 7.6-8.0 was used and then was switched later in the study to TBS with a pH of 7.4-7.6. The TBS of more basic pH produced a gel, which appeared weaker, and less stable. When performing experiments the gel formed at the higher pH was easily removed from the system and easily broken down when handled. When using the TBS solution of lower pH, the gel appeared stronger, more stable and when handled, appeared more fiber-like and organized.

Factors which benefit the formation of fibrin gel *in vivo*, i.e. aprotinin, factor XIII and fibronectin, were not used for these *in vitro* experiments. These *in vitro* studies

focused mainly on the delivery technique thus it was not necessary to focus on the biological response of the fibrin gel. Only when studies are moved *in vivo*, will these components be added and the biologic response of the *in vivo* system to the fibrin gel assessed.

Understanding the way particular components influence the structure, stability and overall properties of the fibrin gel is extremely important when working with fibrin. To achieve successful results when using the fibrin gel, the gel must be constructed in accordance to how it should function for particular applications.

CHAPTER III
ENDOVASCULAR DELIVERY TECHNIQUE

III.1 FLOW STUDY SYSTEM

An *in vitro* system, consisting of a glass aneurysm model in a flow loop, was used to simulate a flow environment (Figure 5). A pulsatile pump controlled the flow rate and circulated saline throughout the system at a rate of 120 ml/min. Temperature of the circulating saline remained at room temperature for this study.

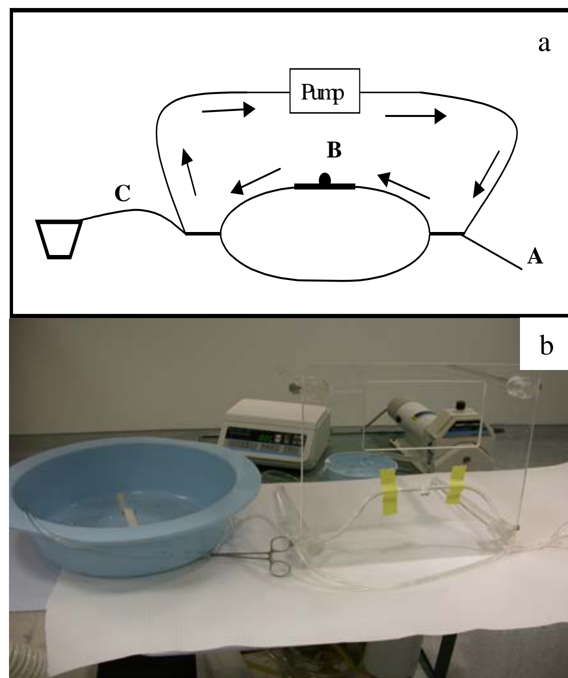

Figure 5: a) **A**. location where endovascular tools were entered; **B**. Aneurysm Flow Loop; **C**. Discharged saline when doing angiographic runs; b) Actual photograph of setup

III.2 INJECTION TECHNIQUE

Initially, a dual microcatheter delivery system was used with in a stasis flow set-up but quickly discarded as the tips of the two microcatheters were difficult to place in close proximity of one another and thus control over the delivery of the two components

was extremely difficult. The stasis flow environment served the initial purpose of testing the feasibility of endovascular delivery of the two components. A coaxial system was used for the remaining experiments and delivered the two components into the aneurysm dome. This method successfully kept the components separated and allowed more precise delivery into the model. Initially a metallic vent tube (Boston Scientific) inside a microcatheter was used, but due to the inflexible nature of the vent tube, a more flexible inner tube replaced this system. Using the inner tube (ID: 3F) of an I.T.C Coaxial Angiographic Catheter set inside a Medtronic Jetstream-327 microcatheter (ID: .027in), better control over the placement of the delivery system inside the aneurysm was achieved. The tip of the microcatheter was steam-shaped, to allow for better placement of the delivery system within the aneurysm (Figure 6). The inner tube was inserted into the microcatheter up to approximately 1mm proximal the microcatheter tip. A standard 3.5mm angioplasty PTCA (non-compliant) balloon was placed across the aneurysm orifice and inflated to 8-10 ATM in order to restrict the flow entering the aneurysm while the components were injected (Figure 7). This was done for the majority of experiments; however experiments were also done without balloon protection.

Figure 6: Picture of glass aneurysm model with steam shaped microcatheter placed in dome

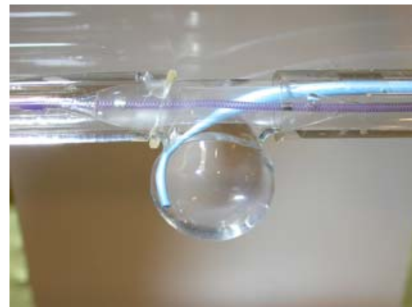

Figure 7: Aneurysm model with inflated balloon

Using a y-connector, the inner tube was introduced into the microcatheter. The dead spaces of both the inner and outer catheters were filled with thrombin and

fibrinogen. Equal amounts of fibrinogen and thrombin were simultaneously injected using 1ml threaded syringes, MTI Cadence Precision Injector, (Figure 8). Thrombin was injected through the inner angio-catheter and the fibrinogen was though the outer microcatheter. By injecting at a slow rate, and due to its rapid formation, allowed for direct injection of fibrin gel into the aneurysm dome. Volume measurements of the aneurysm were performed prior to filling. This was done because of visibility limitations and volume measurements were used to prevent overfilling of the aneurysm. With balloon protection, a volume of .1ml of each solution was injected and without balloon protection, intervals of injecting .1ml of each component was done with angiographic runs in between each injection to determine the amount of gel within the aneurysm. Once the aneurysm became nearly occluded, the amount of injected components decreased to values that suited the amount remaining to be filled.

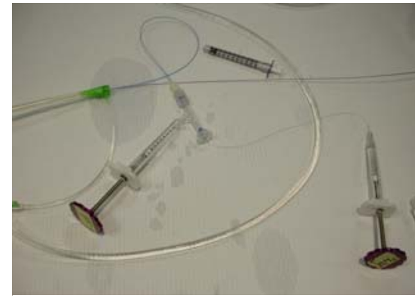

Figure 8: Photograph of y-connector with 2 MTI syringes. The inner catheter was introduce into the microcatheter using the y-connector

Once injection was complete, the microcatheter delivery system was removed. When using balloon protection, after injection the balloon was left inflated for approximately 5 minutes to allow for further solidification and stabilization of the gel, then removed. Angiography was used for obtaining images of the aneurysms and determining the amount of filling that was obtained.

III.3 Results

The main objective of this study was to determine the feasibility of delivering fibrin gel into an intracranial aneurysm by way of a coaxial endovascular technique. In addition, components, which influenced the gelation and overall stability of the fibrin gel,

as well as radiopaque materials, which enabled the fibrin to be visible with fluoroscopy, were explored.

Initial studies were performed in a stasis flow environment using glass and sylgard (see appendix) aneurysm models. These experiments tested variations of the endovascular delivery technique as well as examined the effects that varying protein concentrations of the two components and various radiopaque materials had on the gel. Following success of the stasis environment, experiments were moved into a flow environment in order to examine the effects a flow system had on the stability and formation of the fibrin gel.

