Characterizing Endothelial Damage During Ischemic Stroke Treatment with 3D Cerebrovascular Live Cellular and Finite Element Models

Ana Regina Martinez Dehesa

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Characterizing Endothelial Damage During Ischemic Stroke Treatment with 3D Cerebrovascular Live Cellular and Finite Element Models

by

Ana Regina Martinez Dehesa

A thesis submitted to the Department of Biomedical and Chemical Engineering Sciences of Florida Institute of Technology
in partial fulfillment of the requirements for the degree of

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Abstract

Characterizing Endothelial Damage During Ischemic Stroke Treatment with 3D Cerebrovascular Live Cellular and Computational Models

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Stent retriever mechanical thrombectomy (SRMT) is part of the clinical standard-of-care for acute ischemic stroke (AIS) patients with large vessel occlusion (LVO). Endothelial injury (EI) is widely associated with SRMT. Studies report finding EI markers in over 50% of retrieved clots, suggesting the frequency of EI in MT patients is rather common. The mechanism of EI and its contribution to MT failure and clinical outcomes remain poorly understood because of a lack of techniques to observe EI and study it in vitro. This study aims to investigate the mechanisms of EI induced by SRMT, as well as the effect stent retriever diameter has on the extent of EI after SRMT, by developing and analyzing an in vitro live cellular 3D cerebrovascular model, and an in silico finite element model (FEM). Extent of EI and effect the size of a stent retriever has on resulting EI from SRMT is then analyzed qualitatively and quantitatively both in vitro and in silico.

The in silico FEM consisted of a tubular artery model with a stent expanding and passing through it. The results show a high variability in the shear stresses experienced by the artery depending on the location, and its proximity to the stent and its movement: different points in one same circumferential region of the artery can experience different levels of
shear stress. The results also suggest that the region where the stent expands experiences shear stresses that remain partially present even after the stent has passed, and that the passing of the stent edges results in higher shear stresses compared to the middle body. Lastly, while the stent movement still has an effect on the areas it has passed through, the areas close to stent movement but that do not experience actual contact with the stress remain relatively unaffected with low shear stress values. The 3D cerebrovascular live cell model consisted of tubular artery constructs produced using 3D printing and PDMS casting techniques, with endothelial cells seeded on the inner wall. After a functional and confluent testbed was established, an SRMT was simulated using two different stent sizes. Extent of EI as well and impact the stent size had was then assessed using qualitative and quantitative analysis. A system to seed a confluent layer of endothelial cells with general homogeneity throughout PDMS artery models was successfully developed. The combined results show clear signs of EI caused by both stent sizes, which was seen as areas with significant denudation of cells, and wrapping of cells over each other into 3D structures. Statistically significant difference was achieved between the control condition and the smaller stent size condition for number of nuclei ($p = 0.04$, 95% C.I. = [11.57, 450.88]), and between the control condition and both stent sizes for percent cell coverage ($p = 0.002$, 95% C.I. = [23.58, 72.93]). Additionally, the results show the smaller stent size resulted in a higher degree of EI compared to the larger size. Although more testing is needed to assert the accuracy of the results, this finding is supported by other studies that have found an increased risk of restenosis and other adverse cardiac events with smaller stent diameters.
# Table of Contents

Abstract ........................................................................................................................................ iii  
List of Figures .............................................................................................................................. viii  
List of Tables ............................................................................................................................... x  
Acknowledgement ...................................................................................................................... xi  
Dedication ..................................................................................................................................... xii  
Chapter 1 Introduction ................................................................................................................ 1  
1.1 Stroke Statistics ....................................................................................................................... 1  
1.2 Treatment of Acute Ischemic Stroke ...................................................................................... 2  
  1.2.1 Thrombolytic Agents ........................................................................................................ 2  
  1.2.2 Endovascular Treatments ............................................................................................... 2  
1.3 MT Complications .................................................................................................................. 4  
  1.3.1 Endothelial Injury ........................................................................................................... 4  
  1.3.2 First Pass Effect ............................................................................................................. 5  
1.4 Literature Review ................................................................................................................... 6  
  1.4.1 Cerebrovascular Circulation & Stroke .......................................................................... 6  
  1.4.2 Arteries & Endothelium Biology .................................................................................... 7  
  1.4.3 Endothelial Damage and Endovascular Therapy ............................................................. 9  
  1.4.4 Study Objectives ........................................................................................................... 9  
Chapter 2 Materials & Methods: 3D Cerebrovascular Computational Model ...................... 10  
  2.1 Breakdown of SRMT Computational Model ................................................................. 10  
  2.2 Setting Up Cerebrovascular Model Geometry ................................................................. 11  
    2.2.1 Arterial Model ............................................................................................................. 11
2.2.2 Stent Retriever Model .................................................................................. 11
2.2.3 Material Properties ...................................................................................... 12
2.2.4 Boundary Conditions, Displacement, Analysis Settings ............................. 12
2.3 Post-Processing of Simulation Results ......................................................... 13
Chapter 3 Materials & Methods: 3D Cerebrovascular Live Cellular Model ............. 14
3.1 Arterial Models ................................................................................................ 14
  3.1.1 Material for Arterial Models ................................................................... 14
  3.1.2 PDMS Surface Modification .................................................................. 15
  3.1.3 Tubular Artery Models ......................................................................... 16
  3.1.4 Endothelial Cell Seeding on Arterial Models ......................................... 18
3.2 Stent Retriever Mechanical Thrombectomy .................................................. 19
3.3 Post-MT Microscopy Imaging ........................................................................ 20
  3.3.1 Immunofluorescence Microscopy .......................................................... 20
  3.3.2 Scanning Electron Microscopy Imaging ................................................. 21
3.4 Post-MT Quantitative Analysis ...................................................................... 22
  3.4.1 Statistical Analysis ................................................................................ 23
Chapter 4 Results & Discussion ........................................................................... 25
4.1 Cardiovascular Finite Element Model Simulation ........................................... 25
  4.1.1 Points 1-4: Region Closest to Stent at Beginning of Simulation ............... 26
  4.1.2 Points 5-8: Central Region .................................................................... 28
  4.1.4 General Discussion .............................................................................. 31
4.2 3D Cerebrovascular Live Cellular Model ....................................................... 32
  4.2.1 Cytotoxicity Study .............................................................................. 32
  4.2.2 Regular Immunofluorescence Microscopy Imaging .................................. 32
<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.2.3</td>
<td>Confocal Microscopy Imaging</td>
<td>37</td>
</tr>
<tr>
<td>4.2.4</td>
<td>Scanning Electron Microscopy Imaging</td>
<td>37</td>
</tr>
<tr>
<td>4.2.5</td>
<td>Quantitative Analysis</td>
<td>40</td>
</tr>
<tr>
<td>4.2.6</td>
<td>Statistical Analysis</td>
<td>42</td>
</tr>
<tr>
<td>4.2.7</td>
<td>General Discussion</td>
<td>43</td>
</tr>
<tr>
<td>Chapter 5</td>
<td>Conclusion &amp; Future Work</td>
<td>45</td>
</tr>
<tr>
<td>5.1</td>
<td>Conclusion and Future Works for Computational Finite Element Model</td>
<td>45</td>
</tr>
<tr>
<td>5.2</td>
<td>Future Works for 3D Live Cellular Model</td>
<td>46</td>
</tr>
<tr>
<td>5.3</td>
<td>Final Comments</td>
<td>47</td>
</tr>
<tr>
<td>References</td>
<td></td>
<td>48</td>
</tr>
</tbody>
</table>
List of Figures

