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MICROFLUIDIC PLATFORM FOR IMPEDANCE CHARACTERIZATION OF ENDOTHELIAL CELLS UNDER FLUID SHEAR STRESS

By

Vanessa Velasco

B.S., Florida State University, 2008

M.S., University of Louisville, 2010

A Dissertation

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In Partial Fulfilment of Requirements

for the Degree of

Doctor of Philosophy

in Mechanical Engineering

Mechanical Engineering Department

University of Louisville

Louisville, Kentucky

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Dr. Thomas Berfield, Ph.D.

DEDICATION

This dissertation is dedicated to my
parents, Jaime Velasco Medina and Carmen Medina
and husband, Noel G. Aguilar

ACKNOWLEDGEMENTS

First and foremost, I would like to thank God for providing the motivation, resources, opportunities, and placing helpful people through the course of all my educational training. Without his infinite grace, mercy, and power reaching this goal would have been impossible.

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ABSTRACT

MICROFLUIDIC PLATFORM FOR IMPEDANCE
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FLUID SHEAR STRESS

Vanessa Velasco

June 24, 2016

Endothelium dysfunction has been associated with many pathophysiological processes leading to cardiovascular diseases. Studying endothelium behavior is vital to understand the onset, prevention, and treatment of such diseases. Electrical impedance spectroscopy has been shown to provide a real-time *in vitro* evaluation of cell behavior including cell monolayer permeability. However, the majority of published work has been primarily with static cell culture models or macro-scale models that do not properly represent the physiological sizes, structures, and environmental conditions of human blood vessels.

Within this dissertation, the design, fabrication, characterization, and application of a microfluidic impedance platform is presented for the *in vitro* characterization of HUVECs undergoing different hydrodynamic shear stress conditions (static, 2.5, 17.6 and

58.1 dyne/cm²). Electrodes diameters of 50, 100, and 200 μm were incorporated to monitor different subpopulations sizes of HUVECs. Initial characterization experiments with relevant biological solutions indicated that electrodes smaller than 50 μm in diameter suffered from significant interfacial impedance and were unsuitable for the sensing application.

Impedance spectra (10^2 - 10^6 Hz) were collected for HUVECs at the different shear conditions for 14 hours. Equivalent circuit fits were implemented to derive the different electrical cell monolayer parameters including the trans-endothelial resistance, cell membrane capacitance, constant phase element, and the resistance of cell culture medium. Results confirmed that while the trans-endothelial resistance and cell membrane capacitance were suitable measurements for cell permeability and confluency respectively, the constant phase element did not identify any discernible cell behavior. Resistance of cell culture medium was strongly influenced by cell attachment and values should be extracted from control cell-free measurements. Initial trans-endothelial resistance measurements showed a shear magnitude dependent increase at the sudden onset of flow. This increase was greatest for the largest shear condition (58.1 dyne/cm²). After 14 hours of shear, trans-endothelial resistance measurements were largest for HUVECs sheared at 58.1 dyne/cm² and lowest for the 17.6 dyne/cm² shear condition and the difference showed to be statically significant ($p < 0.05$). Monitored HUVECs were stained for nuclei, F-actin and VE-cadherin. Quantification of immunofluorescence of VE-cadherin showed a similar trend to the extracted trans-endothelial resistance values. Immunofluorescence images of F-actin showed significant cytoskeleton remodeling of sheared HUVECs. While cells sheared at 17.6 dyne/cm² aligned parallel to the direction

of flow, HUVECs sheared at 58.1 dyne/cm^2 were angled in the direction of flow and sometimes even perpendicular to flow direction.

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CHAPTER 1

INTRODUCTION

1.1 Motivation

Endothelium dysfunction plays an important role in the pathophysiological processes of atherosclerosis [1]–[5], thrombosis [5]–[8], inflammation [9]–[12], ischemia [13]–[17], tumor progression [18], [19], and their related complications like cardiovascular and cancer diseases. The endothelium is a single film of connected endothelial cells (ECs) forming the interior layer of all blood vessels [20]. It is involved in many vascular functions including maintaining homeostasis [20]–[23], regulating transport across the vascular wall [7], [24]–[26], and mechanotransduction signaling [22], [24], [27]–[29]. Research shows that endothelial cell behavior is strongly influenced by their microenvironment including their interaction with the underlying substrate, the extracellular matrix (ECM), and biochemical and biomechanical cues. The effects of mechanical forces on endothelium function have been of specific interest in many investigations [1]–[6]. As blood flows through the circulatory system, endothelial cells are subjected to two mechanical forces: a normal pressure force and a tangential shear force. The vessel wall cushions the normal force, whereas the endothelial cells absorb all the hydrodynamic shear stress [1]. As endothelial cells sense shear stress on the apical side, they transmit signals to the cytoskeleton by transmembrane mechanosensors located

the on the cell surface [7]. These biochemical signals act as communication avenues between the endothelium and the underlying smooth muscle cells, generating modifications in the arrangement and function of the endothelium and the vessel itself [4]. Generally, shear stress causes endothelial cells to adapt by re-orienting and aligning in the direction of flow. These structure changes are often accompanied by modulations in barrier function which is regulated by cell-cell (adherent and tight junctions) [8] and cell-substrate junctions (cell adhesion molecules) [4], [9]. Static EC cultures have either irregular tight junctions or lack tight junctions altogether [10] leading to increased permeability [11], [12]. Endothelial cells under laminar shear stress express an increased level of occludin tight junction proteins [13], [14]. When the junctions are disrupted the endothelium integrity is compromised which enables the passage of circulating foreign agents to the vascular wall and eventually lead to aforementioned pathophysiological conditions.

1.2 Problem Definition

Understanding the relationship between shear stress and barrier function is important to gain a better prospective of the underlying mechanisms involved in endothelium pathology and how to treat and prevent them. *In vitro* flow models such as parallel plate flow chambers [15]–[18] and cone-plate apparatuses [19]–[23] have long been used for the purpose examining barrier function in endothelial cell cultures. These studies are complemented with measurements of secreted proteins, immunohistological stains, and gene expression levels *after* the cessation of shear stress exposure [24]–[30].

However, valuable information is lost in this approach since endothelial cells responses to shear stress vary from seconds to hours [16] and a real-time approach would be more appropriate for these investigations.

It has been shown that the behavior of cells cultured *in vitro* can be monitored in real-time electrically. Initial methods consisted of applying direct current (DC) voltage across cultured cells and measuring a voltage drop [31]. Later, Giaver *et al.* published the initial findings of what became Electrical Cell-Substrate Impedance Sensing (ECIS) technique where alternating current (AC) potentials are used in combination with fabricated coplanar gold microelectrodes to observe changes in electrical impedance due to adherent cell presence and motility [32], [33]. Since then, ECIS and other impedance systems have been used to measure a wide array of cellular properties such as permeability, changes in shape, toxicity, and cell behavior under flow [16], [20], [32], [34]–[36]. While it has been shown that characterization of cell behavior is frequency dependent [37]–[41] many of the proposed impedance platforms operate at a single frequency and others opt for several selected frequency values. Studies on frequency-dependent cell behavior suggest that at lower frequencies current prefers to flow beneath the cells, through the intercellular junctions, and into the bulk medium resulting in access to information regarding barrier function [42]. At higher frequencies cell-matrix interactions and cell membrane integrity are probed since the current passes through the cell membrane and into the bulk medium [42]. Moreover, measurements acquired over a frequency spectrum can extract extensive monolayer data including interfacial and cell interactions.

In this work, a microfluidic impedance platform is introduced for the electrical assessment of endothelium monolayer kinetics while undergoing fluid shear stress *in vitro*. The platform includes a control cell-free channel that provides information on environmental-induced drifts in the acquired data. The platform design also incorporates three different electrode diameters (200, 100 and 50 μm) in order to evaluate different sizes of subpopulations of endothelial cells within the microchannel. Initial characterization on electrode size based behavior was performed with biologically relevant electrolyte solutions to determine microelectrode sensitivity. The platform was used to capture the impedance spectra (100 Hz-1MHz) of HUVECs under static and different shear (2.5, 17.6, and 58.1 dyne/cm^2) conditions. The electrical parameters of static and sheared monolayers were extracted with an equivalent circuit model in order to compare cell behavior (in particular monolayer permeability) under different shear conditions. Immunostaining of the adherent junction VE-cadherin was performed prior and at the end of each experiment to compare to the quantification of impedance-based endothelial permeability.