A transcatheter system was used in the remaining experiments and provided greater control over the delivery of the gel. Initial tests were performed with the use of a vent tube placed within a microcatheter. Although better control over delivery was apparent with less main vessel gel formation, the vent tube was stiff and hard to position within the aneurysm dome. Removal of the vent tube/microcatheter system posed additional complications because of its stiff nature. The system would often pull out the gel when being removed or detach sections of the gel from the wall, lowering its stability or leave an area around the inflow zone exposed due to the lack of gel formation in that area. The reduced stability was especially important to address, as the complications associated with this reduced stability would become even more critical within a flow environment. Although it was never witnessed in this study, the detached sections of the gel could easily become dislodged and occlude a distal vessel within a flow environment.

To overcome complications associated with the inflexibility of the vent tube, the vent tube was replaced with an inner tube of an I.T.C Coaxial Angiographic Catheter set.

The new inner tube made the delivery system much more flexible and easier to maneuver within the flow system. This more flexible catheter system improved the positioning ability of the delivery system within the aneurysm dome and yet maintained the characteristic of controlled delivery of both the fibrinogen and thrombin into the aneurysm (Figure 9). When the delivery system was removed, the fibrin gel would not detach from the aneurysm wall and the region at the inflow zone was less exposed (Figure 10). For a portion of the studies, a small area of the inflow zone remained slightly exposed to the incoming flow, which was due to the catheter system being positioned through the inflow zone. However, it appeared through observation, when the catheter system was placed more centrally through the aneurysm orifice, the distal neck of the aneurysm appeared well protected by fibrin gel.

Figure 9: Picture of aneurysm model post fibrin injection and after delivery system removal

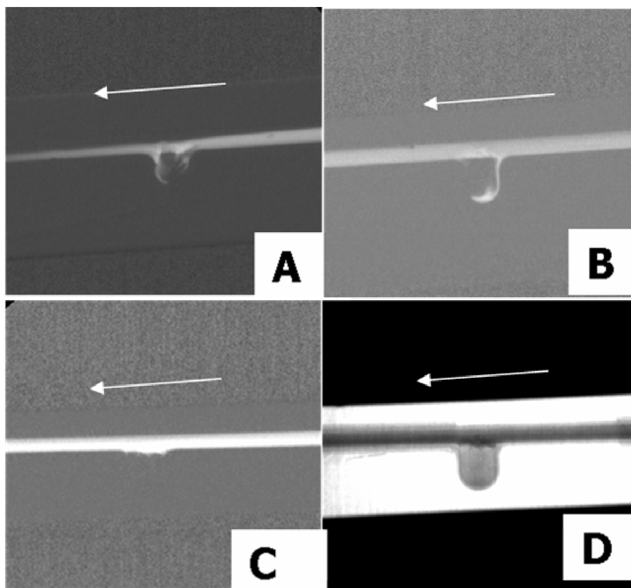

Figure 10: Angiographic images of fibrin gel with balloon protection. Direction of flow is marked with an arrow. A, B, and C are subtracted images; the white represents contrast media and the black within the aneurysm represents fibrin gel. In A, there is a small amount of contrast entering the aneurysm dome; In B, there is a canal at the proximal neck where contrast enters; In C, the aneurysm is completely filled with no contrast media entering. D is an unsubtracted image of C; the black represents the contrast media and the white in the aneurysm represents the fibrin gel formation.

Although the majority of experiments performed used balloon protection, preliminary experiments in which balloon protection was not used, were also carried out.

Using the same flow environment and setup, fibrin was injection into the aneurysm dome without a balloon to seal the neck. The fibrin gel was injected in stages at a volume of .05ml, with angiographic runs done between each injection. The angiographic runs allowed visual guidance as to how much of the aneurysm was embolized and estimate the

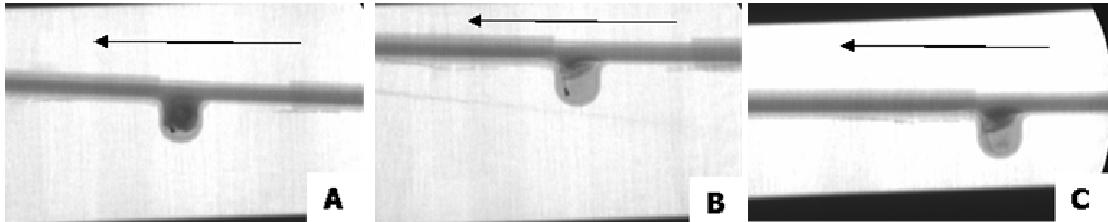

Figure 11: Angiographic images after delivery of fibrin gel using partial filling technique and no balloon protection. Direction of flow is marked with an arrow. These are unsubtracted images with the black representing contrast media and the white in the dome represents the fibrin gel. A) After first .05ml injection, there is a small amount of fibrin formed at the top of the dome; B) after fourth .05ml injection, there is apparent fibrin formation with the vessel remaining patent; C) After removal of the delivery system, the gel remained intact. The gel appeared homogeneous and stable with the vessel patent.

amount of fibrin that should be further injected (Figure 11).

These experiments produced positive results. It took double the amount of components to fill the aneurysm, but there were no visible signs of emboli throughout the system besides the aneurysm dome. However, when using no balloon protection, the distal neck, or inflow zone, was left unprotected. In one occasion, there was a small protrusion of gel into the main vessel, which was due to overfilling of the dome. This protrusion appeared to be forming along the direction of the flow and thus prevented the aneurysm from being completely occluded.

The results of the injection technique were not altered by the used of the glass aneurysm model. Although the glass surface is negatively charge, by using a saline flow system, the saline neutralizes and counteracts any effects the surface charge would have [43]. Therefore, the net negative charge had no effect on the stability of the gel within the model.

III.4 Discussion

Feasibility studies were initially done within a stasis environment to determine if the fibrin gel could be injected into an aneurysm dome endovascularly, in a controlled manner with no parent vessel occlusion. The stasis environment was unrealistic of a physiologic environment and although it posed problems itself, it provided a basis for understanding delivery techniques, protein concentration effects, radiopacity materials and overall handling and understanding of fibrin.

Preliminary studies were unsuccessful due poor delivery techniques, which resulted in overfilling of the aneurysm and main vessel occlusion. The stasis environment of the system contributed to the main vessel occlusion because when the two components leaked out of the aneurysm, there was no dilution of the components and allowed them to coagulate within the vessel. However, by limiting the amount of fibrin injected to values just below the volume of the aneurysm dome and using a better delivery system, consistent filling was achieved and experiments were moved into a flow environment.

Upon entering a flow environment, problems were initially encountered but quickly overcome. Overfilling of the aneurysm was avoided due to injection volumes less than or equal to that of the aneurysm sac and vessel occlusion was avoided due the flow environment. The flow system diluted any leaked components to such low concentrations that they did not react with one another. However, this may change within a physiologic environment as fibrinogen is always in the bloodstream and if a large amount of thrombin would leak out of the system, there's the possibility it would react and emboli form.