Figure 1 Illustrative images of a LVO patient. 67-year-old male presenting 4 hrs after onset with middle cerebral artery (MCA) occlusion. (A) Head computerized tomography without hemorrhage as cause of stroke syndrome. (B) CTA showing right MCA occlusion (white arrow). (C) MRI showing a small established core infarct. (D) Anteroposterior view, catheter angiogram showing ICA occlusion. (E) Complete recanalization following MT (F) 24 hr MRI showing arrest of infarct growth following reperfusion [19]..........................3
Figure 2 Mechanical Thrombectomy Devices: Aspiration and Stent Retriever [24] .......... 4
Figure 3 Structure of the Arterial Wall: Diagram [39] and 100x Microscope View [40] ..... 7
Figure 4 Multiple functions of endothelium [43] ................................................................. 8
Figure 5 Stent Retriever Geometry [54] ............................................................................. 11
Figure 6 Computational Model of Artery and Stent At Beginning of Simulation........... 13
Figure 7 Endothelial cells with Collagen I Coating (above), and endothelial cells with Fibronectin Coating (below).................................................................................... 16
Figure 8 (A) 3D printed PLA 4x5 array of 2 cm rods (B) Resulting PDMS tubular construct (2.5 mm ID, 6.9 mm OD, wall thickness of 1.1 mm, and length of 14 mm (C) Setup of 3D printed array lined up with 96 well plate after pouring PDMS in wells........ 17
Figure 9 Trevo XP ProVue Stent Retriever, Stryker, 2016; images courtesy of Stryker Dimensions: A = 190, 180 mm; B = 36, 40 mm; C = 20, 25 mm, D = 3, 6 mm;................... 19
Figure 10 Fluorescence microscopy of Human Pulmonary Microvascular Endothelial Cells. Blue = DAPI, Green = Phalloidin (Actin), Red = VE-Cadherin Antibody. Data by Daniel Bourquain, Robert Koch Institut, Berlin, Germany [70]......................................... 21
Figure 11 Quantitative Analysis Using ImagePro Software for Number of Nuclei (left) and Cellular Coverage (right)................................................................................................. 23
Figure 12 Flowchart Summary for 3D Cerebrovascular Live Cellular Model............... 24
Figure 13 Contour Plot for Arterial Shear Stress during SRMT expansion (1) and retrieval (B and C)......................................................................................................................... 25
Figure 14 Scatter Plot Showing Shear Stress Levels for Points 1-4 Located Closest to Stent at Beginning of Simulation with Corresponding Region Highlighted in Arterial Model...27
Figure 15 Scatter Plot Showing Shear Stress Levels for Points 5-8 Located in Central Artery with Corresponding Region Highlighted in Arterial Model........................................29
Figure 16 Scatter Plot Showing Shear Stress Levels for Points 9-12 Located Closest to Stent at End of Simulation
4.1.3 Points 9-12: Region Closest to Stent at End of Simulation
........................................................................................................................................30
Figure 17 Cell Number After 24-hour Exposure to Elastic50 Extract Medium Based on AlamarBlue Assay Data........................................................................................................32
Figure 18 Immunofluorescence Microscopy Imaging Results for All Conditions...........34
Figure 19 Volumetric View of Confocal Imaging at 10X for All Conditions...............35
Figure 20 Confocal Immunofluorescence Microscopy Imaging Results for All Conditions ........................................................................................................................................36
Figure 21 Preliminary Study Including Control (above) and Smaller Stent Size (below) Scanning Electron Microscope Images........................................................................38
Figure 22 Scanning Electron Microscopy Imaging Results for All Conditions at 100X ....39
Figure 23 Immunofluorescence Microscopy Imaging Post-Processing Column Graph of Average Number of Nuclei Relative to 10X Image for Control, MT Small, and MT Large Based on DAPI Signal Nuclei Counting through ImagePro Software ......................40
Figure 24 Immunofluorescence Microscopy Imaging Post-Processing Column Graph and Donut Chart of Average Number of Nuclei Relative to 10X Image for Control, MT Small, and MT Large Based on Phalloidin Signal Intensity through ImagePro Software............41
List of Tables

Table 1 Arterial Material Properties ................................................................. 12
Table 2 Conditions for Live Cellular Model Study ............................................ 20
Table 3 Descriptive Statistics for Number of Nuclei in All Conditions ............... 43
Table 4 Descriptive Statistics for Percentage Cellular Coverage in All Conditions ...... 43
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This dedication is to my beloved grandfather, who embodied the epitome of strength and integrity. He was one of my biggest supporters in my educational pursuits, and I sorely will miss his presence during my graduation. I feel immensely fortunate to carry his example with me for the rest of my life. His memory continues to inspire me to strive for excellence in all that I do.

Abue, esto es para ti.
Chapter 1
Introduction

1.1 Stroke Statistics

Each year, over 12.2 million people worldwide will suffer from a stroke, of which approximately 6.5 million won’t survive, making stroke the second leading cause of death globally. The economic burden this poses is estimated at approximately US$700 billion annually. It is a major and growing global health problem, with the number of incidences increased by 70% over the last 20 years [1]–[3].

Acute ischemic strokes (AIS) make up a significant proportion of all strokes, accounting for over 62% of all cases and for approximately 3.3 million deaths each year [1] An AIS is the result of a narrowing or blockage to an artery that supplies blood to the brain. The blockage can be caused by the formation of a clot within a cerebral blood vessel (thrombosis), by the movement of a clot from elsewhere in the body to the brain (embolism), or by severe narrowing of the artery (stenosis). This leads to severely reduced blood flow (ischemia), resulting in a rapid reduction in neurological function [4], [5].

Large vessel occlusion (LVO) is the obstruction of large, proximal cerebral arteries and can account for up to 46% of AIS [6]. Due to the larger vasculature involved, LVO stroke is associated with greater symptoms after onset, larger infarcts, and worse outcomes [7]. LVO’s are responsible for three-fifths of dependency and more than nine-tenths of mortality after AIS [8]. In general, an LVO is referred to as an acute blockage of one or more of the intracranial internal carotid artery, proximal posterior, middle, and anterior cerebral arteries, intracranial vertebral artery, and/or basilar artery [9].
1.2 Treatment of Acute Ischemic Stroke

1.2.1 Thrombolytic Agents

All current treatment strategies for AIS aim at restoring blood flow as fast as possible after symptom onset. The rapid diagnosis and management of a stroke is essential to decrease its impact on the patient. Time is especially critical when it comes to the current initial treatment for AIS, early reperfusion through intravenous administration of tissue plasminogen activator (tPA) to break up the clot. This is the ideal treatment, as this limits the extent of tissue damaged due to infarction [10]; unfortunately, a very limited percentage of stroke patients can consider this treatment option because of the extremely narrow timeframe: it is only a viable option up to 3 hours after symptom onset. By the time the patients are evaluated, the majority have passed this 3 hour window. Additionally, although early reperfusion has shown to be advantageous, a mere 3% to 8.5% of eligible stroke patients who are eligible for t-PA treatment receive it, largely because of the risk of intracranial hemorrhage. More importantly, treatment with IV t-PA alone in patients with LVO is associated with low recanalization rates [11], [12].

1.2.2 Endovascular Treatments

Over the past recent years, reports of mechanical thrombectomy's superiority over intravenous thrombolitics have propelled endovascular intervention to the front line of acute ischemic stroke (AIS) management. From the start of mechanical thrombectomy (MT) with the Merci device (Stryker) and the first-generation Penumbra aspiration system (Penumbra Inc), modern techniques have consistently shown successful recanalization and enhanced clinical outcomes [13]. Endovascular therapy has become the standard treatment for large-vessel occlusion (LVO) acute ischemic stroke (AIS) [7]. MT has proven to be highly beneficial for treating AIS patients with LVO, improving patient outcomes and reducing hospitalization time and costs [14]–[16]. This treatment aims at restoring bloodflow by recanalizing the occluded cerebral artery using mechanical devices [12], and has the capacity to rapidly remove large, proximal clots, resulting in higher reperfusion rates compared to t-PA alone [17]. Mechanical thrombectomy (MT) is part of the current
clinical standard-of-care for AIS patients with LVO [18]. Currently, two of the most widely used and effective MT approaches for AIS treatment are (1) MT with stent retrievers, and (2) MT with direct aspiration.

![Illustrative images of a LVO patient.](image)

**Figure 1** Illustrative images of a LVO patient. 67-year-old male presenting 4 hrs after onset with middle cerebral artery (MCA) occlusion. (A) Head computerized tomography without hemorrhage as cause of stroke syndrome. (B) CTA showing right MCA occlusion (white arrow). (C) MRI showing a small established core infarct. (D) Anteroposterior view, catheter angiogram showing ICA occlusion. (E) Complete recanalization following MT (F) 24 hr MRI showing arrest of infarct growth following reperfusion [19].

The stent retriever mechanical thrombectomy (SRMT) method involves making a small incision (usually in groin area), and mechanically removing the thrombus using a self-expandable stent crimped at the end of a flexible wire in a microcatheter, which is threaded in the occluded vessel across the thrombus. Once appropriately positioned, the microcatheter is withdrawn, deploying the stent retriever. The clot is then trapped in the stent struts and pulled along with the stent towards a receiving catheter.