1.3 Research Objectives

The purpose of this work entails the design, fabrication, characterization, and application of a microfluidic platform that encompasses an impedance sensor, flow-through scheme, and automated data acquisition system. The platform attempts to mimic the arterial structure, size, and environment. Endothelial cell behavior under various hydrodynamic shear was evaluated electrically using such a design. The specific objectives include:

1. Design/optimize and fabricate an impedance chip/platform
2. Characterization of the impedance chip
3. *In vitro* analysis of HUVEC culture under physiologically relevant hydrodynamic shear conditions (0, 2.5, 17.6, and 58.1 dyne/cm²)
4. Investigate platform validation by conducting a comparison study of impedance versus microscopic characterization techniques of HUVEC permeability

1.4 Dissertation Structure

This dissertation is organized in six chapters where Chapter 2 presents relevant endothelium background including the effects of shear stress, monolayer permeability, its role in pathology and concludes with a review of several *in vitro* endothelium models. Chapter 3 introduces impedance spectroscopy as a tool to monitor cell behavior, as well as, the underlying theory and equivalent circuit modeling utilized to extract behavior parameters. This chapter also discusses frequency as a relevant parameter in impedance detection. Chapter 4 discloses the microfluidic impedance platform design, components, and initial characterization performed to identify variations in electrode performance, as well as, determine the effects of temperature and humidity, and cell culture flowrate. The sensitivity of different electrode diameters is also addressed. Chapter 5 details impedance spectra collected from HUVEC cultures in the absence of induced shear and under shear conditions (2.5, 17.6, and 58.1 dyne/cm²). This is presented in conjunction with electrical parameters detailing cell behavior extracted from circuit model fitting. Within this chapter, the validation of the microfluidic impedance platform was deliberated by comparing a microscopy technique for permeability measurement through

immunostaining of the adherent junction, VE-cadherin with impedance based permeability measurements. Finally, Chapter 6 includes a concluding statement of the novel findings and limitations in this work, in addition to a brief description of the future work that can proceed from this project.

CHAPTER 2

THE ENDOTHELIUM

2.1 Structure and Function

A monolayer of endothelial cells, known as the endothelium (Fig. 2.1), forms the inner lining of all blood vessels (arteries, veins and capillaries). Their structure and function depend on the vascular location and their microenvironments [5],[6]. In arterial and venous vessels endothelial cells are underlined with layers of smooth muscle cells and sheets of elastin. Combined they form the mechanical support of the vessel and mediate blood flow. The exterior of these blood vessels are composed of a network of collagen fibers that act as a protective coating and connects the vessels to nearby tissues.

In the endothelium, individual endothelial cells are linked to each other as well as the underlying tissue by multi-protein complexes (Fig. 2.2) known as intracellular junctions and cell adhesion molecules (CAMs). Intracellular junctions (gap, adherent, and tight junctions) link neighboring endothelial cells to one another and provide channels for the transportation of macromolecules through the endothelium. One of the most important junctions that will be discussed in this work is VE-cadherin, an adherent junction found on all vascular segments. This junction provides the major adhesion force between adjacent cells and regulates endothelium permeability [8]. CAMs, like integrins, anchor

the cell to the extracellular matrix (ECM, network of protein and polysaccharides) and play an important role in the mechanotransduction produced by shear stress [9][4].

Figure 2.1: (A) Schematic of a normal human artery structure including the endothelium lining, smooth muscle cells and the exterior network of collagen fibers [45]. (B) The cellular composition of a blood vessel wall: the tunica intima, media and adventitia [46].

The endothelium has a myriad of functions including acting as a physical barrier between flowing blood and underlying tissues, selectively filtering blood-borne components, participating in intracellular signaling, and transducing mechanical forces to biochemical signals [4-6]. As a barrier, it controls flux of solutes, fluid, and macromolecules, continuously maintaining a specific gradient on both the lumen and underlying tissue [47]. It maintains vascular health by regulating vascular tone, vascular growth (angiogenesis), hemostasis and blood clot formation [16]. Vascular tone is controlled by the endothelial cell production of vasodilators and vasoconstrictors which cause the smooth muscle cells to contract and relax [48]. Endothelial cells also participate

in the inflammatory response by the expression of adhesion molecules, chemokines and cytokines.

Figure 2.2: A diagram of endothelial cell junctions which attach adjacent cells to each other and to the ECM [49].

Perhaps one of the most important functions of the endothelium is its participation in mechano-transduction signaling pathways. Due to their anatomical location, endothelial cells are constantly being subjected to various hemodynamic loadings caused by blood pressure and flow. These mechanical stimuli include changes in hydrostatic pressure, cyclic stretching, and of specific interest in this project, shear stress [13]. As endothelial cells sense these mechanical cues from their environment, they induce biological responses at the molecular level eventually leading to changes in cellular structure and function.

2.2 Effects of Shear Stress

As blood flows through the circulatory system, endothelial cells are subjected to two mechanical forces: a normal pressure force and a tangential shear force (Fig. 2.3). The pressurized, pulsatile nature of blood flow exerts a force on the vessel wall, causing the arteries to expand and contract. The vessel wall cushions most of this normal force, whereas the endothelial cells absorb all the shear stress [50]. As endothelial cells sense shear stress on the apical side, they transmit signals to the cytoskeleton by transmembrane mechanosensors located on the cell surface [7]. These biochemical signals act as communication avenues between the endothelium and the underlying smooth muscle cells, generating modifications in the arrangement and function of the endothelium and the vessel itself [4].

Shear stress has been shown to modify membrane potential, protein expression, transcription factor activation, gene regulation, gene synthesis, and of great importance in this work cell morphology [16]. These changes effect endothelial permeability [51], vasoregulation [52], and arterial remodeling [53]. As a result, endothelial cells play an important role in pathophysiological processes of atherosclerosis, thrombosis, and their related complications like cardiovascular diseases [54].

One of the most studied effects of shear stress on endothelial cells is their morphological adaptation [55]–[57]. Their response is strongly dependent on the shear stress magnitude and fluid flow pattern that they experience in their microenvironment. In veins, shear stresses are low, ranging from 1-6 dyne/cm². Endothelial cells in these regions are polygonal in shape and significantly more permeable compared to arterial endothelial cells. Arterial shear stresses vary from 10-70 dyne/cm² [52]. In these vessels,

endothelial cells are ellipsoidal in shape and their elongation aspect ratios depend on the vessel geometry [9]. Typically, blood flow is laminar in the vasculature, but some disturbed flow has been observed in considerably curved arteries such as bifurcations and in areas of stenosis. For the scope of this project, the discussion will mainly focus on the effect of laminar shear stress on the arterial endothelium.

Figure 2.3: Shear Stress (τ) is the lateral force acting on the endothelial cells. Pressure (P) is the force that acts perpendicular to the arterial wall, stretching and contracting underlying structure [58].

In vivo and *in vitro* studies have demonstrated that in static or low shear conditions ($< 5 \text{ dyne/cm}^2$), endothelial cells display a cobblestone arrangement. As they experience stronger shear they align and elongate in the direction of flow [59], [60]. According to DePaola *et al.*, endothelial cells begin to adapt to fluid flow in less than five

hours. In their adaptive process they reorganize their intracellular junctions, begin to position themselves parallel to flow, and form adhesion plaques [16]. However, it takes 24-48 hours before cells fully orient and stretch along the fluid flow. A real-time microscopic observation of cells (also known as morphodynamics) revealed that endothelial cells undergo four phases when exposed to shear [61]. Phase I consists of static and low shear conditions where cells are randomly oriented, forming a cobblestone geometry. In this phase, there is an only moderate locomotion and zig-zag movement (fluctuations) among endothelial cells. Phase II takes place at the start of shear. It involves an increase in locomotion and a decrease in fluctuations. After some period of time, Phase III originates as cells begin to align. Within hours, Phase IV is observed when cells are elongated. As endothelial cells begin to morphologically adapt to their hemodynamic environment, modifications in permeability and cell-ECM interactions are observed which have been linked to the progression of cardiovascular pathology [54].

2.3 Permeability

The vascular endothelium is an important interface between flowing blood and the vessel wall. It acts as a semi-permeable barrier controlling the passage of macromolecules, solutes, and fluid. It does so with the purpose of maintaining gradients of proteins and molecules between the lumen and the subendothelium tissues [49]. Components can travel across the endothelial monolayer either by a paracellular or transcellular mechanism. Transcellular movement allows for large molecules and ions to travel through cells themselves whereas paracellular transportation involves the movement of smaller molecules (< 6 nm in diameter) between intracellular junctions

holding adjacent endothelial cells together [52]. The regulation of this molecular transport across the endothelium is referred to as the barrier function. Barrier function is dependent on the vascular type, location and the junctional integrity of the endothelium, the size of the transported component and of specific interest in this project shear stress [9], [49].

Endothelial cells differ in morphology depending on their location in the vascular system. Accordingly, the endothelium also displays a variation in permeability contingent on component being transported and the vessel type and region [49]. Arteries, veins, and capillaries found in the brain, heart, skin and lungs are less permeable due to the lack of fenestrations. Fenestrated endothelium of capillaries located in the exocrine and endocrine glands, as well as some of the renal tubules, have a high level of exchange [43]. Permeability studies of extracted cells and intact vessels have shown that the endothelium at capillaries and post-capillary venules are more porous than venous and arterial endothelium, where arterial endothelial cells are the least penetrable [43], [62]. At the same time, the endothelium display affinities for different components depending on their position in the vasculature. For arterial lung segments, limited liquid flux is observed compared to venous and capillary sections. Whereas investigations using cultured pulmonary microvessel endothelial cells exhibited far more restrictive behavior to albumin than larger vessels [63], [64].

The type, integrity, and distributions of cell-ECM junctions as well as intracellular junctions play an important role in permeability. Experimental comparison of ECM proteins in microvessel and larger vessel endothelia (HUVECs) revealed a difference in gene expression for the kind and quantity of ECM proteins. For

microvessels genes synthesized laminin, collagen 4 α 1, collagen 4 α 2, and other collagen 4 α -binding proteins. In large vessels genes expressed fibronectin, collagen 5 α 1, collagen 5 α 2 and osteonectin. Additionally, microvessel endothelial cells were found to express higher levels of ECM regulating genes than large vessel endothelial cells [65]. Examinations on extracted endothelium of microvessels, veins, and arteries from the lungs reported that endothelial cells are far better regulated and developed in microvessel than in large vessels which can contribute to the dissimilarities in endothelium penetrability observed [63], [64], [66].