Despite these challenges, within both a stasis and flow environment, successful filling of the aneurysm was achieved. This initial success illustrated that the coaxial

system was superior to the initially-tried dual-microcatheter system and that it provided better control over the delivery of the gel into the aneurysm. In addition, the transcatheter method of delivery allowed for better mixing of the two components at the tips of the system, which allowed for better coagulation times.

Despite the small risk associated with thrombin leakage, a physiological environment will further benefit the use of fibrin gel within aneurysms. Although the temperature of the saline was not physiological, previous work has stated that the clotting time of fibrin would decrease with increasing temperature [32]; therefore we assumed that when moving into a physiological environment, our clotting time would only improve. If this holds true, the risk of high concentrations of thrombin leaking out becomes even smaller, as the thrombin would react quicker with the fibrinogen and have a less likelihood of actually leaking out of the system. Additionally, any of the small cracks that appeared within the gel during the filling would not pose any threat to the stability either. When in a living environment, we postulate that these small cracks would likely self-thrombose, due to the limited flow entering them and the thrombogenic surface.

Experiments done without balloon protection were preliminary but produced fibrin formation within the aneurysm. Using the partial filling technique, the gel appeared homogeneous and stable. Despite the success in fibrin formation within the aneurysm, the unprotected distal neck illustrates that using no neck protection when filling the aneurysm would not produce a beneficial result. The distal neck (inflow zone) has been found to be a point of growth of an aneurysm due to the high levels of shear stress that occur there from the continuous impingement of flow. For a treatment to be successful in preventing

rupture and regrowth of an aneurysm, protection of the inflow zone is critical. It would be more beneficial to perform these experiments without the hindrance of a balloon, as it would make the procedure simpler. Alternatives to balloon protection but still altering the flow with the aneurysm would be to try stent protection across the neck of the aneurysm and then inject the fibrin gel. The use of a stent would allow for disruption of the flow entering the aneurysm, which would allow more complete occlusion of the aneurysm by the fibrin gel.

III.5 Conclusion

These initial results conclude that the coaxial system is able to deliver fibrin gel in a controlled fashion, thereby partially occluding the aneurysm from the continuous flow. The use of a coaxial endovascular delivery technique proved to be successful in delivery fibrinogen and thrombin into an aneurysm dome. It produced fibrin formation, with successful occlusion of the aneurysm. Upon removal, the flexible delivery system did not disrupt the gel formation or detach it from the model wall. There was minimal leakage of material from the aneurysm and the structural integrity of the gel was able to withstand high flow rates. Overall, the results of this *in vitro* study illustrated that when delivered properly and adequately opacified, fibrin gel can be delivered into aneurysms using a catheter based approach.

CHAPTER IV
ADDITION OF STENT

IV. 1 Motivation

When injecting fibrin gel into the aneurysm dome, it is important to have neck protection to promote stasis of the gel. The majority of experiments tried have used balloon protection. Balloon protection is beneficial since its seals off the aneurysm from the blood flow and creates a stasis-like environment for the fibrin to form. A major setback to use of the balloon, however, is that it can only be inflated for a short amount of time before damage to the brain tissue from lack of blood flow occurs. In addition, when using no balloon protection, although fibrin formation occurred within the aneurysm, the distal neck remained unprotected. The distal neck or inflow zone is a critical point in the geometry of aneurysms and thought to be were the growth of the aneurysm occurs and thus it is crucial that for a treatment to be successful it must protect the distal neck area. The purpose of this initial experiment was to test the addition of a stent and determine if the stent disrupted the flow within the aneurysm enough to allow for fibrin formation.

IV.2 Methods

Canine fibrinogen of 16 mg/ml and bovine thrombin of 200 U/ml solutions were made as previously described. The same *in vitro* flow system and coaxial delivery system was used as previously described with a flow rate of .18 L/min. A 3.5mm balloon expandable AngioStent was used in replace of a balloon.

The coaxial delivery system was placed within the aneurysm first, and then the balloon expandable stent was inflated. The balloon was then removed and the system ready for injection.

IV.3 Results

Both components were injected simultaneously. Initially, .2ml was injected with an angiographic run following. The angiogram showed a solid, ball like structure within the aneurysm dome (Figure 12). The vessel was patent and the aneurysm was almost completely occluded. Another .05ml of each component was injected which produced excellent occlusion of the aneurysm (Figure 13).

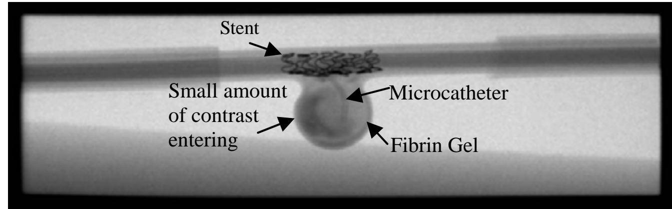

Figure 12: Unsubtracted image of aneurysm model with stent. This image illustrated the microcatheter positioning and the fibrin within the dome. The black represents the contrast media and the white represents the fibrin.

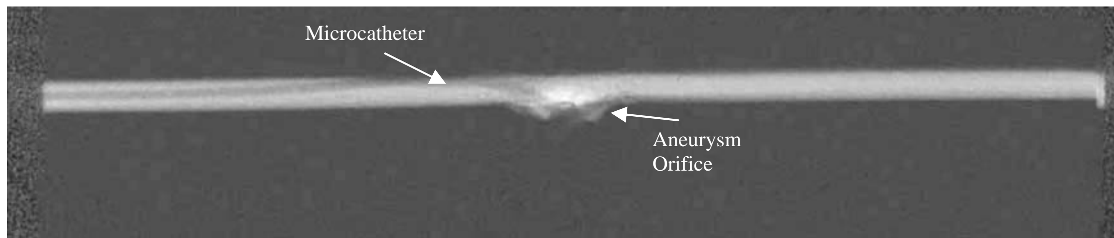

Figure 13: Digital subtraction image. This subtracted image illustrated the successful filling of the aneurysm dome after stent removal. The microcatheter is still intact. The white represents the contrast media. There is no contrast media entering the aneurysm dome.

IV.4 Discussion

The use of a stent in replacement of a balloon produced very good results. When the two components were injected, they did not appear to leak out of the system, since the aneurysm was almost completely occluded after the first injection. When using the partial filling technique, in which no balloon was used, it took double the amount of fibrinogen and thrombin to create a solid mass within the aneurysm and yet, the aneurysm was unable to be fully occluded.

The addition of a stent has illustrated great potential to be used in these experiments. Not only did the stent disrupt the flow to allow for formation of fibrin but it also adds protection to the vessel from any particle of fibrin that may come loose. Although, this was just a preliminary study, its successful results have illustrated that the addition of a stent could be very beneficial to the success of fibrin as a treatment for intracranial aneurysms.