The thrombectomy with aspiration works using large bore catheters to remove the clot by suction: the catheter tip is placed right by the clot and negative pressure is applied, resulting in clot aspiration into the catheter [20]. Neurointerventionalists have yet to settle
on which MT approach is superior over the other [21]–[23]. Although some of the initial information pertains to both approaches, this work focuses on stent retriever mechanical thrombectomy.

Figure 2 Mechanical Thrombectomy Devices: Aspiration and Stent Retriever [24]

1.3 MT Complications

Although the effectiveness and safety of stent retriever thrombectomy devices has been fully established for AIS patients with LVO, device related vessel damage, such as vasospasm, perforation, and dissection, occurs sometimes. In addition to the risk of vessel wall injury, angiographically occult vessel ruptures caused by mechanical stretch during stent retrieval could be associated with vessel wall permeability impairment, as well as blood-CSF barrier disruption after SR thrombectomy [25]. Other complications, such as thrombus embolization to distal vessels because of clot disruption are also possible during any moment of the MT procedure.

1.3.1 Endothelial Injury

While rarely considered in device development, endothelial injury (EI), which refers to damage to the monolayer of endothelial cells that constitutes the inner cellular lining of all blood vessels, is widely associated with MT, and poses a significant concern for the safety and efficacy of this treatment approach. Histological and immunocytochemistry analysis of
clots retrieved during MT has been an approach used to comprehend the mechanics of an LVO stroke, but these clots are rarely studied for EI markers, and so the mechanisms and consequences of this complication are largely unknown. Nevertheless, the evidence pointing towards the prevalence of this issue is certainly cause for concern.

Recent reports suggest that arterial damage from EI is often caused by devices used in both MT approaches (SR and direct aspiration), although the mechanisms of injury likely differ. Vascular injury extending into the medial layer has been reported in swine models, suggesting this type of histological vascular injury caused by thrombectomy devices could potentially occur in real clinical settings [26]. There have also been reports of vascular injury in animal models and in in vitro live cell platforms that vary depending on the thrombectomy device (type, diameter, length) [27], [28].

The risk for endothelial injury should not be taken lightly, as studies report finding different EI markers in 22-58% of retrieved clots suggest the frequency of EI in MT patients is rather common. EI could play a significant role in reperfusion injury (tissue damage that follows restoration of blood flow after a period of ischemia [29]), restenosis (reduction in lumen diameter after endovascular intervention), or re-occlusion post-MT (subsequent reocclusion of target vessel after initial recanalization has been achieved [30]), which would likely result in a poor outcome. This is supported by studies that have reported follow-up MRI/MRA analyses of post-MT blood vessels that show imaging markers characteristic of vessel injury and inflammation being associated to a higher risk of re-occlusion or future restenosis [25], [31]. Although endovascular stroke treatment (EVT) has revolutionized the treatment of AIS, still almost 2 out of 3 patients with an AIS will have an unfavorable outcome and become functionally dependent [14], [32], [33].

1.3.2 First Pass Effect

As previously mentioned, almost 2 out of 3 patients with AIS have an unfavorable outcome and become functionally dependent. In addition to this concerning statistic, the success rate for revascularization after EVT is less than 50%, and success rate on the 1st attempt, or First Pass Effect (FPE), is less than 30%. More specifically, FPE refers to achieving a full
recanalization with a single thrombectomy device pass. FPE is a recently emerged measure for newer generation thrombectomy devices. Achieving FPE is of much importance, as multiple thrombectomy device passes may promote arterial endothelial injury, potentially reducing clinical efficacy and safety. Given the significance FPE has on achieving a positive patient outcome, as thrombectomy device design continues to improve, the main angiographic objective should be to accomplish full recanalization in a single pass [34].

The safest and most effective method for a MT would be to achieve FPE without causing EI. However, even though EVT is a widely practiced procedure, there are no established metrics for this to be achieved in clinical practice. The mechanism of EI and its contribution to MT failure and clinical outcomes remain poorly understood. This is perpetuated by a lack of techniques to observe EI and study it in in vitro biological systems. It is thus evident there is still great improvement to be achieved for EVT, which can only be done by enriching the current knowledge and understanding on the mechanisms and interactions that take place in EVT, as well as the causes of negative outcomes. Due to ethical considerations, as well as the patient-specific and interventionist-experience-based nature of EVT, conducting a systematic in vivo study with a detailed sensitivity analysis of each individual aspect of the procedure is difficult, if not impossible. In this aspect, in vitro and in silico studies have significant potential to help improve and further optimize current understanding of the procedure [35].

1.4 Literature Review

1.4.1 Cerebrovascular Circulation & Stroke

The brain, despite constituting only 2% of the body's mass, utilizes approximately 50% of the body's glucose, making it the most energy-demanding organ in the human body. As a result, it logically follows that the brain requires substantial blood perfusion, which it indeed receives. The brain is among the most perfused organs in the body, receiving arterial blood from two primary sources: the anterior circulation originating in the internal carotid arteries and the posterior circulation originating in the vertebral arteries. These arteries enter the cranium and branch out extensively, supplying blood to all regions of the
brain, both superficial and deep. Any disruption in this blood supply, whether within or outside the cranium, increases the likelihood of cerebrovascular disorders, including the most common and infamous one: stroke [36]. Stroke is a complex condition that results from various disruptions, diseases, and injuries to the arterial wall. The risk factors for stroke are closely linked to modern society's problems, including smoking, obesity, hypertension, diabetes, and inflammation resulting from systemic diseases or recurring infections [37].

1.4.2 Arteries & Endothelium Biology

Blood vessels are usually composed of three layers: the tunica intima, tunica media, and tunica adventitia. The tunica intima consists of a layer of endothelial cells lining the lumen of the vessel, as well as a subendothelial layer made up of mostly loose connective tissue. Maintaining the integrity of the intima is of utmost importance as any harm to it can result in atherosclerosis or clotting. The endothelium is a unique type of epithelial tissue consisting of a thin and fragile lining in all blood vessels, the heart, and the lymphatic system. It can be argued that the endothelium is the most crucial aspect of these structures. [38].

![Figure 3 Structure of the Arterial Wall: Diagram](image1) ![and 100x Microscope View](image2)

For a long time, the endothelium was believed to be relatively inactive, serving only as a physical barrier between the circulating blood and underlying tissues. As time passed, it was discovered that despite its single-layer structure, the endothelium plays a crucial role
in maintaining bodily homeostasis. Figure (4) provides an overview of the various functions of the vascular endothelium, which include regulating vessel integrity, vascular growth and remodeling, tissue growth and metabolism, immune responses, cell adhesion, angiogenesis, hemostasis, and vascular permeability. The endothelium plays a critical role in regulating vascular tone, which involves controlling tissue blood flow, inflammatory responses, and maintaining blood fluidity [41]–[43]. Any disruption to these physiological and pathological processes leads to endothelial dysfunction, which can result in numerous diseases such as atherosclerosis, hypertension, and diabetes [44]. The vascular endothelium is thus a critical contributor to the development of atherosclerosis and cardiovascular disease. On the other hand, conditions such as angioplasty, stenting, diabetes, hypertension, and immunological damage can result in vascular injuries, which can lead to endothelial dysfunction and damage [43], [45], [46].

**Figure 4 Multiple functions of endothelium** [43]

The healthy vascular endothelium synthesizes various molecules that possess antithrombotic properties, such as nitric oxide, prostacyclin, tissue plasminogen activator, thrombomodulin, heparin-like molecules, and tissue factor pathway inhibitor. The intercellular junction complexes of the endothelial cells play a crucial role in maintaining the integrity of the endothelial barrier, regulating signal transduction and controlling endothelial permeability.
1.4.3 Endothelial Damage and Endovascular Therapy

Under normal circumstances, vascular endothelial cells act as a robust barrier against thrombosis, lipid uptake, and inflammation. However, after endovascular intervention, the regenerated endothelium is lacking in terms of integrity and function, characterized by poorly formed cell junctions, decreased expression of antithrombotic and antiatherogenic molecules, and reduced nitric oxide production. Delayed arterial healing with poor endothelialization is the primary cause of late (1 month – 1 year post MT) and very late stent thrombosis. Stent-induced flow disturbances lead to complex spatiotemporal shear stress, which can change the endothelium phenotype from quiescent to inflammatory and increase its thrombogenicity. A comprehensive understanding of vascular biology is thus essential for all cardiologists, particularly interventional cardiologists, as the maintenance of a functional endothelium is crucial for long-term vascular health [47].