Recent work has shown shear stress produces modulations in barrier function. Shear stress has been associated with increased molecular absorbance. As confirmed in a case of cultured bovine aortic endothelial cells where increased albumin flux was measured at the onset of shear (10 dyne/cm²) and returned to baseline values once the cells were no longer exposed to shear stress [67]. Similarly, the type and magnitude of shear stress endothelial cells are exposed to impacts their permeability function significantly. Cells under low or lack of flow exhibit higher permeability [13]. Static cell cultures or at low shear often have irregular tight junctions or can even lack tight junctions all together [10]. In contrast, endothelial cells under laminar shear stress express increased levels of tight junctions [13]. For cells exposed to disturbed flow like those located in branched regions of vasculature, the cell monolayer are far more penetrable to macromolecules like dextran as opposed to cells located in regions where laminar shear stress is observed [48], [35]. This is often a result of higher cell turnover [68], higher mitosis [69], and low distribution of VE-cadherin and b-catenin [48] which

have all been linked to a disturbed flow pattern where flow is low and no longer unidirectional.

2.4 Role in Pathology

The endothelium is a pivotal organ due to its involvement in numerous critical physiological functions, disease pathologies, and even the treatment of illness. Normally, the endothelium maintains vascular homeostasis by controlling molecular flux to subendothelium tissues, vascular remodeling, thrombosis, inflammatory response and vascular smooth muscle contraction. When irregularities are observed in these endothelium functions, particularly in the inflammatory response, diseases emerge. Variants in endothelium permeability, cell signaling, gene expression, wound-healing, and inflammation response have all been reported to induce clinical complications like atherosclerosis, cardiovascular disease, neurological disorders, and tumor progression [70], [71].

In inflammation, atherosclerosis and cardiovascular disease, the endothelium is converted from a deactivated to a host defense phenotype [72]. Nitric oxide (NO), a free radical that inhibits vascular contraction and growth, in addition to harmful platelet aggregation and leukocyte adhesion, decreases and ROS (reactive oxygen species) production increases [73]. Significant up-regulation of ROS for prolonged periods causes cell damage. In inflammation, it's a foreign host that triggers this response. While in atherosclerosis and cardiovascular disease, risk factors such as hypertension and diabetes set off the defense response. If the cardiovascular risk factors are repeatedly present or the endothelium remains activated, the self-preserving anti-inflammatory system

depletes. Thereupon, the endothelium becomes dysfunctional. Endothelial cells become loosely connected to one another and ultimately detach, leaving unprotected regions where any component (lipoprotein, leukocyte, and collagen) can infiltrate or attach [72]. The endothelium also implicates proper wound healing. As lesions are created, endothelial cells in the near surroundings migrate to close the void [74]. If the endothelial cells' motility is abnormal and the monolayer remains subconfluent, foreign components can cross the endothelium, compromise vascular homeostasis, and the progression of disease follows [75].

Recently, experimental studies have shown that shear stress effects are involved in atherogenesis. Though laminar shear stress is required to maintain proper vascular physiology, disturbed flow conditions where shear stress is low and oscillatory promotes the progression of pathogenesis. Disturbed flow patterns are usually observed at vessels with significant curvature or branching. The combination of this vessel geometry and altered hemodynamic forces stimulates atherosclerotic lesions and in due course plaques. Altered gene expression, cytoskeleton arrangement, wound repair, vasoreactive, oxidative, and inflammatory states at the vascular endothelium have been linked with these abnormal and non-laminar flow conditions [3]. Low shear stress ($< 5 \text{ dynes/cm}^2$) down regulates NO production and with it vasodilation, and cell repair [2]. *In vivo* observations on rabbit carotid arteries show that a reduction in shear stress also has adverse effects on endothelium homeostasis and remodeling [48]. In the study, blood flow was reduced by 70% for two-weeks, causing the artery diameter to shrink by 21%. In order to rule out that this was not a result of the smooth muscle cells contracting, a

smooth muscle cell relaxant was added but no change was seen in the contracted vessel [76].

Even though the endothelium participates in the pathological process, it also plays a role in the treatment of disease. As a barrier, the endothelium regulates the molecular flux to sub-vascular tissues. Any pharmaceutical agent ingested circulates the blood and must be transported across the vessel wall for systematic or organ-specific effects [77]. There is no doubt the endothelium is in charge of critical functions and thus a suitable subject of study in disease pathology and treatment.

2.5 Models

Models help us understand endothelium functions and properties during physiological and pathological conditions. Animal models have long been used for this purpose but they are very expensive and the use of them brings up many ethical issues. As a result, many have focused on developing *in vitro* models for various chemical and electrical endothelia assessments. Development of *in vitro* endothelium models requires careful consideration to design parameters such as size, flow conditions, visualization, and end-point cell analyses.

A. Fluid Dynamic Models

Initial *in vitro* endothelium models were often enlarged versions under static conditions. It is not until recently that emerging microfabrication technology has allowed for the recreation of virtual vessels on a chip [78]–[81]. These chips are usually referred

to as microfluidic devices. In these chips, vessels can be made of soft flexible materials and perfusion systems simulate the hemodynamic forces cells undergo. Simple soft lithography techniques involving polydimethylsiloxane (PDMS) easily replicate complex multichannel networks like those found in the vascular tree. Microfluidic devices also provide an approach for multiplexed analysis of gene expression, hemodynamic loading conditions, and therapeutic agents, all in a single chip. The small length scales of microfluidic systems guarantee low Reynolds number and steady laminar flow conditions. Unlike macro-scale systems, microfluidic devices require only small volumes of reagents and avoid large bulky setups. Despite all the benefits of microfluidic systems, traditional chemical analyses including immunostaining, mRNA and protein extraction, and molecular secretions are more difficult due to the minute volumes involved in these devices. When designing a microfluidic device, it is important to determine appropriate geometries to extract the needed reagent for the desired analysis.

Another important design factor for endothelium models is how the wall shear stress will be applied. Blood is pulsatile and does not have a constant viscosity. This makes replicating physiological conditions in a model with predictable fluid parameters a challenging task. Consequently, most cell culture flow systems aim for simplified configurations and controlled hemodynamic conditions where fluid flow is predictable [9]. As mentioned previously, experimental setups, especially microfluidic platforms, have low Reynolds numbers (Re) indicative of laminar flow where streamlines are steady and organized. They also assume constant viscosity. As a result, the Navier Stokes equation [7] is simplified to:

$$\mu \left(\frac{\partial^2 v}{\partial y^2} + \frac{\partial^2 v}{\partial x^2} \right) = \frac{\partial p}{\partial z} \quad (2.1)$$

Where v is flow velocity, μ is fluid viscosity, ρ is fluid density, p is pressure, and $\mathbf{f}$ are forces acting on the fluid. Despite some oversimplifications, microfluidic models do represent the laminar behavior of blood flow in the vessels. It is only in stenosis and in branching regions that fluid behavior becomes unsteady [82]. For many fluidic systems, wall shear stress is applied using a parallel plate configuration. Parallel plate flow systems are composed of two plates arranged one above the other with a gap in between. The gap is usually small, less than 1 mm. In these systems, shear stress (τ_{PP}) is expressed as follows:

$$\tau_{PP} = \frac{6\mu Q}{WH^2} \quad (2.2)$$

where Q is the fluid flowrate, H is the height, and W is the width of the flat plates/channels. For this mathematical model, flow must be fully developed and plates are presumed infinitely wide (there is no significant shear stress at the side walls). This assumption holds true only if the cross-sectional ratio, $\alpha=h/w$ is small ($\alpha < 1$) and the length of channel must exceed the entrance length, L_c :

$$L_c = ah \cdot Re \quad (2.3)$$

where a is an empirical proportionality constant. In circular tubes a is 0.06, while in rectangular tubes a is 0.08. Once these parameters are satisfied, the fluid flow velocity profile (Fig. 2.4) is parabolic between flat plates and the shear stress profile is the greatest at the plate surfaces but becomes negligible as you approach the corners of the channel [83], [84].

Figure 2.4: (A) A diagram of the calculated velocity and shear stress profile along the x-y-z axes in a parallel plate flow chamber [84]. (B) The velocity and shear stress profile along the x-y plane [85].

Alternatively, fluid shear stress within microchannels can also be calculated using the rectangular duct approximation [86], [87] :

$$\tau_{RC}(x) = \mu \frac{Q}{q} \sum_{n=0}^{\infty} \frac{(-1)^n a \pi}{(2n+1)^2} \left(\frac{2}{\pi}\right)^3 \tanh \left[(2n+1) \frac{\pi h}{2a} \right] \cos \left[\frac{(2n+1)\pi x}{2a} \right] \quad (2.4)$$

where a is half of the total width of channel ($W=2a$) and h is half of the total height of the channel ($H=2h$), and x is the coordinate value across channel width. A complete derivation of Eq. (2.4) and defines coordinate convention can be found in the Appendix. Unlike the parallel plate Poiseuille approximation, the rectangular duct estimation accounts for the shear stress of side walls.