CHAPTER V
Direct Fibrin Injection

V.1 Motivation

The purpose of the direct fibrin injection experiments was to test the feasibility of this method. Direct fibrin injection would provide numerous benefits to the procedure, including less complication with the delivery of components, less risk of thrombin leakage into the bloodstream and increased viscosity of the material to allow for additives.

V.2 Methods

Using a single 1ml threaded syringe, MTI Cadence Precision Injector, (Figure 14), fibrinogen of 15 mg/ml and thrombin of 200 U/ml were combined. Fibrinogen was initially drawn into the syringe with a volume of .5ml. Using a 1ml syringe with a long needle tip, .5ml thrombin was injected into the fibrinogen. The two component mixture then gelated for approximately 20 minutes. After the 20 minutes, the syringe contained both fibrin gel and saline.

Figure 14: Photograph of 1ml MTI Cadence Precision Injector threaded syringe

Following gelation, using the Medtronic Jetstream microcatheter (ID .027in) and balloon protection (inflated 8-12 ATM), the fibrin was injected directly into the aneurysm dome.

V.3 Results

Once injection of the fibrin began, the gel entered the aneurysm in an inconsistent manner. The injection rate of the fibrin remained consistent but due to the saline / fibrin

mixture within the syringe, the fibrin would enter the aneurysm dome in stages. When fibrin would enter the aneurysm model, it was a burst of fibrin; it did not fill the aneurysm slowly. This resulted in less control over the volume of fibrin which was injected.

The fibrin entered the aneurysm model in a string-like fashion and was not homogeneous throughout the aneurysm dome (Figure 15). Complete filling of the aneurysm was unable to be attained, due to this string-like structure. There were many channels for contrast to enter the aneurysm. The fibrin did however, appear stable and was not easily removed when subjected to high flow rates.

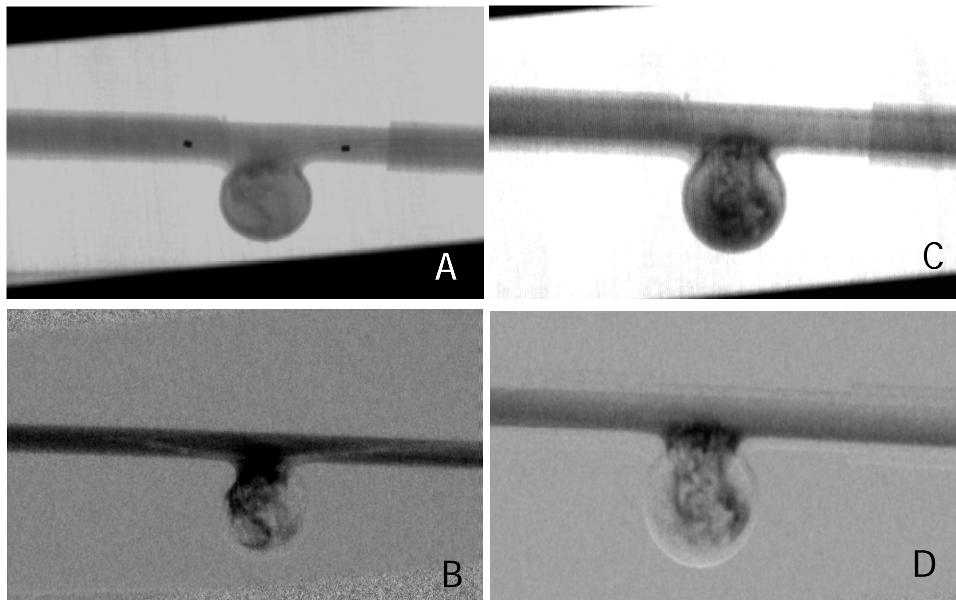

Figure 15: Digital Subtraction Images. A) Unsubtracted image illustrating the structure of direct fibrin injection; filling does not appear homogeneous throughout; B) Subtracted image of A; the black represents the contrast media and the white within the dome is fibrin. The non-uniformities are more apparent; C) Unsubtracted image of direct fibrin injection. Again, the fibrin does not appear homogeneous; D) Subtracted image C illustrating the crevasses in the fibrin in which contrast media can enter the aneurysm.

V.4 Discussion

The aneurysm model became occluded by direct injection of fibrin gel. However, this method of direct fibrin injection provided less control over the delivery of the gel, as

the fibrin did not enter the aneurysm dome in a unified manner. Due to this sporadic entry, it was very difficult to know the volume of fibrin that was being injected. The string-like form of the fibrin allowed for numerous channels to exist within the mass, which allowed leakage of contrast or saline into the dome. We postulate, however, that within a physiologic environment, these channels would self-thrombose.

V.5 Conclusion

Direct fibrin injection would, in theory, be very beneficial to this form of treatment. It reduces the risks associated with thrombin leakage and injection of one component is simpler then injection of two components simultaneously. These initial experiments with direct fibrin injection produced positive results but clearly still need further study. Although the fibrin remained stable within the aneurysm when subjected to high flow rates, concern is still present with the sporadic manner it has when being injected. Additionally, the string-like nature may not withstand a physiological system's fibrinolytic system and be digested at a much faster rate. These are questions and concerns which would be further understood once this form of delivery of fibrin was test *in vivo*. Overall, these preliminary direct fibrin experiments brought new ideas and insight as to alternative forms of delivery of this biologically active liquid embolic to achieve successful results.

CHAPTER VI
RADIOPACITY

VI.1 Motivation

For this embolic agent to be successful when using as an endovascular treatment, radiopacity is an important aspect that needs to be addressed. Although the material being radiopaque is not a necessity to the success of the fibrin gel, it would greatly improve the ease and control over delivery. The purpose of these preliminary radiopacity experiments was to test a variety of radiopaque materials and to evaluate if the addition of these materials allows for the delivery of the fibrin gel to be visible with fluoroscopy and improve the overall filling of the aneurysm.

VI.2 Methods

Various radiopaque materials were used in attempt to make the gel visible with fluoroscopy. Tantalum powder, Barium Sulfate ($BaSO_4$) and Oxilan™ (ioxilan) contrast media were combined with fibrinogen, thrombin or both and tested independent of one another within a stasis environment. Visipaque® (iodixanol) contrast media was combined with the thrombin and tested in a flow environment. Visipaque was also tested with two component fibrinogen and thrombin injection and with direct fibrin injection.

Tantalum powder was combined with 15-mg/ml fibrinogen. The tantalum powder was mixed into the fibrinogen solution. The two were lightly shaken to combine. Once shaken, the solution was then drawn into the MTI syringe.

Barium sulfate was combined with 20 U/ml thrombin. After the two were combined, the solution was then drawn into the MTI syringe.

Equal amounts of .2ml of Oxilan™ contrast media was combined with both thrombin and fibrinogen. Both were simultaneously injected using the MTI syringes.