1.4.4 Study Objectives

This study aims to contribute towards scientific literature on investigating the mechanisms of EI induced by stent retriever thrombectomy. We hypothesized that developing and analyzing an in vitro live cellular 3D cerebrovascular model, as well as an in silico finite element model, will provide insight on the EI extent as well as mechanisms that take place during a SRMT, and will thus “open the door” to long-term development of methods to predict EI magnitude for a given MT strategy, all for the goal of more effective approaches to the treatment of AIS patients with LVO. Furthermore, we hypothesized that parameters such as stent retriever diameter and length play part in the extent of EI during SRMT. To test these hypotheses, the study has 3 aims: 1) Develop an in vitro live cellular 3D cerebrovascular model representing an artery and its inner endothelial lining, and simulate an in vitro stent retriever mechanical thrombectomy (2) Develop an in silico cerebrovascular model representing the artery and stent retriever, and simulate an in silico SRMT, and lastly, 3) Assess the effect the size of a stent retriever has on the extent of the EI resulting from MT both in vitro and in silico.
Chapter 2
Materials & Methods: 3D Cerebrovascular Computational Model

The methods for this work are divided in two parts, Chapter 2 describes the methods for the computational finite element model, and Chapter 3 covers the methods for the 3D cerebrovascular live cellular model.

2.1 Breakdown of SRMT Computational Model
The SRMT mechanically removes the thrombus using a Nickel-Titanium (NiTi) self-expandable stent at the end of a flexible wire, initially in a crimped configuration in a microcatheter, which is threaded through the artery to the blockage site and positioned across the thrombus. Once in position, the microcatheter is withdrawn, resulting in the stent retriever being deployed. The clot is then trapped in the stent struts and pulled along with the stent towards a receiving catheter [48]. Studies show MT complications such as perforation and embolization are thought to arise mostly during stent retrieval [49]. The computational model in this work focuses on investigating the EI that occurs during MT, more specifically, during the expansion and retrieval of a stent. Since some of the EI is potentially caused by a combination of clot-stent interactions, the model will initially include only the artery and the stent, to first understand the EI that is caused by the stent expansion and retrieval.

ANSYS 14.5 (Ansys Canonsburg, PA) was used for finite element model development and post processing of the results. The initial computational model consisted of an artery represented by a tubular model and a stent. The stent geometry was made to have an initial interference with respect to the artery, which allowed to represent the expansion of the stent: the artery is radially stretched by the stent, representing the expanded state of the stent. The stent retrieval was then simulated by introducing a displacement of the “expanded” stent geometry in the axial direction of the artery. The simulation was ran
followed by post-processing of results which allowed a numerical approximate of the shear stress values experienced by the artery model during the expansion and retrieval of the stent model.

2.2 Setting Up Cerebrovascular Model Geometry

2.2.1 Arterial Model

The arterial model was made to have a tubular geometry with dimensions based off the dimensions of an MCA (mean diameter 3 mm [50], mean thickness for atherosclerotic artery > 1 mm [51], [52]), which as previously mentioned, is one of the most common sites for LVO [53]. The artery model consisted thus, of a horizontally lying cylinder, with inner diameter of 2.5 mm, outer diameter of 6.9 mm, wall thickness of 1.1 mm, and length of 205 mm.

2.2.2 Stent Retriever Model

The stent geometry used for the computational model was obtained from the Cardiovascular Stent course available through Ansys Innovation Courses [54]. The geometry was created in SolidWorks, saved as a Parasolid file and imported to Ansys Mechanical.

Figure 5 Stent Retriever Geometry [54]
2.2.3 Material Properties

The material properties of the artery were based on a previous study by Lally et al. This model describes the behavior of the artery using a five-parameter, third-order, Mooney-Rivlin hyperelastic constitutive equation. The constants for this model were developed by fitting the five-parameter Mooney Rivlin expression to uniaxial and equibiaxial tension tests of human femoral arterial tissue data (see Table 1) [55], [56].

This model assumes the stent to be made of 316LN stainless steel. The Poisson ratio is 0.3 and the Young Modulus is 200 GPa.

Table 1 Arterial Material Properties

<table>
<thead>
<tr>
<th>Hyperelastic Coefficients (MPa)</th>
<th>Incompressibility (MPa⁻¹)</th>
<th>Density (kg m⁻³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C10</td>
<td>C01</td>
<td>C20</td>
</tr>
<tr>
<td>0.0189</td>
<td>0.00275</td>
<td>0.59</td>
</tr>
</tbody>
</table>

2.2.4 Boundary Conditions, Displacement, Analysis Settings

Standard earth gravity was added in the appropriate axis. A frictional contact was set with a friction coefficient of 0.2 between the artery and the surface edges of the stent geometry, with the stent as the contact body, and the inner wall of the artery as the target body; large deflection was turned on. An initial contact tool analysis allowed to determine the pinball radius which was set to 0.015 mm, with ramped effects. The artery and stent were constrained in the rotational directions. The artery was constrained axially at the distal ends, and the stent as well with the exception of the axis where the displacement would take place which was left free.

To model the expansion of the stent, the stent was assigned an initial ramped interference of 0.01 mm that would result in the artery being radially stretched by the stent. This initial interference was solved in a one-second initial step at the beginning of the simulation. An axial displacement of 7.5 mm was set for the stent to be simulated over the course of the
next 5 seconds, ending the simulation with a one-second end break, bringing the total theoretical length of the simulation to 7 seconds. The first interference step was solved with a time step of 0.04 seconds, while the remaining 6 steps were solved using a time step of 0.01 seconds.

A mesh density study was performed with three element sizes (0.075 mm, 0.1 mm, and 0.125 mm) and showed convergence and similar results (<5% difference) for all three element sizes. The element size of 0.1 mm was used both for the artery and the stent.

2.3 Post-Processing of Simulation Results

The shear stress was retrieved for the entire body of the artery. Following this, three regions were selected from the artery, and four points were selected per region (12 points in total). The first region was located on one of the ends of the artery, the end closest to the stent at the beginning of the simulation. The second region was chosen in the center of the artery. The third region was on the other end of the artery, the end closest to the stent at the end of the simulation. The four points were chosen to be along different points of the artery’s circumference for each of their corresponding regions. This was done to gather shear stress data on several different areas of the artery to see the effect location had on the extent of the damage caused by the SRMT. Figure 6 shows what the computational model looked like at the beginning of the simulation.

![Computational Model of Artery and Stent At Beginning of Simulation](image-url)
Chapter 3
Materials & Methods: 3D Cerebrovascular Live Cellular Model

3.1 Arterial Models

3.1.1 Material for Arterial Models

The first task was to find a material that met the necessary requirements to successfully develop the 3D cerebrovascular model. Ideally, the material could be 3D printed to replicate models based off patients’ actual vascular geometries. The chosen material also had to be able to be used as a surface for endothelial seeding and for a stent retriever thrombectomy simulation. Lastly, the material had to be transparent enough to be imaged through, in order to assess the extent of arterial damage as a result of EI. For this reason, the transparent elastomeric photopolymer resin Elastic 50 [57] was initially considered.

To assess the feasibility of using this elastomeric resin as a surface to seed endothelial cells, an initial cytotoxicity study using the Alamar Blue assay was conducted following standard procedures [58]. First, extracts of the polymer were obtained (0.1 mg/mL) from 24, 48, and 72-hour incubations in fibroblast growth medium containing 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin at 37 °C and 5% CO$_2$. Semiconfluent fibroblast NIH 3T3 cells seeded on a 24 tissue culture polystyrene well plate were then exposed to the treated growth medium for 24 hours at 37 °C and 5% CO$_2$. Alamar Blue assay was then conducted to study the effect of the Elastic 50 polymer on cell metabolic activity, viability and growth. After 24 hours of the cells being in contact with the Elastic 50 extract media, the extract growth medium was aspirated, AlamarBlue solution was diluted from 10X to 1X in growth medium and added to each of the wells for all timepoint conditions. The fibroblasts were then incubated in the Alamar Blue mix at 37 °C and 5% CO$_2$ for 4 hours. After this, the Alamar Blue solution was collected for all timepoints and 100 μl was added to the wells of a clear bottom 96-well plate. The plate was read using the
SpectraMax M2e reader with an excitation wavelength of 555 nm and an emission wavelength of 595 nm. The next material considered was PDMS since this material has been widely used as a surface for seeding endothelial cells [59], has relatively simple fabrication techniques (although 3D prints are not possible), and is transparent.