B. Previous Endothelium Studies

In vitro endothelium systems have facilitated the study of shear stress effects and their implications on endothelial cell biology. Chemical and electrical methods have been used to monitor the barrier's function. What follows is a brief overview of shear stress studies including both chemical and electrical approaches of endothelium characterizations.

Information regarding endothelium integrity and health has been accomplished by characterizing cell-matrix interactions using adhesion assays. Adhesion assays quantify the bonding strength between adherent cells and their plating substrates under shear stress. One approach which is an overall assessment of how well a cell type bonds to a particular surface and quantifies the amount of cells that detach as a result of flow [9]. It is usually performed to determine if a material or coating is suitable for cell attachment, growth, migrations, and the limiting flow conditions that cells can endure. Another approach, which is more specific, attempts to measure the force between each cell attachment and the underlying substrate [9]. This method can be cumbersome since it requires significant effort to visualize and identify individual focal bonds between the cell and substrate.

Migration assays determine cell motility over time. In these studies, cells can be analyzed individually or as a population, on two dimensional surfaces or three dimensional surfaces like gel scaffolds [9]. For endothelial cells, these assays provide information of how cells adjust to fluid flow and try to maintain confluency when other cells or components transverse the endothelium or when lesions are formed. For studies focused on how cells like leukocytes and cancer cells cross the endothelium, endothelial cells are cultured on a porous membranes and a chemo-attractant is added to the opposite side. Migrating cells are tagged with fluorescent probes and tracked as they are encouraged to permeate the barrier by the chemotaxis [88]. Other migration assays track the movement of cells on a surface using microscopy techniques such as fluorescent tags, time-lapse videos, or the recently notable method of morphodynamics [9], [61]. As mentioned previously, these assays are performed to examine the effects of shear stress, surface coatings, and surface stiffness. One particular study demonstrated the strong migratory response of shear stress gradients of porcine aorta endothelial cells. Cells were exposed to a shear stress of 20 dyne/cm^2 for 48 hours in a 1.5 mm wide microchannel. Using time-lapsed video microscopy, they observed that the cells move away from the gradient shear stress they experienced at the outer edges. Instead, endothelial cells concentrated at the center of the channel length and elongated laterally [89].

Other important migration assays worthy of mentioning are wound healing assessments. As mentioned previously, abnormal flow patterns can cause lesions and endothelial cells are programmed to migrate to close the open wound. The most common assessment of this type is the scratch assay. Confluent endothelial cells are scratched and cells are observed as they move to maintain confluency [90]. Bovine aortic endothelial

cells (BAECs) were studied to see how shear stress variations (3 or 19 dyne/cm²) regulated wound closure. Image analysis showed how cells sheared at 3 dyne/cm² migrated at a very slow rate, unable to close the wound after 24 hours. While cells sheared at 19 dyne/cm², migrate at a fast pace almost closing the wound within 24 hours [91]. An alternative to a scratch assay, is one involving hydrodynamically focusing a stream of protease trypsin over a confluent layer of endothelial cells. Like the previous study discussed, endothelial migration was observe to increase under flow stimulus. This method offers the unique advantage of possible gradient generation [92].

Many groups have studied shear stress effects on endothelial cell behavior using *in vitro* systems. *In vitro* systems can either be acute or chronic shear stress models. Acute models are those where shear stress application is sudden and held for seconds to several hours. Chronic shear stress models are those where endothelial cells are cultured under (constant or variable) flow for a period of days or even weeks. Chronic shear stress models are more representative of *in vivo* conditions [50]. Whereas acute shear stress models represent *in vivo* condition of newly formed vessels or the restoration of blood flow in an obstructed vessel. Despite this fact, most of literature work is based on acute models due to the sensitivity and length of chronic shear stress models.

Perhaps the most recognized work has materialized from the adaptations in endothelial cell morphology and structure in response to shear stress. As mentioned in a previous section, endothelial cells exposed to laminar shear for 24-96 hours, align and elongate in the direction of flow. The majority of the systems expose cells to physiological shear values which range between 10-40 dyne/cm² where flow is steady and constant. One of the first endothelial shear stress response studies using microfluidic

channels consisted of etched trenches on silicon substrates [93]. Bovine aortic endothelial cells were cultured in array of microchannels with different widths ranging from 25-225 μm . For the cells in the 200 μm wide channels, fluid flow applied at 20 dyne/cm^2 for 16 hours, encouraged cell orientation to be the same direction as the fluid flow. However, under static conditions cells also become aligned in the direction of channel length and the alignment was greater with decreasing microchannel width. This study showed how important it is to determine the correct channel width for a microfluidic based *in vitro* device involving endothelial cells.

Some effort has also been placed in examining the morphology changes of endothelial cells undergoing pure oscillatory or pulsatile flow which are closer to *in vivo* hemodynamic conditions. One group accomplished this by creating on-chip actuating valves with a method referred to as *Braille* pin actuation, to generate pulsatile shear stress levels. Researchers found that human dermal microvascular endothelial cells (HDMECs) exposed to a pulsatile shear of $<1 \text{ dyne/cm}^2$ remained randomly oriented but the morphology of cells exposed to shear stress of 9 dyne/cm^2 were stretched and aligned along the flow path [94]. Endothelial cells experiencing disturbed flow including regions of redirected flow, high shear, stagnation (no flow) and low shear that vary with the cardiac cycle are also studied using *in vitro* devices. A simple approach used to reproduce disturbed flow patterns implemented a modified parallel plate or cone-and-plate system which included protruding steps at the bottom surface. The step changes generate recirculation patterns which are merely a couple micrometers in length. Unfortunately, only a few cells experience the disturbed flow, making immunostaining analysis difficult. Although exposing cells to gradient shear stress can minimize the number of

experimental studies performed and can be easily achieved with modified systems and flow patterns, this is ultimately undesired because it can be challenging isolating cellular shear stress response from local variations.

All the *in vitro* devices discussed thus far required some type of microscopy technique, either by fluorescent probes, end-point immunostaining, or time-lapse video. Researchers have long been suggesting that it would be more advantageous to monitor cell behavior electrically in real-time. One publication demonstrated the detection of NO produced by pulmonary artery endothelial cells by incorporating carbon-ink microelectrodes for amperometric detection. Some advantages of this approach are that it is a real-time measurement and it reduces the NO amount lost due to auto-oxidation [95]. However, detection limitation (low concentrations of NO), convection, diffusion, and chemical gradients, make it very difficult to monitor NO produced by cells under flow. Recently, a study showed a parallel-plate flow chamber with two compartments separating the NO recording electrode and bovine aortic endothelial cells (BAECs) under flow by a Transwell membrane. Cells were sheared at ramped controlled steps of 0.1 dyne/cm² to 6, 10, or 20 dyne/cm² produced a temporary decrease in NO followed by increase to a new steady state [96].

C. Permeability Assessment

In vitro endothelial permeability assessments have been heavily pursued due to its implications in disease processes. In these assessments, the structural integrity of endothelium junctions are measured by quantifying the flux of molecules from the apical (blood flow side) to the extra-cellular matrix side. These studies have been used on static

and, of specific interest in this project, sheared cell cultures. Commonly, permeability assays are performed using one of two methods. The first is a chemical approach and involves tagging a specified concentration of traversing molecules with fluorescent probes. Endothelial cells are cultured on Transwell membranes and submerged in media-filled wells. The tagged molecules are added to one side of the membrane, tracked and quantified at the other side of the membrane using a microscope for visualization [9],[77]. Albumin and calcium permeability across an endothelium monolayer as a function of shear stress was monitored using this approach. It was found that at the onset of shear stress albumin and calcium permeability increased [67], [97]. When the flow ceased albumin concentration returned to baseline values demonstrating that flow levels do regulate permeability [67].

The second most common permeability assay is an electrical approach. Similarly, endothelial cells are cultured on a Transwell membrane and submerged in a media-filled well. Electrodes are placed on either side of the membrane and direct current (DC) is applied across a cultured endothelium. The voltage drop produced by the monolayer's presence and confluency is measured. Ohm's law is used to determine a numerical representation of endothelial permeability known as the transendothelial electrical resistance (TEER) [9]. Some shortcomings of this method include the low resistance of endothelial cells which are in the range of $< 5\Omega/\text{cm}^2$ and isolating the accumulative resistance of the membrane pores, cell culture media, and connection cables organized in series [16]. Later, an alternative electrical permeability measurement technique emerged, called impedance sensing. This technique eliminated the need for porous membranes and

involved the use of planar micrometer sized electrodes through which AC currents would be applied.

2.6 Conclusions

In this chapter, an overview of the endothelium was provided. Besides describing the vital role the endothelium has in many vascular functions and disease mechanism, the effects of shear stress on the endothelium behavior were thoroughly discussed. *In vitro* endothelium models and how they are used to study monolayer permeability were also reviewed. Despite the many innovations in these models, there is still a need for physiologically relevant and real-time techniques for comprehensive assessments of endothelial cell behavior.