Visipaque® contrast media was combined with the thrombin only. 20%, 40% and 50% concentrations were used. A higher concentration of thrombin was used, 200 U/ml, to negate the affects the Visipaque® may have on the coagulation rate. Thrombin would be drawn into the MTI syringe initially, then the amount of Visipaque®. The two were shaken lightly to combine within the syringe. For the two component injection, the thrombin / Visipaque® combination syringe was attached to the inner catheter and the fibrinogen was attached to the outer microcatheter. When the direct fibrin injection was used, the thrombin / contrast media was injected into the fibrinogen by means previously described in Chapter IV.

VI.3 Results

Radiopaque materials, Oxilan™ and Visipaque® contrast media, barium sulfate and tantalum powder, were used in attempt to make the gel visible with fluoroscopy. Barium sulfate did not combine well with the fibrinogen and thrombin, leaked into the main vessel and in addition, would not integrate well within a physiologic environment, thus was omitted from further testing (Figure 16A). Tantalum, an inert material, was too heavy and unable to be thoroughly mixed within our solutions. Due to the inviscous nature of the fibrinogen and thrombin, the tantalum particles descended to

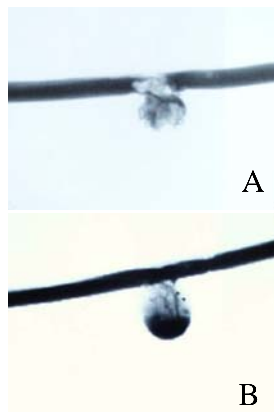

Figure 16: Digital Subtraction Image of the addition of A) Barium Sulfate to the components and B) Oxilian Contrast media. The black represents the contrast media and the white represents the fibrin / radiopaque component mixture. In A) injection of the mixture was difficult and as shown, the Barium Sulfate leaked in the main vessel. In B) the contrast media was too dense and pooled at the dome of the aneurysm.

the bottom of the syringe and settled almost immediately. Oxilan™ contrast media mixed sufficiently with the two components and was visible with fluoroscopy during injection. When injecting, the contrast media appeared very dense and would pool at the dome of the aneurysm (Figure 16B). In addition, the fibrin gel did not appear stable, was not homogeneous and was easily removed when subjected to high flow rates. Further use of Oxilan™ at that point in the experiments was discontinued due to the lack of successful results.

Visipaque® contrast media was tried within a flow environment. Injection of fibrin into the aneurysm was done until it appeared filled from visible measurement using fluoroscopy for both the two component injection and the direct fibrin injection. The gel did appeared stable within the aneurysm and was left exposed to the flow environment for more then 15 minutes, with no change occurring. Filling of the aneurysm was successful and the gel appeared homogeneous and was able to withstand high flow rates. However, when using the two component injection method (coaxial delivery), the contrast agent did not form within the fibrin and the Visipaque® would sink to the top of the dome. This gave misleading results when using fluoroscopy; the aneurysm would appear half filled with fibrin (Figure 17A), however when an angiographic run was performed, it would illustrate complete filling of the aneurysm (Figure 17B). When using direct fibrin injection, Visipaque® remained within the fibrin and thus filling

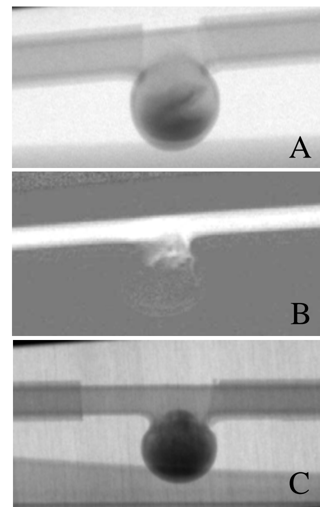

Figure 17: A) Unsubtracted image of coaxial delivery of fibrinogen and thrombin; the aneurysm appears ¾ filled; B) Subtracted image following angiographic run. This demonstrates how the aneurysm was almost 100% filled. C) Unsubtracted image of direct fibrin injection with 40% Visipaque contrast media added to the thrombin. The black represents the fibrin / contrast mixture in the dome.

of the aneurysm was more accurately displayed with the fluoroscopy (Figure 17C). Unfortunately, the direct fibrin still was difficult to inject, due to its viscous nature and entered the dome in a discontinuous manner.

VI.4 Discussion

None of the initial radiopaque materials used in the stasis environment produced successful results and further use was discontinued. The stasis environment may have contributed to part of the problems with the radiopaque materials and the resulting vessel occlusion however, discontinued use of the materials was more based on the delivery of the mixtures into the aneurysm model and the effects the radiopaque materials had on the stability of the gel. The tantalum wasn't able to be tried in delivery. Since tantalum is an inert material, it should have little effect of the gelation of the fibrin gel; however, for it to produce successful results, the tantalum would need to be uniform to some extent throughout the components. The fibrinogen and thrombin were too inviscous to support the tantalum particles. The barium sulfate was very difficult to work with and did affect the stability of the fibrin gel The fibrin gel was very easy to remove, delivery control was very difficult and the main vessel became grossly occluded by the fibrin / barium sulfate mixture.

Visipaque® was more successful then the previous radiopaque materials but still posed its own problems. Both the two component injection and the direct fibrin injection were visible with fluoroscopy with the addition of Visipaque® and obviously the higher the percentage of contrast the more visible the injection. However, for the two component injection, the contrast media did not form within the fibrin gel and would sink to the top of the dome. This fact made injection more difficult because the accuracy of filling was

greatly decreased since the contrast would descend to the dome and the fibrin would continue to fill the aneurysm. The gel did, however, appear stable and homogeneous. When using the direct fibrin injection, the Visipaque® was integrated well with the fibrin and produced more accurate results using fluoroscopy visualization. Despite encouraging results with the improved radiopacity, the direct fibrin gel entered the model in a string-like fashion, resulting in a non-homogeneous formation, as what occurred when there was no radiopaque material added. Thus, the problems associated with direct fibrin still occurred and because of this it is more difficult to assess the affects the Visipaque® had on the stability of the gel.

Previous studies have reported that the addition of contrast media, or any non-gelling particles, affects the coagulation rate and the strength of the fibrin [33, 38]. These studies also stated that an increase in thrombin concentration should negate these affects. Richling [38] also suggested the addition of factor XIII to the fibrin gel to combat the affects radiopaque materials have on the coagulation rate. For this preliminary study, factor XIII was not used but would be when testing in an *in vivo* survival study.

VI.5 Conclusion

Radiopacity is a key element in advancing the success of fibrin gel for treatment of intracranial aneurysms. Although our results are not very encouraging, this is still an area that need to be further explored.

CHAPTER VII
Preliminary *In Vivo*

VII.1 Motivation

When testing a biologically active liquid embolic, understanding how it interacts in a physiological environment is critical. Four vein pouch aneurysms were created on the carotid arteries of a canine. Using endovascular techniques, the aneurysms were injected with fibrin gel. Following injection, angiographic runs were done hourly to observe the fibrin gel. The purpose of this preliminary, non-survival animal study was to gain further insight as to the delivery of the fibrin and the stability of fibrin once inside the aneurysm.

VII.2 Methods

VII.2.A Animal Model / Aneurysm Creation

One mongrel dog was used for this study and was IACUC approved. Four vein pouch aneurysms were created, 2 on the right common carotid artery (RCCA) and 2 on the left common carotid artery (LCCA).