3.1.2 PDMS Surface Modification

It is widely known that surface modification techniques such as plasma treatment [60] of the PDMS surface results in a more hydrophilic surface, which allows for a much improved cellular attachment. Unfortunately, this resource is not available at Florida Tech. Nevertheless, there are still techniques that can significantly improve endothelial cell attachment, such as surface coatings, among some of these, Collagen I [61] and Fibronectin [62]. In order to assess which of these two options would result in the higher endothelial cell attachment to PDMS, a preliminary 2D study was conducted. Flat discs of PDMS were prepared using the Sylgard PDMS kit and the specified instructions and a 12 well plate as the casting surface. The 12 well plate was initially coated in polyvinyl alcohol (PVA) before adding the PDMS mixture to facilitate lifting the samples after curing. The constructs were left to cure in a desiccator (to avoid the formation of bubbles) for 48 hours at room temperature. After this, the constructs were soaked in ethanol to remove any excess PVA and sterilized by autoclave. The flat disc models had a diameter of 1.5 cm and an approximate thickness of 2 mm. In accordance to standard procedure in our lab, the concentrations used were 4 ug/cm² for the fibronectin coating, and 4 mg/mL for the collagen I coating. After the PDMS discs (n=3 for each condition) were coated with their respective coating, endothelial cells (HUVECs) were seeded at a density of 800,000 cells/mL. After two days of growth, the samples were fixed with 4% formaldehyde, permeabilized with 0.1% Triton X, stained with DAPI and Phalloidin for 30 minutes and then washed 3x with PBS. The samples were then imaged with immunofluorescence microscopy. The images show the fibronectin coating resulted in a higher cellular attachment compared to the collagen I coating, which is why the fibronectin coating was used for the rest of the project (Figure 7).
At this point, a decision had been made for the material and the coating to be used (PDMS and fibronectin). This had to now be translated from a 2D setting to a 3D setting to be able to produce tubular arterial models seeded with endothelial cells. The goal was to obtain a tubular structure with a confluent monolayer of endothelial cells to be able to replicate a SRMT and assess the post-procedure EI. The structure of the tubular models was thus to be initially very simple and short in length.

Tubular arterial models were prepared using 3D printing and PDMS casting techniques. The PDMS material silicone constructs were prepared using the Sylgard PDMS kit and the
specified instructions (10:1 ratio for base and curing agent, respectively [63]), a 96 well plate, and a 3D printed rod array that was designed on Onshape, based on the geometry of the 96 well plate in order to have an exact fit. More specifically, it consisted of a 4x5 array of 2 cm rods whose center lined up exactly with the well centers of the 96 well plate, 3D printed using PLA. Figure 8 shows the setup details.

Figure 8 (A) 3D printed PLA 4x5 array of 2 cm rods (B) Resulting PDMS tubular construct (2.5 mm ID, 6.9 mm OD, wall thickness of 1.1 mm, and length of 14 mm (C) Setup of 3D printed array lined up with 96 well plate after pouring PDMS in wells

During preparation, the 96 well plate and the rod array were initially coated in polyvinyl alcohol (PVA) and left to dry before adding the PDMS mixture to facilitate lifting the tubular samples after curing. After removing all bubble formation from the PDMS mixture using a dessicator, the tubular models were left to cure at room temperature for 48 hours. After this, the constructs were soaked in ethanol to remove any excess PVA and sterilized by autoclave. The tubular models had an inner diameter of 2.5 mm, outer diameter of 6.9 mm, wall thickness of 1.1 mm, and length of 14 mm. The dimensions of the models were attempted to be based off the dimensions of one of the most common sites for LVO [53], the middle cerebral artery (MCA, mean diameter 3 mm [50], mean thickness 0.6 mm [51]). Studies have reported atherosclerotic MCA’s to have a maximum thickness of over 1 mm [52].
3.1.4 Endothelial Cell Seeding on Arterial Models

As previously mentioned, an initial coating study determined fibronectin to be the optimal coating when compared to collagen I in terms of endothelial cell attachment. The fibronectin coating was added to the tubular models after they had been fully cured and sterilized by autoclave. This was done by diluting stock fibronectin solution in PBS to obtain a coating solution with a concentration of 4 ug/cm² (standard lab procedure). The tubular structures were then loaded with the fibronectin coating solution and refrigerated overnight.

After coating the PDMS tubular models with fibronectin and refrigerating overnight, confluent Human Umbilical Vein Endothelial Cells (HUVECs, Passage 4) were passaged and seeded on the luminal surface of the PDMS arterial models at a density of 1*10⁶ cells/mL [59]. This was done by washing the endothelial cells with PBS, followed by lifting the cells with Trypsin/0.25% EDTA and a three-minute incubation period. After this, the trypsin was neutralized at a 1:1 ratio with fresh complete endothelial cell culture medium, and the cell solution was centrifuged at 1100 rpm for 4 minutes. After this, the cells were stained with Trypan blue and counted using a hemocytometer. The cell solution was then diluted with complete endothelial cell culture medium based on a volume that would fill the constructs’ inner lumen. The dilution was calculated based on the amount of cells counted with the hemocytometer and the desired density of 1*10⁶ cells/mL. After removing the fibronectin coating solution, the tubular constructs were loaded with the diluted cell solution with concentration of 1*10⁶ cells/mL. The volume of each construct being 100 ul, there would theoretically be approximately 100,000 cells per construct. The constructs were then incubated at 37°C and given a half rotation every 2 hours for the first 4 hours after seeding. After 24 hours, fresh complete endothelial cell medium was used to flush the constructs’ channels and added to their surrounding (24 well plate). The endothelial cells were given a 3-day growth period, with media changes and flushing of the construct channels every 2 days.
3.2 Stent Retriever Mechanical Thrombectomy

After the cell seeding, the arterial constructs were divided into three conditions: control (no mechanical thrombectomy/MT), and treated samples, where two different stent retriever sizes were used (n=3 for each size condition). Therefore, the three conditions were: 1) control, 2) small SR, and 3) large SR (refer to Table 2). After the 3-day growth period had passed, the SRMT was performed on the samples for the two treated conditions, using two different sizes of SR (Trevo XP PROVUE 3x20 mm and Trevo XP PROVUE 6x25 mm [64] seen on Figure 9), both used in clinical practice. The mechanical thrombectomy was performed in such way that the stent retriever was expanded and then slowly passed through the full length of the artery model at a velocity of approximately 2 cm/s. After the mechanical thrombectomy was performed with the two different stent sizes, all constructs were post-processed for qualitative and quantitative analysis.

Figure 9 Trevo XP ProVue Stent Retriever, Stryker, 2016; images courtesy of Stryker
Dimensions: A = 190, 180 mm; B = 36, 40 mm; C = 20, 25 mm, D = 3, 6 mm;
E = 0.015, 0.018 in
Table 2 Conditions for Live Cellular Model Study

<table>
<thead>
<tr>
<th>Controls (no MT)</th>
<th>MT Small Size</th>
<th>MT Large Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>N=3</td>
<td>N=3</td>
<td>N=3</td>
</tr>
</tbody>
</table>

3.3 Post-MT Microscopy Imaging

3.3.1 Immunofluorescence Microscopy

After performing the mechanical thrombectomy for the appropriate conditions, all samples were fixed and stained for immunofluorescence imaging. The samples were fixed with 4% formaldehyde, permeabilized with 0.1% Triton X, stained with DAPI and Alexa Fluor Phalloidin stains for the cells’ nuclei and cytoskeletons, respectively, with VE cadherin antibody and Alexa Fluor 633 as a secondary antibody. The constructs were then imaged at 10x magnification using regular fluorescence and confocal microscopy. The stained constructs were additionally imaged using confocal microscopy in hopes to better capture the three-dimensionality of the arterial models using the Nikon C1Si multi-spectral laser scanning confocal microscope [65].

The Role of DAPI, Phalloidin, and VE Cadherin

DAPI and phalloidin are powerful stains that are widely used in cell imaging. The DAPI stain is used to visualize the cells’ nuclei by staining nuclear DNA. Measuring the number of nuclei is the most straightforward way to measure cellular confluency or attachment. Phalloidin derivatives are commonly used to stain actin filaments in fixed cells and tissues, allowing thus the visualization of the cytoskeleton of the cell, which is essential to have an idea of the cell’s morphology and structure.