CHAPTER 3

IMPEDANCE SENSING

In vitro models can help us understand the onset of endothelium dysfunction related diseases at the cellular level. Emerging microfabrication technology has improved the development of these *in vitro* models with the recreation of virtual vessels on a chip, while perfusion systems simulate endothelial cell hemodynamic forces. Yet, many of these endothelium models rely heavily on microscopic analyses. As a result, scientists are required to purchase very sophisticated and costly microscope setups. Though, these setups combine the optics and the incubation chamber which allows them to observe the cells while the experiment is ongoing [61], they still lack the temporal and spatial resolution to capture all the rapid and minute cell behavior changes. Another approach used in endothelium *in vitro* studies entails end-point biological analysis where researchers wait for the allotted time of the experiment to observe the effects of hemodynamic forces on the cells [98]. This limits the scientist to observe the before and after affects while omitting any changes occurring intermediately. In addition, this method often requires pre and post-processing of cells with markers that have the potential to adversely modify cells. To observe cells intermediately other scientists elect to pause the experiment and disconnect the chip from the perfusion system to observe the

cells, leading to possible contamination and disruption of the experiment as a whole (for example bubble formation which can shear off the cultured cell layer). To address these issues some have suggested using impedance biosensors to monitor cell behavior. Impedance biosensors have the ability to detect and analyze a variety of cellular reactions in real time due to a wide range of chemical, biological, and physical triggers and do so with high sensitivity and temporal resolution and no need of labels [99], [100].

The behavior of cells cultured *in vitro* have been monitored by their electrical properties in the past. Initial methods consisted of applying direct current (DC) voltage across cultured cells and measuring a voltage drop [31]. However, DC currents have the potential to cause damage to both cells and the electrode sites [42]. Later, electrochemical impedance spectroscopy (EIS) techniques emerged making use of alternating current (AC) signals. Unlike DC signals, the AC signals used in EIS do not cause adverse effects to the cells or electrode sites and provide a more elaborate cell assessment due to frequency dependent cell behavior [37].

EIS techniques are usually categorized in two different groups: non-faradaic and faradaic. These techniques are distinguished by the type of current flow that takes place at the electrode-electrolyte interface. In faradaic systems, measurements rely on charge transfer across the interface as a result of an oxidation-reduction reaction. As cells attach to the electrode surface, they obstruct the redox probes and increase the resistance at the electrode surface (charge transfer resistance, R_{ct} or faradaic resistance) which transduces to cell confluency [101], [102]. One of the major pitfalls of faradaic systems lies in the lack of reproducibility as a result of small and typically unscreened changes at the interface impedance caused by the recognition mechanism [101].

In non-faradaic sensors, there is no charge transfer, instead capacitive current takes place. This capacitive current indicates the movement of ions, adsorption/desorption, re-orientation of solvent dipoles, etc., at the electrode-electrolyte interface [103]. This approach is simple and has been found in numerous applications (sensing of cell viability, attachment, morphology, proliferation and toxicity studies) [100]. Even though this technique becomes less sensitive when cell confluency is low, non-faradaic sensors provide a label-free, quantitative and dynamic measurement of cell activity and environmental changes [101]. For the purpose of this dissertation, non-faradaic impedance spectroscopy will be the primary technique discussed and used to detect cell monolayer behavior.

Perhaps the most well-known non-faradaic platform was pioneered by Giaver *et al.* who published the initial findings of what later became Electrical Cell-Substrate Impedance Sensing (ECIS) systems. In this platform, fabricated co-planar gold microelectrodes were used in conjunction with AC potentials and changes in electrical impedance due to adherent cell presence and motility were observed [32], [33]. When AC potentials were applied, the cell monolayer presence and structure would convolute the current, causing impedance changes. As cells cover the electrode surface, they restrict current to flow through the small spaces beneath the cells and in between cells (Fig. 3.1). Equivalent circuit models are then used to fit the acquired data and deduce the different electrical parameters that define the physical parameters (membrane capacitance and barrier function) of the cellular monolayer [34], [104]. In order to understand how these non-faradaic impedance sensors work, a proper background is provided in the proceeding sections.

Figure 3.1: (A) Schematic of ECIS system. (B) Diagram of current path on a cell-free electrode and a cell covered electrode. Current can either travel through the paracellular spaces or in transcellular fashion [37].

3.1 Theory

Impedance is a measurement of a circuit's ability to resist electrical current flow when a voltage is applied. Though it sounds similar to resistance, impedance is dependent on frequency and has both a magnitude and phase. In electrochemical impedance, small (1-25 mV) and defined AC voltage signals are applied. The AC current responses are measured. Small excitation signals are used for two purposes: (1) they guarantee the response to be pseudo-linear, meaning that, at the same frequency, the response is the same type of waveform as the applied signal except shifted in phase and (2) they allow for a non-invasive and damage free assessment of cells.

In an impedance measurement, the excitation AC voltage signal can be expressed as:

$$E_t = E_0 \sin(2\pi ft) \quad (3.1)$$

where E_t is voltage at time t , E_0 is the magnitude of voltage and f is frequency in hertz.

The AC current (I_t) signal with respect to time is defined as:

$$I_t = I_0 \sin(2\pi ft + \phi) \quad (3.2)$$

where I_0 is the magnitude of current response and ϕ is the phase-shift angle. Applying

Ohm's law, we define impedance (Z) as a magnitude Z_0 (or $|Z|$):

$$Z = \frac{E_0 \sin(2\pi ft)}{I_0 \sin(2\pi ft + \phi)} = Z_0 \frac{\sin(2\pi f)}{\sin(2\pi f + \phi)} \quad (3.3)$$

Applying Euler's relationship, impedance (Z) can also be expressed in complex form:

$$Z(2\pi f) = \frac{E}{I} = Z_0 e^{j\phi} = Z_0 (\cos \phi + j \sin \phi) \quad (3.4)$$

where $j = \sqrt{-1}$ [105]. Complex impedance can often be described as two components: the

real (Z') and imaginary impedance (Z'') component:

$$Z = Z' + iZ'' \quad (3.5)$$

$$Z' = \text{Re}(Z) = Z_0 \cdot \cos \phi \quad (3.6)$$

$$Z'' = \text{Im}(Z) = Z_0 \cdot \sin \phi \quad (3.7)$$

$$Z_0 = \sqrt{(Z')^2 + (Z'')^2} \quad (3.8)$$

$$\phi = \tan^{-1} \frac{Z''}{Z'} \quad (3.9)$$

where the Z' component arises from resistance (in-phase) elements and Z'' component arises from capacitive and inductive (out-phase) elements. Because complex impedance is a function of frequency, complex impedance can also be defined as:

$$Z(2\pi f) = Z_0 \cdot e^{i\phi(2\pi f)} = Z'(2\pi f) + iZ''(2\pi f) \quad (3.10)$$

For complex impedance measurements, both Z_0 and ϕ are collected and fitted to an equivalent circuit model or mathematical model.

3.2 *Equivalent Circuit Models*

The interpretation of measured impedance is done by fitting the data to a circuit model. Each component of the circuit model is representative of a characteristic in the cell monolayer, measuring system or environment. A simple model used in impedance analysis is found in Fig. 3.2 [34]. In this circuit, the capacitor (C_{cl}) and resistor (R_{cl}) in parallel describe the behavior of the transcellular (membrane) and paracellular barriers respectively; these parameters are usually expressed per unit of electrode area. The trans-endothelial resistance (R_{cl}) symbolizes the tightness of cell-cell junctions. These values can range from 3 -1000 $\Omega\text{-cm}^2$ or higher depending on cell type [20]. C_{cl} , also referred to as the membrane capacitance, which represents both the apical and basolateral membranes as parallel capacitors, has been reported to be approximately $1\mu\text{F}/\text{cm}^2$ for most cell types [106]. Most circuit models require a confluent cell layer to provide accurate parameter values. Though they do produce changes in impedance, subconfluent cell monolayers or partially uncovered electrodes cause an artificial inflation in C_{cl} and measured data differs substantially from calculated impedance.

The other components in the circuit model, C_{El} and R_{bulk} are parameters of the measurement system and environment. These parameters alone define a cell-free electrode-electrolyte system. R_{bulk} symbolizes the resistance of cell culture bulk medium.

C_{El} describes the interactions between electrolyte and the electrode surface and it is referred to as the capacitance of the electric double layer. When a metal is introduced into an electrolyte, like cell culture medium, there is a negative charge accumulation at the electrode surface. This charge accumulation creates an electric field that attracts cations from medium, together they form this electrical bilayer known as the electric double layer [107].

Figure 3.2: An illustration of equivalent circuit model. The C_{El} describes the interfacial capacitance between electrolyte and electrode. The bulk resistance (R_{bulk}) is indicative of the conductivity of the cell culture medium. Both C_{cl} and R_{cl} represent the cell behavior. C_{cl} is the cell membrane capacitance and R_{cl} the transendothelial resistance [34].

Recently, more circuit models have been suggested for impedance analysis. They usually include improvements on parameter selection/inclusion in order to accurately depict the complex dynamics of the monolayer characterization system. One of the

additions that has enhanced the accuracy of this analysis technique is the representation of the capacitance of the electric double layer as a constant phase element (*CPE*) [77]. Under ideal conditions, the electrical bilayer of charges at the electrode surface is modeled as parallel plate capacitor and impedance of this capacitor (Z_c) is defined as:

$$Z_c = \frac{1}{j2\pi fC} \quad (3.11)$$

where C is the ideal capacitance. However, in most cases, electrode surface roughness and porosity, passivation composition, non-uniform potential and current distributions create deviations from ideal capacitor behavior [108], [109]. This electrolyte-electrode interface behavior can be more accurately quantified by a *CPE* impedance (Z_{CPE}):

$$Z_{CPE} = \frac{1}{Y(j2\pi f)^n} \quad (3.12)$$

where Y is the magnitude of the *CPE*, the phase angle is equivalent to $-90^\circ n$ and n is the power factor ranging between 0-1. When $n=1$, the impedance of the *CPE* is equivalent to that of an ideal capacitor; if $n=0$, the impedance of the *CPE* is equal to that of an ideal resistor. Although Z_{CPE} represents the interfacial behavior between electrolyte and metal surface in most common electrochemical systems, it also has been shown to change in the presence of adhered cells. It was found that Y and n vary when BAECs were cultured on thin gold films [77], [108]. These changes were contributed to differences in the small volumes of electrolyte that were trapped beneath the cell and above the electrode, as well as, the close proximity of cells to the electrode surface.