The canine was initially anesthetized with a combination of 0.2 mg/kg Acepromazine and 0.02 mg/kg Atropine delivered intramuscularly. Following transport to the lab, 2.2 mg/kg Telezol was administered intravenously, to allow for intubation. After intubated, the animal was connected to the anesthesia machine and maintained with 1.5-2% isofluorane in oxygen.

Under sterile conditions, a longitudinal incision was made in the midline of the neck. A portion of the right external jugular vein was isolated, ligated and a segment of approximately 12cm was harvested and placed in a dilute heparinized/saline solution. Next, the RCCA was exposed and cleaned for anastomotic surgery. A small longitudinal

incision was made with an 11 blade to promote entry of the 5mm arterial punch and a hole was created. The artery was then flushed with heparin and saline in preparation for the attachment of the vein pouch. A 2-3 cm segment of the harvested vein was grafted to the carotid using 6'0 prolene suture to create the first aneurysm. The top of the vein graft was ligated using Braunamed tie to seal off the dome with a purse-string technique. Following completion of the first aneurysm, the second aneurysm on the RCCA was created following the same method described at a location approximately 20mm proximal. The left common carotid artery (LCCA) sidewall aneurysms were created using the method described above.

Following the aneurysm creation, the aneurysms were allowed to heal for approximately 4 weeks. At the 4 week time point follow up angiography was done to ensure that all four aneurysms were patent. The canine was again anesthetized using the same technique previously described. After the animal was anesthetized and prepared sterile fashion, a 5F sheath was introduced to the femoral artery via the modified Seldinger technique. A 5F diagnostic angiographic catheter was advanced into the carotids and angiograms were done by injection of non-ionic contrast material. The size and patency of the aneurysms were then determined (Figure 18). All four aneurysms were found to be patent (Figure 19). The volumes of the aneurysms on the RCCA were approximately .47ml distal and .54ml proximal. The volumes of the LCCA aneurysms were approximately .40ml distal and .65ml proximal. Following the imaging, the catheter and

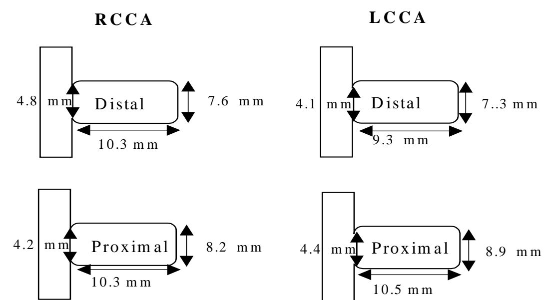

Figure 18: Schematic drawing of the 4 aneurysm models with approximate dimensions in mm.

femoral sheath were removed and the femoral wound compressed until the bleeding stopped, approximately 20 minutes. The dog recovered without incident.

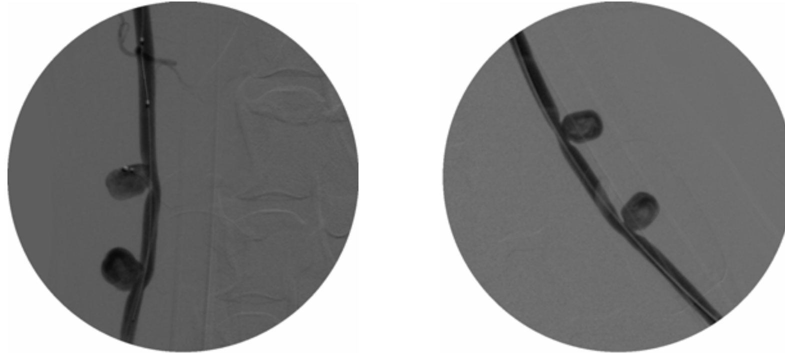

Figure 19: Digital Subtraction Images. A) Unsubtracted image of the two aneurysms created on the RCCA; B) Unsubtracted image of the two aneurysms created on the LCCA. All four aneurysms are patent.

VII.2.B Materials

Fibrin gel was created as previously mentioned. Five 1ml MTI Syringes were filled in total. Two syringes were filled with approximately 19 mg/ml fibrinogen, two were filled with 160 U/ml thrombin and the fifth syringe was filled with a mixture of thrombin and Visipaque contrast media, with a 1:1 ratio.

VII.2.C Injection Technique

The animal was anesthetized as mentioned previously. After the animal was anesthetized and prepared in sterile fashion, an 8F sheath was introduced to the common femoral artery via the modified Seldinger technique. Although sterile techniques were used to prep the animal, it was not necessary to work in a completely sterile environment, as this was a non-survival study.

An 8F-guide catheter was used for entering the target vessels. The coaxial delivery system was the same as previously described. Balloon protection was used for

all four aneurysms however a semi-compliant Equinox™ balloon (MTI) was used to reduce the risk of vasospasm.

Starting with the distal aneurysm on the RCCA, the coaxial delivery system was placed within the aneurysm dome. It was decided that both aneurysms on the RCCA would be injected with the thrombin / contrast mixture. The dead space of the Medtronic Jetstream microcatheter (ID: .027in) was filled with .75ml fibrinogen. The dead space of the I.T.C inner catheter (ID: 3F) was filled with .1ml thrombin/contrast mixture. Using fluoroscopic guidance, the aneurysm was injected with both components. The components were injected in stages, with angiographic runs done in between to gain a visual as to how much of the aneurysm was being filled with fibrin. Injection of the two components into the proximal aneurysm on the RCCA followed the same method as done for the distal aneurysm.

When switching to the LCCA, the delivery system was removed to be cleaned. Once removed and washed with saline, the inner catheter was unable to be placed within the outer microcatheter, thus both the I.T.C inner catheter and Medtronic Jetstream microcatheter were replaced with new ones before continuing on with the LCCA. It was decided that both aneurysms on the LCCA would be injected with fibrinogen and thrombin only. This would allow for a comparison of the physiological response to the fibrin created with and without the addition of contrast media. As with the RCCA, filling of the distal aneurysm on the LCCA was performed first. The procedure followed that of the RCCA with the two components being injected in stages. Angiographic runs were performed in between injections for visual guidance as to how much of the aneurysm was

filled by the fibrin. The proximal aneurysm was filled in the same manner as the distal aneurysm.

Once filling of all four aneurysms was complete, follow up angiographic runs were done every hour for five hours.