Lastly, VE cadherin is a transmembrane cell-cell adhesion molecule localized at the intercellular boundaries of endothelial cells that plays a significant role in their biology, by regulating the cohesion and organization of intercellular junctions, as it facilitates homophilic adhesion, functioning similarly to a traditional cadherin [66], [67]. Besides its adhesive functions, VE-cadherin also regulates important cell processes such as cell
proliferation and apoptosis [68]. Since it can be argued that VE-cadherin is the most important adhesion molecule for the formation of vascular structures and the stability of their cellular junctions [69], it was chosen as a functional biomarker during the staining process, to confirm not only the attachment of the endothelial cells to the PDMS surface, but also their functionality. Figure 10 shows what typical VE-cadherin (red), phalloidin (green), and DAPI (blue) stains look like on endothelial cells.

![Image of endothelial cells with staining](image_url)

**Figure 10** Fluorescence microscopy of Human Pulmonary Microvascular Endothelial Cells. Blue = DAPI, Green = Phalloidin (Actin), Red = VE-Cadherin Antibody. Data by Daniel Bourquain, Robert Koch Institut, Berlin, Germany [70].

3.3.2 Scanning Electron Microscopy Imaging

Scanning Electron Microscopy (SEM) imaging was performed after performing a serial dilution dehydration with pure isopropyl ethanol (50%, 65%, 75%, 85%, 90%, 95%, 100%; 5 minute soak per concentration) on the previously fixed samples. After this, the samples were sputter coated in gold for 120 seconds with the Denton Vacuum Desk II Sputter Coater [71] and then imaged at 100X using the JEOL JSM-6380LV Scanning Electron Microscope [72]
3.4 Post-MT Quantitative Analysis

After gathering the qualitative data through different types of microscopy, a quantitative data analysis was performed to further assess the EI caused by the SRMT performed on all PDMS arterial models. Two metrics were chosen and measured for all constructs relative to the imaged area: number of nuclei and cellular coverage percentage (based on the cytoskeleton). Both measurements were made using the ImagePro software and its Measure/Count/Size function, although the number of nuclei required some manual counting as well.

As previously mentioned, all samples were fixed and stained with DAPI and Alexa Fluor Phalloidin stains for the cells’ nuclei and cytoskeletons, respectively. All images taken with immunofluorescence microscopy are in fact obtained by the merging of several images, each corresponding to a specific channel imaging a specific stain. The quantitative analysis is done by using these individual images, more specifically, the number of nuclei is measured using the DAPI channel, and the percent coverage is measured using the phalloidin (or GFP) channel. The ImagePro software allows the user to specify the range of signal that should be taken into consideration. In the case of percent cellular coverage, the range is selected in such way that considers the area where cytoskeleton is present. This can be achieved without the need for any major manual adjustment. On the other hand, in the case of nuclei counting, the range is selected in such way that only considers the nuclei; however, after this, manual adjustments such as splitting, drawing, or toggling objects on/off, are made in order to get a more accurate count (in some cases combined with manual counting). Figure 11 depicts what the different channel (DAPI/GFP) images look like in the software after performing the necessary adjustments.
3.4.1 Statistical Analysis

The results were expressed as average ± standard deviation for N, with sample size of n=3 for all assays and tests. One-way ANOVA with Tukey post-hoc test for comparison of the three conditions (control, MT small, MT large) was employed for average number of nuclei and for average percent cellular coverage. Statistical significance was set at p < 0.05.

Figure 13 provides a brief flowchart summary for the methods of this first stage.
Figure 12 Flowchart Summary for 3D Cerebrovascular Live Cellular Model
Chapter 4 Results & Discussion

4.1 Cardiovascular Finite Element Model Simulation

The contour plot results show varying arterial shear stress depending on the location relative to the stent. The higher shear values correspond to the points in the artery directly in contact with the stent struts. Nonetheless, the surrounding areas still experience some shear stress, even after the stent has passed over an area, the area remains at a partial shear stress up to the end of the simulation (Figure 14).

![Contour Plot](image)

Figure 13 Contour Plot for Arterial Shear Stress during SRMT expansion (1) and retrieval (B and C).
4.1.1 Points 1-4: Region Closest to Stent at Beginning of Simulation

The results for points 1-4 located in the first region (Figure 15, closest to stent at beginning of simulation) show a linearly increasing shear stress for the first second of the simulation, which corresponds to the expansion of the stent and the radial stretch of the artery, represented by the initial interference as it is rampally resolved.

After the first second, once the stent displacement begins, the shear stress dips at different levels followed by a spike between seconds 1 and 2 for all points except for point 3, which keeps on increasing until it reaches the spike and joins the other points at approximately 1.5 seconds before they all gradually decrease. This time (between 1 and 2 seconds) shows the most variance in the plot, and corresponds to when the stent is passing over.

The different levels of dip can be explained by the stent’s geometry: some points of the artery were in contact with the stent (like point 3), which caused the shear stress to further increase (after already increasing because of the stent expansion), while other points were not in contact with the stent, which explains the different levels of dipping and spiking once the stent came in contact.

After this, the shear stress values become significantly consistent for all points, as the overlap in the graph shows (Figure 15) starting from 2.1 seconds up to the end of the simulation. This would suggest that any difference in the extent of damage that took place while the stent was passing over the region is averaged out while the stent passes over the rest of the artery.

The next 5 seconds of the simulation correspond to the stent passing over the rest of the artery. During this time, the shear stress values for the first region stay high and show a lot of variance (Figure 15 spikes). The shear stress values then stabilize for all points (spikes stop) at second 6 of the simulation, which corresponds to the end break during which the stent stands still for a second before the simulation ends.
This can be interpreted as the region still being affected by the stent’s nearby movement (spikes reappear) while it passes the rest of the artery, and the region remaining with a partial shear stress up to the end of the simulation, even after the stent has passed over it.

![Points 1-4 Shear Stress (MPa) Over Time](image)

*Figure 14 Scatter Plot Showing Shear Stress Levels for Points 1-4 Located Closest to Stent at Beginning of Simulation with Corresponding Region Highlighted in Arterial Model*
4.1.2 Points 5-8: Central Region

The initial expansion of the stent caused an increase in shear stress in the first region which was maintained throughout the simulation, and which on the other hand is not seen in the central region; the shear stress values stay low between seconds 0 and 1. This suggests that the region where the stent is expanded will experience an initial increase in shear stress that is maintained while the stent is slid through the rest of the artery, and that is not equally experienced in the rest of the artery. The scatter plot for the central artery region has much more variance and “activity” compared to the other two plots, which makes sense as this is the region that spends the most time in contact with the stent. This high variability can be seen in Figure 16 as spikes and dips all throughout the simulation (unlike the first region which stabilized for all points after 2 seconds of running the simulation).

The effect that the stent movement has on the arteries can be seen the most between seconds 3 and 6 of the simulation. This region is where the highest levels of shear stress were found, the highest shear stress corresponding to point 6 (approximately 815 Pa). A general spike-dip-spike can be seen for all points between seconds 4 and 5 of the simulation. This could perhaps be caused by an increased shear stress exerted by the edges of the stent compared to the center body, which would mean an increase in shear stress (spike) when the first end of the stent comes in contact with the region, followed by a decrease in shear stress (dip) when the center body of the stent is passing over it, and lastly, an increase in shear stress again when the other end of the stent passes over (second spike).

Lastly, the shear stresses stabilize at second 6, which corresponds to the end break during which the stent stands still for a second before the simulation ends. While the first region ended the simulation at a consistent shear stress of approximately 450 Pa, the central region ended it between 440 and 740 Pa. The higher shear stress values could be caused by points in the region that remained closer to the stent’s edge up to the end of the simulation.
Figure 15 Scatter Plot Showing Shear Stress Levels for Points 5-8 Located in Central Artery with Corresponding Region Highlighted in Arterial Model
Figure 16 Scatter Plot Showing Shear Stress Levels for Points 9-12 Located Closest to Stent at End of Simulation
4.1.3 Points 9-12: Region Closest to Stent at End of Simulation

The third and last region, located at the end of the artery, shows very low activity compared to the other two plots (Figure 16). For the majority of the simulation, all 4 points remain at close-to-zero shear stress values. It is only between seconds 5 and 6 of the simulation that a sudden and steep increase in shear stress values occurs for point 9, and a very slight increase for the rest of the points, which have overlapping plots all throughout the simulation. Lastly, as seen with the two previous plots, the values stabilize and stay at a fixed value. In this case, point 9 ends at approximately 42 Pa, while the rest of the points end at 3.86 Pa. The shear stresses for this third region are significantly lower than the other two regions, with a minimum difference of approximately 400 Pa. This would suggest that this region did not, or barely did, come in contact with the stent, and that the low levels of shear stress seen between seconds 5 and 6 are because of the stent’s proximal movement. Applied to a SRMT, this would mean that the portion of the artery that is proximal to the stent but does not come in contact with it, experiences only minor levels of shear stress compared to the artery region the stent passes through.