Investigators have modified the circuit models to omit assumptions on cell shape and geometry as was done by the initial model presented by Giaver *et al.* They proposed a circuit model with two pairs of parallel resistors-capacitors connected in parallel as

depicted in Fig. 3.3 [110]. The intercellular space, specifically the solution in between cells, is defined by a capacitor and resistor in parallel. The other set of capacitor and resistor in parallel represent the transcellular behavior through the cell membrane. Like the other mentioned circuit models, the cell characteristic parameters were organized in series with the medium resistance and interfacial polarization at electrode. Though more elaborate circuits have been recommended, it is believed that a simple model with just a few elements may provide a closer estimate of the electrical parameters of cell monolayer structure and function.

Figure 3.3: An equivalent circuit model of an impedance platform where the impedances are broken up between the paracellular behavior (Z_{sol}), transcellular behavior (Z_c) and electric double layer (Z_p) [110].

3.3 *Frequency Response*

Impedance sensing can be recorded in different ways: simple impedance, complex impedance, and at single, multiple, or spectrum of frequencies. Choosing the appropriate acquisition mode is an important design feature when developing an impedance system. It determines what cell behavior information you can extract from your data. It also defines how often you electrically probe the cellular sample, as well as, the extent of data collection and subsequent analysis that will be required to extract electrical parameters.

Some systems implement the acquisition of simple impedance. In this format, only the magnitude of impedance is acquired. This approach requires less sophisticated equipment and data analysis is straightforward. Other designs record complex impedance which includes both the magnitude and phase quantities. Using this information capacitance values, which indicate cell attachment and spreading, can be estimated. This method definitely provides more data to form a more comprehensive conclusion of cell behavior but it does require more data acquisition and analysis.

For impedance platforms the frequency mode is a critical design feature. The value at which frequency samples depends on the specific cell type under investigation [41]. The initial ECIS system sampled multiple frequencies ranging from 22 Hz to 90 kHz for fibroblast cells WI-38 and WI-38 VA13 [32]. However, most of the results discussed were acquired at 4 kHz which was determined as the most sensitive measurement frequency. This frequency value was extracted from normalized plots of impedance, resistance and capacitance as a function of frequency. The normalization quantities are ratios of cell-covered electrode to cell-free electrode measurements ($|Z|_{cell-covered}/|Z|_{cell-free}$, $R_{cell-covered}/R_{cell-free}$, $C_{cell-covered}/C_{cell-free}$) [32], [99]. The frequency value at

which the difference between cell-free and cell-covered electrodes measurements are the largest is the frequency value at which cells impede the current flow most effectively. Thus, this frequency is denoted as the monitoring frequency. For ECIS systems and endothelial cells, this frequency value is also approximately 4kHz [41]. The acquisition of impedance at a single frequency has since become a common practice in many impedance characterization platforms. In this measurement mode the acquired impedance is presented as a function of time.

Alternatively, impedance measurements are recorded as a function of a specified frequency range. Studies have shown that characterization of cell behavior is frequency dependent [37]. At lower frequencies, current prefers to flow beneath the cells, through the intercellular junctions, and into the bulk medium (Fig. 3.4B, red arrows). In this frequency range, the barrier function can be accessed. At higher frequencies, cell-matrix interactions and cell membrane integrity are probed (Fig. 3.4B, dashed blue arrows). At these values current can easily pass through the cell membrane and into the bulk medium. For example, in the ECIS systems, the monitoring frequency of 4 kHz captures cell motility and morphological modifications taking place at the sub and inter-cellular areas. At 32 or 40 kHz, the resistive behavior in the sub and inter-cellular gaps no longer dominate. Instead, the capacitive behavior of the cell membranes is easily captured as the current couples through the cell membrane [99]. The specific frequency range for each cell behavior aspect depends on the design of the impedance sensor system. Yet, it is obvious that by incorporating a comprehensive frequency sweep more detailed information can be extracted from the acquired data, including interfacial and cell interactions, allowing for a more sensitive assessment of the endothelium cell monolayer.

This method, however, has been overlooked as a result of longer data collection time intervals (decreased temporal resolution) and the substantial data analysis that it requires.

Figure 3.4: A diagram representing a (A) cell-free and (B) cell covered electrode with the respective equivalent circuit models where R_{bulk} is the resistance of the cell culture medium, CPE describes ion behavior at the electrolyte-electrode interface and Z_{cl} is the impedance attributed to the cell layer. In addition, the diagram also includes depictions of current flow beneath cellular clefts and adjacent cells at low frequencies (red arrows), between adjacent cell, as well as, some through the cell itself at intermediate frequencies (dashed black arrow), and finally, through the cells at high frequencies (dashed blue arrows) [99].

3.4 Graphical Representations of Impedance Spectroscopy

Impedance spectra can be visualized in many different ways. The most common method to display impedance data is a Bode plot (Fig. 3.5A) which constitutes of two complementary plots where one is a log-log representation of impedance magnitude ($|Z|$) and the other is a semi-log representation of phase shift (ϕ) as function of frequency. In Bode plots, phase shifts are produced by capacitive and inductive elements; while, resistive elements cause voltage and current to be in phase. This can be further visualized in Table 3.1 which provides the impedance and phase shift contributions of ideal circuit elements.

For any electrode-electrolyte system, a negative slope is observed at low frequencies in the impedance magnitude ($|Z|$) plot and is accompanied by phase angle of approximately -90° , both of which represents the electric double layer capacitance. At high frequencies, the impedance magnitude ($|Z|$) plot flat lines while the phase angle increases nearly reaching 0° ; an indication of a resistive element, in this case, the solution resistance. When cells are present, the Bode plot changes significantly at the mid frequency range values. A new plateau and peak is formed in the impedance magnitude ($|Z|$) plot and phase plot respectively characterizing the cellular monolayer at the electrode surface. The plateau's height, slope and width distinguish the cell type specific morphological changes [99]. Unlike the solution resistance peak, the phase peak pertaining to the cells usually lies between -90° and 0° . This is due to the fact that cellular monolayer is characterized by a parallel combination of a resistance (paracellular pathway) and a capacitance (cell membrane) component.

The Nyquist plot (Fig. 3.5B) is yet another depiction of complex impedance in Cartesian coordinates. In this type of plot, the real (Z' , horizontal axis) versus imaginary (Z'' , vertical axis) components of complex impedance are shown. Unlike the Bode plot, where frequency increases from left to right, frequency in the Nyquist plot increases from right to left. One disadvantage of this plot is the difficulty to correlate the same frequency value between multiple plots lines since they do not lie on the same coordinate.

Although not discussed in this work, Other possible complex impedance data representations include admittance ($Y=1/Z$), permittivity ($\epsilon=Y/(i \cdot \omega \cdot C_C \cdot \epsilon_o)$) and modulus ($M=1/\epsilon$) where C_C is the cell constant, ϵ_o is the permittivity of free space, and ω is the angular frequency and is equivalent to $2\pi f$.

Equivalent Circuit Element	Parameter	Impedance $ Z $	Phase Shift ϕ
Ohmic Resistor	R	R	0
Capacitor	C	$(i \cdot \omega \cdot C)^{-1}$	$-\pi/2$
Inductor	L	$i \cdot \omega \cdot L$	$+\pi/2$

Table 3.1: Impedance and phase shift contributions of ideal circuit elements [99].

Figure 3.5: Illustration of graphical representations of complex impedance of cell-free and cell (HUVECs) covered electrode (200 μm in diameter) in a (A) Bode plot and (B) Nyquist plot. For the Bode plot, the solid plots are quantifications of $|Z|$ while the dashed plots are representative of the phase shift, θ . The blue dashed arrows indicate the resistive cellular monolayer while the dashed green arrow indicates the cell culture media resistance.

3.5 *Design Aspects*

Design aspects used in impedance spectroscopy can vary on the sensing application and environment. Each component in an impedance platform plays a role. The substrate is the foundation of the sensor, the electrode is the transducing element, and the passivation layer acts as an insulator, preventing noise in the signal. For adherent cell characterization platforms, the critical performance parameters include: substrate and passivation layer material, as well as, electrode material, geometry and configuration. In the following sections, these critical parameters are discussed in more detail.

A. Substrate Material

The substrate acts as the infrastructure for the deposition of metal electrodes, wires, and connections, cell growth, and passivation layer. Ideally, the substrate material should have a low dielectric constant in order to minimize stray capacitance [111] and reduce the possibility of noise. The substrate must be biocompatible, as well as, compatible with electrode fabrication techniques. Other considerations for substrate selection involve the transparency of the material for the purpose of improving cell visibility. Impedance platforms have been constructed on a whole range of substrates including glass [20], [77], [101], [107], [111]–[125], glass epoxy (printed circuit board, PCB) [41], [126], [127], silicon/silicon dioxide [107], [128]–[132], silicon/silicon nitride [133], [134], polystyrene [32], [135], polycarbonate [136]–[139], and polycarbonate membranes [36], as well as, nitrocellulose membranes [36]. Most recently, due to the increasing interest in flexible electronics some impedance sensors are composed of polyethylene byproducts [140]–[142] and parylene [143].