VII.3 Results

VII.3.A Right Common Carotid Artery: Distal Aneurysm *(Figure 20)*

Initially .15ml of each component was injected. Once injecting, the tip of the inner catheter moved out of the outer microcatheter, so the tips were not close to one another. Injection stopped, as it appeared that contrast was entering the main vessel. The balloon was deflated and it appeared that the contrast washed away. An angiographic run was done and filling appeared good, although not complete. Gel formed on the inflow side of the aneurysm and dome and the main vessel remained patent. The balloon was reinflated and .12ml more of each was injected. An angiogram was taken and the aneurysm appeared 75% filled. There appeared to be a small formation of gel between the vessel wall and the balloon. An additional .05ml of each component was further injected. The balloon was then deflated and moved slightly forward. It appeared when the balloon was moved, the fibrin mass within the aneurysm dome moved with it. When angiography was performed the aneurysm was well filled with the fibrin gel. There was a portion of the aneurysm open at

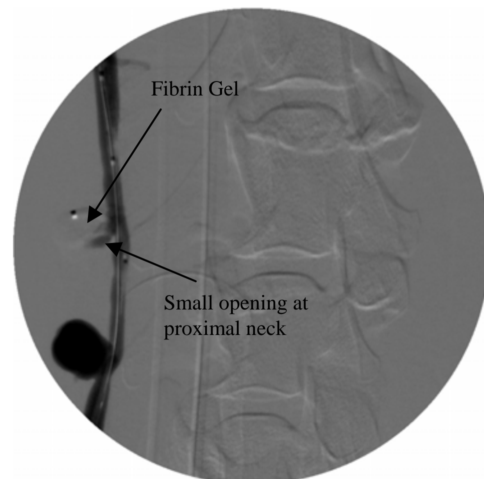

Figure 20: Subtracted image of distal aneurysm. The black represents contrast media and the white in the aneurysm dome illustrates fibrin gel. Arrows mark the fibrin gel and the opening at the outflow zone.

the proximal neck, but the distal neck was well covered. Removal of the delivery system and balloon did not disturb the gel within the distal aneurysm.

VII.3.B Right Common Carotid Artery: Proximal Aneurysm *(Figure 21)*

Initially .2ml of each component was injected. The injection of the thrombin was visible with fluoroscopy due to the addition of the contrast. After injection, the balloon was deflated and an angiographic run done. The aneurysm was partially filled and the balloon was reinflated and .125ml more was injected of the components. An angiographic run following the second injection showed a large formation of fibrin within the main vessel. This fibrin formation did not occlude the vessel entirely and blood still flowed easily. Although the aneurysm was not completely occluded, injection stopped due to the formation of fibrin in the main vessel. The balloon and delivery system were removed and the gel in the proximal aneurysm and in main vessel was not disrupted.

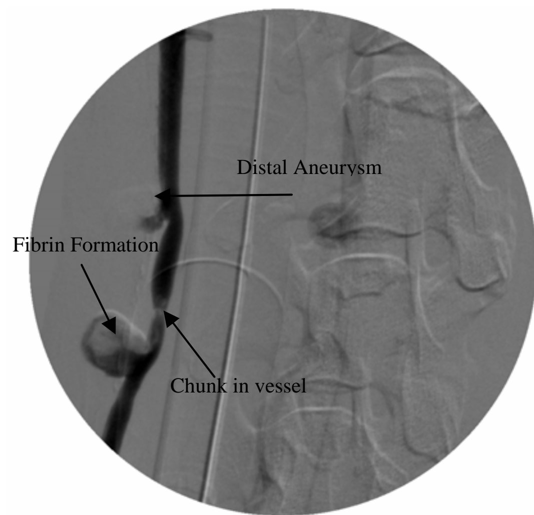

Figure 21: Subtracted image of the proximal aneurysm. The black represents contrast media and the white in the aneurysm dome illustrates fibrin gel. Arrows mark the fibrin gel and the fibrin formation within the vessel.

VII.3.C Left Common Carotid Artery: Distal Aneurysm *(Figure 22)*

Injection of .2ml of fibrinogen and thrombin (no contrast) was done initially. After a short time interval, the balloon was deflated and an angiographic run performed. The angiogram showed partial filling of the aneurysm with no visible fibrin formation within

the vessel. The balloon was inflated and .05ml of each was further injected. An angiogram following injection showed that the aneurysm was approximately 95% occluded. Following the angiogram, another .05ml of each component was injected. The aneurysm appeared well filled however there was a small channel open at the distal neck. In addition, proximal leakage into the main vessel occurred.

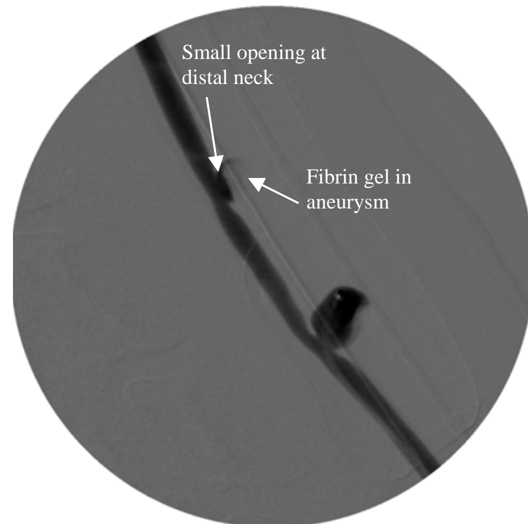

Figure 22: Subtracted image of the distal aneurysm. The black represents contrast media and the white in the aneurysm dome illustrates fibrin gel. Arrows mark the fibrin gel and the small opening at the distal neck.

VII.3.D Left Common Carotid Artery: Proximal Aneurysm *(Figure 23)*

.2ml of each component was injected initially. An angiogram illustrated good filling of the aneurysm with only a small portion remaining open. Another .025ml of each component were injected which resulted in more occlusion of the aneurysm, however, the distal and proximal necks still remained unprotected. An additional injection of .025ml followed and resulted in little change in the filling of the aneurysm. A final injection of .025ml of each still resulted in little change in filling of the aneurysm with the distal and proximal necks remaining open.

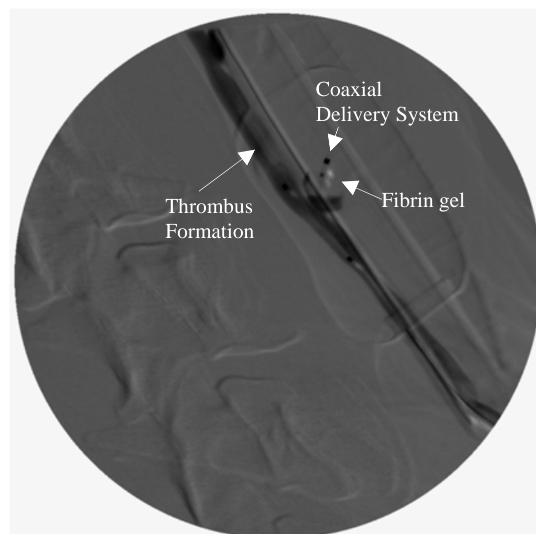

Figure 23: Subtracted image of the proximal aneurysm. The black represents contrast media and the white in the aneurysm dome illustrates fibrin gel. Arrows mark the fibrin gel. The coaxial delivery system is still in the aneurysm dome.

VII.3.E Follow Up Angiography Post Fibrin Injection

Angiographic runs were performed hourly for 5 hours following filling of all four aneurysms. The small opening at the proximal neck of the distal aneurysm on the RCCA appeared to become slightly larger. The remaining 3 aneurysms remained the same. The fibrin formation within the main vessel located at the proximal aneurysm on the RCCA remained intact and did not prevent blood flow. When an angiogram was taken of the distal vessels at the 3hour mark, a distal external carotid artery branch occlusion was found (Figure 24).