4.1.4 General Discussion

The combined results of all three regions show a high variability in the shear stresses experienced by the artery depending on the location and its proximity to the stent and its movement. The first region only had one general “burst” of activity, because it only experienced the shear stress caused by one single end of the stent. On the other hand, the center region has a much more “active” plot, with two bursts of activity, because this region experiences the passing of both ends of the stent. The plot for this region could also suggest that there are higher shear stresses resulting from the passing of the stent edges compared to its middle body. This is supported by other studies that report an “edge effect” seen on implanted stents, with higher incidences of restenosis for areas proximal to the stent edge [73]–[75]. Additionally, the results suggest that the region where the stent expands will experience shear stresses that remain partially present even after the stent has passed. The results also show different points in one same circumferential region of the artery can experience very different levels of shear stress because of the stent geometry;
some points will be in high contact with the stent, while others will not. Lastly, the stent movement still has an effect on proximal areas it has already passed over. However, the areas close to stent movement but that do not experience actual contact with the stress remain relatively unaffected with low shear stress values.

4.2 3D Cerebrovascular Live Cellular Model

4.2.1 Cytotoxicity Study

As Figure 18 shows, the results show the extract from the 48-hour incubation had a decreased cytotoxic effect compared to the 24-hour, 72-hour, and 1 week time points. However, given how essential it was to achieve a functional endothelial cell test bed during this early stage of the project, the negative impact was strong enough to discard the idea of using the material for the study. Given that PDMS has been widely used in studies involving endothelial cell seeding, it was selected as the material to be used for the rest of the study.

![Figure 17 Cell Number After 24-hour Exposure to Elastic50 Extract Medium Based on AlamarBlue Assay Data](image)

4.2.2 Regular Immunofluorescence Microscopy Imaging

The immunofluorescence imaging results show, firstly, an overall general success in terms of establishing a system for developing a functional endothelial cell model test bed on the PDMS tubular constructs, as shown by Figure 19 (A-F). The control constructs achieved
significant cellular coverage overall, and Figure 19 (B, D, F) shows significant expression of VE-cadherin, which indicates the functionality of the endothelial cells, since they have a sturdy enough attachment to the surface of the PDMS to be able to form intercellular junctions. There were a few spots throughout the control constructs with a lower cellular coverage, but overall, a significant part of the constructs are covered in a confluent layer of functional endothelial cells.

The results also clearly show damage to the endothelial cell layer of the constructs that were treated with SRMT, which is what was expected based on the initial hypothesis. This damage can be seen when comparing the treated conditions with the controls. Although the control samples do have certain areas with fewer cells, the majority of the construct surface has significant coverage. On the other hand, the treated samples show an overall decreased cellular coverage area, as well as what appears to be the wrapping of the cells over each other and into 3D structures in certain regions (especially seen on Figure 19 L, M and O). In addition to this, the treated samples showed prevalent denuded zones where the endothelial cells seem to have been stripped away when compared to the rest of the cellular layer.

There was overall more denudation seen for the smaller size stent, with plenty of regions throughout the smaller size condition’s constructs with very limited cellular coverage, and plenty of regions throughout the larger size condition’s constructs with cellular coverage similar to the control samples. Most of the 3D wrapping structures were seen for the larger size, although still seen on the smaller. It is important to note the cellular coverage was not entirely consistent throughout the constructs, which could have had an impact on the extent of the damage relative to initial cellular coverage for the treated conditions.
Figure 18 Immunofluorescence Microscopy Imaging Results for All Conditions
Figure 19 Volumetric View of Confocal Imaging at 10X for All Conditions
Figure 20 Confocal Immunofluorescence Microscopy Imaging Results for All Conditions
4.2.3 Confocal Microscopy Imaging

The confocal microscopy imaging was done as a supplement to the regular IF microscopy in order to better visualize the cells. The 3D nature of the tubular models made it difficult to get a good focus throughout the construct using regular inverted IF microscopy, resulting in blurry spots in some of the images. On the other hand, confocal microscopy allows for the collection of multiple focal planes through Z stacking, which provides three dimensional data because of the greater depth of field. However, confocal microscopy is a much slower process than regular IF microscopy, with certain images taking up to one hour to produce, thus making it a perfect supplementary qualitative data to the data collected from regular microscopy, which was collected with sample sizes appropriate for statistical analysis. The confocal results firstly confirm that a system to seed a confluent layer of endothelial cells with general homogeneity throughout PDMS artery models was successfully developed. The results are also consistent with those found with regular IF microscopy: the control samples show a significantly higher cellular coverage compared to the treated samples. The treated samples (both sizes) have several regions of partial or complete cellular denudation. The first image for the larger size stent condition (Figure 20, lower left side) is the best visualization of the lifting and wrapping of the cells over each other, creating 3D structures as well as denuded zones throughout the construct. Overall, the highest denudation regions were seen for the MT Small condition.

4.2.4 Scanning Electron Microscopy Imaging

The preliminary results obtained for a study including control and the smaller stent size do suggest damage to the surface of the treated samples that is not present in the control samples (Figure 21). There are visible scratch marks in the surface of the construct that could have been a result of the stent passing. However, the results from the scanning electron microscope that include both stent sizes were not as clear as the previous microscopy approaches. The processing of the samples could have accounted for some of the damage seen in the images; some peeling of the cellular layer could have occurred after dehydrating the samples. In addition to this, the gold sputter coating could have modified the surface of the constructs in such way that made it harder to visualize individual cells.
Figure 21 Preliminary Study Including Control (above) and Smaller Stent Size (below) Scanning Electron Microscope Images
It was not possible to distinguish individual nuclei or even individual cells. There was no clear sign of a higher cellular coverage for the control condition compared to the other conditions, as confluency was not visualized clearly. Nevertheless, there were signs of damage for the treated samples, seen firstly on the right image for the MT Small condition (Figure 22), where there seems to be a scratch on the surface of the construct which looks like it could have been caused by the stent. Evidence of damage is then seen on the left image for the MT Large condition as well, where there appears to be partial lifting and peeling of the cellular layer all in a single axis line, suggesting it could have been a result
of the stent passing. Overall this method of imaging was not as informative as the other ones used.

4.2.5 Quantitative Analysis

The quantitative results from the post-processing of the immunofluorescence microscopy images support the findings from the previous qualitative analysis. Both metrics considered, number of nuclei and percentage of cellular coverage, were the highest for the control conditions (averages of 310 nuclei and 62.76% cellular coverage relative to 10X image). The results for the treated conditions are consistent with the findings found in the qualitative analysis, as both average number of nuclei and percentage of cellular coverage were higher for the constructs treated with the larger stent size stent MT compared to the smaller size (averages of 113 nuclei and 12% cellular coverage for the larger size stent, versus 79 nuclei and 7% cellular coverage for the smaller size stent).

![Figure 23 Immunofluorescence Microscopy Imaging Post-Processing Column Graph of Average Number of Nuclei Relative to 10X Image for Control, MT Small, and MT Large Based on DAPI Signal Nuclei Counting through ImagePro Software](image)
Figure 24 Immunofluorescence Microscopy Imaging Post-Processing Column Graph and Donut Chart of Average Number of Nuclei Relative to 10X Image for Control, MT Small, and MT Large Based on Phalloidin Signal Intensity through ImagePro Software
4.2.6 Statistical Analysis
A one-way ANOVA was performed to compare the effect of stent size on number of nuclei as well as percentage cellular coverage.

The results for the one-way ANOVA analysis regarding mean average nuclei showed there was a statistically significant difference in number of nuclei between at least two groups (F(2, 6 df) = 6.0671, p = 0.03). Tukey’s HSD Test for multiple comparisons found that the mean value of number of nuclei was significantly different between the control condition and the MT Small condition (p = 0.04, 95% C.I. = [11.57, 450.88]). There was no statistically significant difference between control and MT Large (p=0.07), or between MT Small and MT Large (p = 0.88).