B. Passivation Layer Material

The passivation layer defines the electrode geometry and electrically isolates the wires from the sensing area. Both the type of passivation layer material and thickness are important design aspects in minimizing parasitic capacitances at higher frequencies [109]. Similar to the substrate material, the passivation layer must have a low dielectric constant while still being chemically inert and biocompatible. Passivation layers used in impedance platforms include silicon dioxide [123], [144], [145], silicon nitride [118], [122], [128], [133], [144], [146], a multilayer combination of silicon dioxide and nitride [121], parylene [143], polyimide [134], photoresist [41], [130], parafilm [138], polymer paste [142] and dry-film [126]. Other suggested passivation layers are silicon carbide, TEFLON, ceramic, or any kind of polymer or thermal plastic [147]. A range of insulator layer thicknesses can be found in the literature, usually between 150 nm – 5 μ m. The larger the thickness of the layer, the less parasitic capacitance in the passivation layer is observed.

C. Electrode Material

The electrode layer behaves as the transducing element of the sensor, as well as, a cell growth support. It is perhaps the most published design aspect of microelectrodes and impedance sensors. Like the substrate and passivation layer, the electrode material should be biocompatible and resist fouling. The electrode material must have a low impedance and also be compatible with lithography and patterning techniques. Gold [40], [41], [77], [107], [109], [112], [121], [122], [126], [129]–[132], [136], [138], [139], [141], [147], [148], platinum [125], [134], [142], indium tin oxide (ITO) [118], [124], [140], [146],

iridium [120], [128], [147], polysilicon [149], and carbon [150] have all been used as electrode materials. The electrode material can be sputtered, evaporated, deposited by PECVD, or even screen printed. Electrodes such as ITO which are optically transparent provide an additional benefit of allowing for an optical and electrical assessment of cells simultaneously.

Electrodes can either be bare or surface treated. This surface treatment is often performed in order to increase surface area and minimize impedance at the electrode-electrolyte interface (electric double layer). This is particularly important in applications where the dimensions of the electrode are limited to the cell population probed and increasing the area of the electrode is disadvantageous [107].

Several surface treatments have shown to reduce the impedance at the electrode cite and decrease current density. One example is the deposition and growth of a porous metal coating on top of the electrode through electroplating. This type of deposition has been found to decrease electrode impedance as much as two orders of magnitude as in the case of platinum black [151], [152]. Other surface techniques involve adding a porous conducting polymer [126], patterning the surface with micro-sized features[107], etching the surface to create a rough topography [122], as well as, chemical modifications like activated iridium [107].

D. Electrode Configuration

Measurement setups (Fig. 3.6) can differ due to experimental parameters and conditions. Some systems consist of planar surfaces (Fig. 3.6B) while others are

composed of porous membranes (Fig. 3.6A) in which cells are cultivated on. For impedance systems involving filters, the membrane separates two fluid compartments mimicking the apical and basal side of cells. In these setups, one electrode is placed in each fluid compartment and the resistance or impedance is measured across the entire cell layer [153]–[155]. This type of system has been used in many applications including examining endothelia and epithelia barrier regulation of blood brain barrier [156], renal [157], and drug delivery [158]. In spite of this, such setups suffer from certain disadvantages. For example, in the chopstick electrode systems, the electrical measurement is greatly influenced by the position of the electrodes. As a result, the user can have discrepancies in the acquired data due to failing to replicate the identical electrode position in multiple samples. In addition, the chopstick electrode arrangements are susceptible to inhomogeneous electric fields across the cell layer which often leads to a systematic overestimation of the electrical measurement[42], [159].

Another typical electrode configuration consists of two (Fig. 3.6B) or four pair of planar electrodes. Planar electrodes are integrated with the substrate by thin film deposition. In these systems, electrodes act as the growth substrate and the signal transducer. For the two pair electrode configurations, there are several different electrode geometries that exist. One of the most used electrode configuration (Fig. 3.6D) consists of a small circular (diameter 30-250 μm) or rectangular sensing (SE) or working electrode (WE) and common counter electrode (CE) [34], [120], [160], [161]. As current is applied, the voltage (or vice-versa) is measured between the electrodes. In these systems, the surface area of the CE is designed at least 100-200 times larger than the SE. In doing so, the interactions at the CE are considered negligible and the overall

impedance is due to the cell behavior at the SE [77] which simplifies data analysis. Alternatively, it is also possible to have two small working electrodes (Fig. 3.6E), each provides an individual contribution to the overall impedance measurement. For these coplanar electrode arrangements, electrode geometry governs electrode polarization impedance due to accumulation of ions/molecules on electrode surface. The polarization impedance increases significantly and dominates the impedance frequency spectrum as the electrode surface area decreases, leading to a diminution of detection sensitivity.

Four electrode pair configurations systems use two pairs of electrodes to pass current while the other two measure voltage. Characteristically, the current and voltage measurement electrodes are placed intermediately from one another in a linear fashion. Unlike the two pair electrode configuration, four pair electrode impedance sensors do not encounter notable electrode polarization impedance owing to the separation of current and voltage measurement electrodes. Despite this, this electrode arrangement is unsuitable for large micro-electrode arrays necessary for reproducibility and spatial resolution.

Figure 3.6: Illustration of impedance measurement setups [99]. Cells can either be cultivated on: **(A)** porous membranes which separates two fluid compartments above and below the filter. Each fluid compartment has one electrode by which potential and current flow is measured. **(B)** Planar setups have electrodes directly integrated into the substrate. For planar electrode systems, electrodes can consist of **(C)** interdigitated electrodes (IDE), **(D)** a small working electrode (WE) and a larger counter electrode (CE) or **(E)** two same sized WE.

E. Electrode Geometry

Sensing electrodes are small, usually in the micrometer range (diameter 30-250 μm) [99]. This ensures that impedance is not dominated by the solution resistance. In an electrode-electrolyte interface, there are two main sources of impedance: the solution and Faradiac resistance. For circular electrodes, the solution resistance is proportional to r^{-1} and the Faradiac resistance to r^{-2} where r is the radius of the electrode. It is only when the electrode radius is small enough that the impedance is primarily due to the interactions at the electrode surface (Faradiac resistance) versus the current through the electrolyte (solution resistance) [37]. Microelectrodes increase the signal to noise ratio by limiting the assessment range to the adhered cells and nearby microenvironment. Proper microelectrode geometry ensures the elimination of background noise and interference without compromising the sensor sensitivity significantly. However, the polarization impedance increases significantly and dominates the impedance frequency spectrum as the electrode surface area decreases, leading to a diminution of detection sensitivity [100]. As result, for significantly small electrode geometry, electrode material must be

carefully selected and modification electrode process must be incorporated to decrease the polarization impedance.

Microelectrodes are either arranged in individual chambers [33], [162] or arrayed within a single chamber [120], [134], [160], [161]. For those electrodes arranged in individual chambers, fluctuations due to cell motion are more readily observed and can be useful in wound healing [41] and cell migration studies. Nonetheless, with singular electrodes spatial resolution is small and data reproducibility can be an issue. The arrayed electrodes improve the spatial resolution by incorporating several singular spot measurements of one cell monolayer. This type of cumulative signal also minimizes discrepancies caused by undistributed cell seeding and growth, in addition to the distortion of the signal due to cell movement. Cell proliferation and barrier investigations have found this multi-electrode arrangement effective [41]. However, the substrate coverage of the electrode array will strongly determine the improvement of spatial resolution, as well as, statistically relevant data [100].

An alternate electrode array geometry often used in impedance spectroscopy is interdigitated electrodes (IDE) (Fig. 3.6C) which consist of co-planar comb-like structures. These electrodes are equally large and individually contribute to the impedance measurement [163], [164]. They are usually patterned to cover a larger surface area of the substrate surface (~70%) [165], [166] and thus, provide an overall assessment of the entire cell population. IDEs have been used in numerous applications including cell-ECM interactions [167], signal transduction assays [77], cell migration through a cell monolayer [168], barrier function[169] and cell proliferation [170].

Similarly, the xCELLigence system (Fig. 3.7) also referred to as the real-time cell electronic sensing (RT-CES) system provides an electrode array which covers approximately ~80% of the surface area [171]. The electrode array is described as a circle-on-line geometry, where rows of circular metal electrodes, 90 μm in diameter, are continuously connected to each other and each row is separated by a 10 μm gap. This system can be used to monitor cell proliferation, viability and cytotoxicity.

Figure 3.7: Image of the xCELLigence circle-in-line electrode array [171].

3.6 Applications

Since the emergence of impedance sensing, these systems have been used for a multitude of applications and adherent cell types. ECM-cell interaction assay, *in vitro* toxicity assay, electroporation assay, cell wound-healing and migration assay, signal transduction assay, tumor metastasis assay, and, most importantly, the study of cells under flow have all been accomplished using impedance based biosensor. Initial

impedance studies were performed on fibroblast cells, but have extended out to MDCKs, BAECs, microvessel ECs, and HUVECs among others [37].