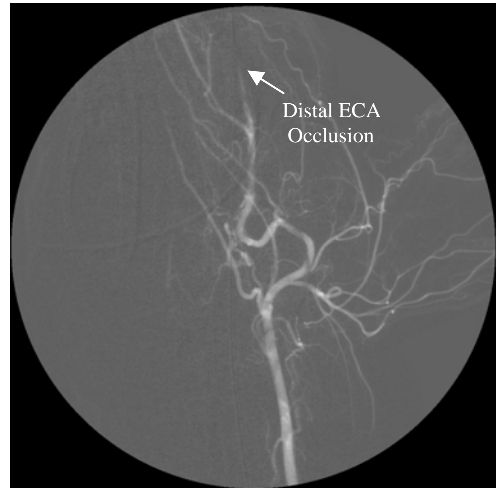

Figure 24: Angiographic Image of Distal Vessel. Shows occlusion of a distal branch of the external carotid artery

VII.4 Discussion

This preliminary experiment gave much insight to how fibrin gel would react when placed within a physiologic environment. The occlusion of the aneurysms was decent despite the fibrin formation in the main vessel. Other factors besides leakage of fibrin into the vessel may have contributed to the vessel emboli that occurred. These factors include that the animal was not heparinized and the guide catheter may have induced thrombus formation.

Despite the thrombus formation within the vessel and the distal emboli, the results of filling the aneurysm appeared successful. The gel remained within the aneurysms for at least five hours and did not appear to be subjected to quick digestion by the animal's own thrombolytic enzymes. This would however occur, and thus is why when doing a

survival animal study, it would be important to include factors such as aprotinin and Factor XIII added to the fibrin gel. These factors resist the digestive enzymes and strengthen the overall gel, thus would result in longer occupancy of the gel within the aneurysm. Delivery of the gel would be further tested in a heparinized environment and would be injected in smaller increments.

VII.5 Conclusion

In vivo testing is a vital part of the progression of fibrin gel as a potential treatment for use in intracranial aneurysms. This preliminary study illustrated that the coaxial delivery system previously described could deliver fibrinogen and thrombin in a manner that results in formation of fibrin gel, occluding the aneurysm. Although there was fibrin formation within the main vessel, future prevention methods could be taken to reduce that risk.

CHAPTER VIII

CONCLUSION AND FUTURE SUGGESTIONS

Fibrin gel is a potential liquid embolic capable of occluding intracranial aneurysms using endovascular techniques. The purpose of this study was to explore the feasibility of this novel treatment idea and develop a technique for delivery of this agent into a cerebral aneurysm. In addition to the delivery technique, other factors such as radiopacity and properties affecting the structure of the fibrin were also explored.

The results of this research illustrated that when delivered properly, fibrin gel potentially can be a viable and effective treatment for occluding intracranial aneurysms. The *in vitro* results concluded that the two components creating fibrin gel could be delivered through a coaxial system and occlude the aneurysm from the oncoming flow, avoid main vessel occlusion and withstand high flow rates. In addition, this coaxial delivery technique, in combination with the use of threaded syringes, allowed good control over the injection of the two components.

These *in vitro* experiments also demonstrated the need for a form of neck protection. When using no balloon protection, the inflow zone of the aneurysm model was left exposed to the oncoming flow. For this treatment to be successful, it must protect the inflow zone. The addition of a stent was found to be a viable alternative to balloon protection. The stent adequately altered the incoming flow to allow for successful occlusion of the aneurysm, including inflow zone protection, by the fibrin gel.

Direct fibrin injection was explored and also produced successful results. The direct fibrin injection was able to fill the aneurysm and withstand high flow rates. This form of delivery is more beneficial to these experiments, as it is a simpler procedure,

reduces the amount of time needed to inject and may reduce problems associated with the two component injection i.e., not injecting simultaneously. This is a method of delivery that still needs further exploration.

The radiopacity of the fibrin gel was experimented with. Our results were not as successful as when injecting the two components without any addition radiopaque materials. The most promising results which were produced were when using direct fibrin injection. This is another area which needs further study.

The preliminary *in vivo* study demonstrated that the use of the coaxial delivery system could inject both components simultaneously when in a physiologic environment. The injected fibrin gel was not digested immediately by the animal's fibrinolytic system and appeared stable. Although a thrombus formation occluded a distal branch, this study opened the door for future experiments.

The overall results of this research provided promising results for continuing research on fibrin gel as a potential alternative treatment for intracranial aneurysms.

Future Recommendations

Further studies vital to the progression of this potential treatment includes *in vivo* testing of this method using survival animal studies and further *in vitro* testing, using models of varying curvatures and geometry and testing of radiopaque materials.

Survival animal studies would answer many questions which remain. Will the cracks within the fibrin gel self thrombose? What is the histological response to the fibrin? And is the fibrin degraded too quickly or is there ample time for new cell and tissue ingrowth?

Curvature and geometry are important factors which need to be incorporated into this study. The flow environment greatly changes with changing geometry and may have different affects on the fibrin gel and its stability.

Radiopacity of the fibrin gel is another aspect to making this a successful treatment. Although not necessary, having visual guidance as to the amount of fibrin filling the aneurysm would enhance the clinician's control over the delivery as well as shorten the time of the procedure.

Chapter IX: Appendix

Sylgard Aneurysm Models:

Initial experiments used sylgard aneurysm models. Sylgard is a silicone elastomer. When the silicone elastomer base is combined with the curing agent, at a ratio of 10:1, the combination forms a liquid substance. Once the liquid stands for a period of at least 24 hours, it becomes a flexible polymer.

To create the aneurysm model, we would initially use Play-Doh to form the shape of the aneurysm. All models were straight, sidewall aneurysms. Once the Play-Doh hardened (approximately 1 day), the liquid mixture of the sylgard would be painted on in thin layers. After each layer the sylgard would be allowed to dry for 24 hours. After about 8-10 sylgard layers, the model would be submerged in water to dissolve out the Play-Doh.

The sylgard models were quickly replaced by the glass aneurysm models. The sylgard models were extremely flexible and due to this flexibility, the walls of the model were elastic, not rigid as we desired. The glass models provided a rigid system, and thus provided better results.

Glass Aneurysm Models:

The glass aneurysm models were purchased from Farlow's Scientific GlassBlowing, Inc. All models were hand blown to the specific measurements that were provided. The following chart is the measurements used for the straight,

Straight SideWall Aneurysm	*Model 1*
Aneurysm Diameter (mm)	10
Orifice Diameter (mm)	6.25
Height from Main Vessel Center to Aneurysm Center (mm)	5.53
Inner Diameter (ID) (mm)	3.25
Length (mm)	100

sidewall glass aneurysm model. To contact Farlow's Scientific GlassBlowing, Inc: Office: (530) 477- 5513 Fax: (530) 477 9241

Chapter X: References

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