The results for the analysis regarding mean percentage cellular coverage showed there was a statistically significant difference in number of nuclei between at least two groups (F(2, 6 df) = 24.0029, p = 0.001). Tukey’s HSD Test for multiple comparisons found that the mean value of number of nuclei was significantly different between the control condition and the MT Small condition, and between the control condition and the MT Large condition (p = 0.002, 95% C.I. = [23.58, 72.93]). There was no statistically significant difference between MT Small and MT Large (p = 1).

Although the effect of stent size on the extent of EI was not explicitly seen with the statistical analysis, it can be deduced, since the only statistically significant difference regarding average number of nuclei was between the control condition and the MT Small condition. This suggests the damage caused by the larger stent was not large enough for the damage to be statistically significant, which would show that the damage caused by the smaller stent was of a larger magnitude than the damage caused by the larger stent. This would be consistent with the rest of the results, as well as with reported data.

The following two tables provide descriptive statistics for both of the analyzed metrics including sample number, mean, standard deviation, and 95% confidence interval bounds.
Table 3 Descriptive Statistics for Number of Nuclei in All Conditions

<table>
<thead>
<tr>
<th>Condition</th>
<th>N</th>
<th>Mean # Nuclei</th>
<th>Std Dev</th>
<th>Lower Bound</th>
<th>Upper Bound</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3</td>
<td>310</td>
<td>128.98</td>
<td>164</td>
<td>455</td>
</tr>
<tr>
<td>MT Small</td>
<td>3</td>
<td>79</td>
<td>50.09</td>
<td>22</td>
<td>135</td>
</tr>
<tr>
<td>MT Large</td>
<td>3</td>
<td>113</td>
<td>62.59</td>
<td>43</td>
<td>184</td>
</tr>
</tbody>
</table>

Table 4 Descriptive Statistics for Percentage Cellular Coverage in All Conditions

<table>
<thead>
<tr>
<th>Condition</th>
<th>N</th>
<th>Mean % Coverage</th>
<th>Std Dev</th>
<th>Lower Bound</th>
<th>Upper Bound</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3</td>
<td>62.76%</td>
<td>9.45%</td>
<td>52.07%</td>
<td>73.45%</td>
</tr>
<tr>
<td>MT Small</td>
<td>3</td>
<td>7.11%</td>
<td>6.08%</td>
<td>0.23%</td>
<td>13.99%</td>
</tr>
<tr>
<td>MT Large</td>
<td>3</td>
<td>14.51%</td>
<td>10.04%</td>
<td>3.14%</td>
<td>25.87%</td>
</tr>
</tbody>
</table>

4.2.7 General Discussion

The combined results of the immunofluorescence along with the quantitative analysis and what can be interpreted from the statistical analysis partially support the initial hypothesis, in that developing and analyzing an in vitro live cellular 3D cerebrovascular model would provide information on the extent of EI, as well that parameters such as stent retriever diameter play part in the extent of EI during SRMT. More specifically, the combined analysis of qualitative and quantitative results shows an apparent higher extent of EI seen for the smaller stent size compared to the larger one. However, there are other factors that could have had an impact in this finding, like the initial cellular coverage, which was not entirely consistent throughout all constructs. Nevertheless, this finding is supported by other studies that have explored the effect stent parameters such as diameter and length have on the extent of endothelial damage caused during a MT, and have found the risk of restenosis and other adverse cardiac events is increased with smaller stent diameters and longer stent lengths [76]. A study investigated the impact of stent oversizing on acute and long-term outcomes after drug-eluting stents implantation. The results showed the use of larger stents to have a positive impact on both procedural and clinical outcomes. In
particular, smaller vessels that are treated with smaller stents were associated with greater adverse events [77]. Another study investigated the impact of stent length and diameter on 10-year mortality after coronary intervention. The results show extensive and small stenting were associated with higher 10 year mortality, compared with coronary artery bypass grafting; concluding that when patients have to be treated with extensive or small stenting, revascularization with bypass grafting should be preferred [78]. Although more testing is needed in order to confirm the accuracy of the results, there are trends that are consistent with findings that have been previously reported.
Chapter 5 Conclusion & Future Work

5.1 Conclusion and Future Works for Computational Finite Element Model

A simple and straightforward finite element model simulating the expansion and retrieval of a stent during a SRMT was developed and analyzed. The main limitation for the model is the material of the stent is assumed to be stainless steel, while the actual stent retriever is made out of Nickel Titanium. The shear stress values are thus not an accurate representation of what occurs in clinical practice. Future modifications would firstly include introducing material properties that are an accurate representation of the stent material. This study assumed the stent to be stainless steel, while the actual stent retriever used for the SRMT simulations was made out of Nickel Titanium. There are several studies that have developed material parameters that accurately represent the Nickel-Titanium alloy that could definitely be implemented in the future to increase the accuracy of the model [79], [80].

Another continuation to this part of the project would be to incorporate a larger initial interference of the stent with respect to the artery as an additional condition in order to model a larger size stent. Once this is achieved, other parameters such as artery geometry can be explored as well, with the possibility of using real cardiovascular geometries with varying degrees of occlusion. There is also the potential of adding a blood clot to the model (with the possibility of varying size, shape, and composition). Other things can be explored such as adding a sheath to the stent model and assessing the impact it has on EI. Although the model is a simplified version of what occurs in real life, it does provide information on the varying extent of EI depending on location and proximity to stent, and it provides a strong base for further improvements and expansion as well.
5.2 Future Works for 3D Live Cellular Model

On the side of the live cellular model, achieving a system to consistently obtain constructs with functional and confluent endothelial cell layers proved to be the biggest challenge of the project, but was partially achieved, although cellular attachment, growth and coverage on the constructs can be greatly improved. As previously mentioned, improving the overall consistency of cellular coverage would prevent the results to be skewed by initial disparities in cellular coverage across conditions, which would ultimately increase the accuracy of the obtained results.

Improving cellular attachment could be achieved by several methods, for example, adding a rotational component to the cell culture. The static cell culture used in the study relied on manually rotating the samples for the first hours after cell seeding, hoping to get as close to a confluent monolayer all throughout the construct as possible. This would be achievable using a rotational device that would allow to slowly and continuously rotate the constructs after cell seeding, all while maintaining sterile conditions and appropriate temperature and CO2 levels (37 °C; 5 % CO2). Several studies have successfully achieved rotational three-dimensional seeding with uniform distribution and growth of endothelial cells [81]–[83], implementing speed rotations of 0.1-3 rpm.

Another aspect of the live cellular model that can be improved in terms of increasing cellular attachment is the hydrophilicity of PDMS. While this study relied on a fibronectin coating to improve cellular attachment to PDMS, there are plenty of other methods that are used to modify the PDMS surface to be more hydrophilic. Studies have used surface modification procedures related to addition of PVA, PDMS-PEG copolymers, polyacrylic acid coating, and many other approaches [84]–[86]. An article that reviews several of the techniques used to make PDMS more hydrophilic, specifically for the application of endothelial cell culture, found that the techniques that compare to fibronectin are the addition of polydopamine, oxygen plasma treatment combined with fibronectin (worked better than fibronectin alone), and TEOS (tetraethylorthosilane) combined with fibronectin.
This would also allow for a more uniform attachment and growth of the endothelial cells on the PDMS surface.

Similar to the computational model, there are several parameters regarding the arterial models that can be modified and explored, such as the diameter, length, and thickness of the tubular constructs. In addition to this, the presence of blood clot analogs with varying size, shape, and composition can also be implemented in order to explore the combined effect the stent and clot have on the extent of EI. The next immediate step in terms of experimental modification would be to attempt implementing a polydopamine surface modification to the PDMS constructs in an attempt to increase hydrophilicity and thus cellular attachment, as well as preparing longer constructs in order to be able to simulate an SRMT over a longer area and thus gather more information on the varying degrees of EI depending on location and proximity relative to stent retrieval.

5.3 Final Comments

Lastly, computational and experimental data could be combined by using data from experimental approaches to validate data from computational simulations. This would imply recreating the same situation both in silico and in vitro, or as close to being the same as possible, simulating different SMRT scenarios and comparing the outcomes between experimental and computational simulations. This would allow the potential prediction of SRMT outcomes based on cardiovascular anatomy and stent geometry and properties.
References


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[55] “An investigation into the applicability of a Mooney-Rivlin constitutive equation for modeling vascular tissue in cardiovascular stenting procedures | Request PDF.”