A. Impedance Analysis and Endothelium Models under Flow

Recently, the study of shear effects on cell structure and function has attracted a lot of attention due to their implications in disease development. A few scientists have seen an opportunity to monitor cell behavior under fluid flow using impedance based sensors due to the fact that it provides a real-time assessment. The following section provides a brief overview on these studies and their findings.

Porcine pulmonary trunk endothelial cells were observed under shear stress produced by a cone-and-plate system [20]. Cells were exposed to a range of shear stresses between 0.5-50 dyne/cm² and impedance measurements were acquired for a frequency spectrum between 10 Hz- 1 MHz for 240- 1020 minutes. It was found that trans-endothelial resistance increases between 2-15% with increasing shear stress values (2-50 dyne/cm²) within 15 minutes of the onset shear. Cell movement decreased during this period. This was trailed by a decrease in trans-endothelial resistance which continued to 20% below the baseline values. During this time, cell shape changed significantly. Within hours, cells began to slow down and the trans-endothelial resistance returned to baseline values.

The ECIS system was used to investigate effect of disturbed flow on bovine aortic endothelial cells [35]. Their experimental set-up consisted of an altered version of a parallel plate flow system where protruding features were added to the substrate of the cultured cells. Endothelial electrical resistance (transendothelial resistance, TER) values

collected were lowest at the adjacent areas of highest shear stress gradient where it is presumed that the cells are most permeable. This study was complimented with dextran permeability experiment which also showed that there was a 5.5 increase in trans-endothelium transport of dextran in areas of high shear stress gradients.

In another study the ECIS system, in combination with a parallel plate flow chamber, was also used [16]. Bovine aortic endothelial cells were exposed to laminar shear stress (10 dyne/cm^2) for defined time intervals and followed by periods where flow was ceased. TER value recorded showed an initial decrease at the onset of shear. After 15 min, TER values begin to climb up to baseline values and slightly surpassing them. Eventually, TER decrease again with time and fluid flow exposure. It was also found that when cells experienced no fluid flow after being exposed for 5 hours, TER would return to baseline values.

3.7 Conclusions

In this chapter, we discussed impedance sensing theory and numerous applications. Regardless of the use of this technique in many research areas, the majority of the current *in vitro* models using impedance characterization only considered static cell cultures [172]–[174]. Also, traditional impedance platforms usually acquire data at a single or a limited range of frequency values [175]. This limits the information that can be gathered from a broad frequency sweep like the one a spectroscopy offers. As a result, the need for a physiologically relevant *in vitro* impedance spectroscopy platform can provide a useful tool to study endothelial cells under hydrodynamic shear stress.

CHAPTER 4

DESIGN AND CHARACTERIZATION OF A MICROFLUIDIC IMPEDANCE PLATFORM FOR THE REAL-TIME, *IN VITRO* ANALYSIS OF ENDOTHELIUM

In this work, a microfluidic impedance platform is introduced for the electrical assessment of endothelium monolayer kinetics while undergoing fluid shear stress *in vitro*. In this chapter, the components of the microfluidic impedance platform which include an impedance sensor, a flow-through scheme, and automated data acquisition system are introduced and their design aspects are discussed. The platform attempts to mimic the arterial structure, size, and environment. A control cell-free channel was incorporated to the design with the propose of providing information on environmental-induced drifts in the acquired data. The platform design also incorporates three different electrode diameters (200, 100 and 50 μm) in order to evaluate different sizes of subpopulations of endothelial cells within the microchannel. Initial characterization of the impedance platform was performed with biologically relevant electrolyte solutions to determine microfluidic sensor sensitivity, electrode size based behavior, parasitic contributions due to electrical connections, variations in performance due the fabrication

process, solution conductivity effects, flowrate effects, effects due to pH changes and bubble effects. Additional characterization was performed with cultured HUVECs to study the sensitivity of monitoring frequency and the effect of individual electrical parameters on impedance spectra.

4.1 Microfluidic Impedance Chip Design

The microfluidic impedance chip (Fig. 4.1 and 4.2) described herein consists of a large and common counter electrode (CE) positioned in between two arrays of independently addressable gold sensing electrodes (SEs). Each array included two 50 μm , three 100 μm , and three 200 μm diameter electrodes. The multiple electrode design element provides an averaged measurement across sites of the cell monolayer. These different electrode geometries were used to test and compare the behavior of different cell population sizes. The CE was designed to be much larger, with a surface area 200 times greater than the SEs. As a result, the impedance of the CE was negligible and the measured impedance was attributed to the SE [77], [176]. All electrodes were connected through patterned wires to contact pads located at the edge of the chip. The spacing and arrangement of these contact pads were designed to fit a commercially available card reader which facilitated the electrical connections to the chip.

In order to generate fluid shear stress, two adjacent channels were incorporated. Each channel contained one array of SEs and part of the CE. One of the channels was defined as a cell-free control, while the other was used for the cellular monolayer measurements. The channels were designed 335 μm in height, 2.0 mm wide and 13 mm long (straight region) with inlet and outlet region positioned at a 45° offset (6 mm long)

to enable fully developed flow at the measurement region. The cross-sectional aspect ratio of this channel geometry was 0.17.

A. Shear Stress Approximation

Shear stress within microchannels can be calculated from either the parallel plate Poiseuille flow or rectangular duct approximations as mentioned in Chapter 2. Both flow approximations were used to determine the appropriate shear stress calculation. The comparison analysis of both shear stress approximations (refer to Appendix) indicated that the rectangular duct approximation resulted in the most accurate shear stress measurement. Table 4.1 displays the yielded shear stress approximations using both the parallel plate and the rectangular duct equations. Fig. 4.3 displays the shear stress and shear gradient across the bottom surface of microchannel design.

τ_{PP} (dyne/cm ²)	τ_{RC} (dyne/cm ²)
2.2	2.5
15.7	17.6
21.2	23.7
31.3	35.0
52.0	58.1

Table 4.1: Shear stress calculations using both the parallel plate Poiseuille flow and flow within a rectangular duct approximation.

Figure 4.1: (A) A top view image of the impedance chip design. The design incorporated two arrays of 50, 100 and 200 μm diameter SEs and a complimentary common CE. Each array of SEs and part of CE is embedded in one of the adjacent microchannels. For the experiments presented here, the left channel was used as a control (cell-free) and the right channel contained the HUVEC monolayer. All electrodes were wired to contact pads located at the bottom of the chip. Contact pads were patterned to fit into a commercial card reader and facilitate electrical connections.

Figure 4.2: (A) A top-view image of the fabricated microfluidic impedance chip. The dashed blue line depicts the position of the PDMS channels. Flow flows from right to left as shown in the diagram. The zoomed image shows the various diameter SEs and adjacent CE. (B) A cross-sectional view of the chip illustrates the control “cell- free” channel and the channel containing HUVEC monolayer.

Figure 4.3: Shear stress (A) and and shear stress (B) gradient across the width of the channel.

4.2 *Fabrication of Microfluidic Impedance Chip*

The microfluidic chip was fabricated in a batch process as illustrated in Fig. 4.4. First, 4in by 4in borosilicate glass substrates were utilized due to the low dielectric constant of this material. These substrates were sputtered with an adhesion film of chrome (Cr, 30nm) and followed by a layer of gold (Au, 90nm). This Cr/Au layer was lithographically patterned to form the contact pads, wires, SEs, and CE. Following, a 1 μ m thick silicon dioxide film was deposited by plasma enhanced chemical vapor deposition (PECVD). This film formed a passivation layer which electrically isolated the noise and impedance not contributed by the SEs [70,72,77]. A second lithography step was used to define SEs and CE geometry as well as open windows at the contact pads. The exposed silicon dioxide windows were then etched by reactive ion etching (RIE). Finally, remaining photoresist was chemically dissolved and the chip was cleaned and prepared for the incorporation of fluidic channels.

The microfluidic channels were formed with PDMS and double sided adhesive (DSA) which are biocompatible and have been shown to be used for other *in-vitro* devices [177]. The double sided adhesive (Adhesive Research, ARseal 90880, ~300 μ m thick) layers were laser cut to define the channel geometry. The adhesive was carefully aligned with the electrode arrays and adhered to the chip to form the side walls of channels. A top PDMS lid with inlets and outlets ports was layered on top to enclose the channel cavities.

4.3 Microfluidic Impedance Platform

The microfluidic impedance platform encompasses the microfluidic chip, a commercially available card reader (Samtec, mini edge card socket, MBI series), and a custom designed printed circuit board (PCB) equipped with relays (Fig. 4.5). The impedance chip was connected via the card reader to the PCB which allowed for the automation of time-dependent measurements on individual electrodes. A custom LabVIEW algorithm controlled both the PCB and impedance analyzer by directing the electrode switching and data collection parameters.

Impedance measurements were acquired with a HP 4294A impedance analyzer at an applied voltage of 10mV and at a frequency range from 100Hz to 1MHz. For time dependent data, measurements were collected at each respective SE every 24 minutes- this was the approximate cycling time to acquire individual impedance measurements from each electrode.

Figure 4.4: Fabrication process flow diagram of the impedance chip. (i) A thin layer of photoresist was spun on 4in x 4in borosilicate glass substrate. (ii) The photoresist was patterned to open windows for SEs, CE, wires, and contact pads. (iii) A thin layer of Cr/Au was sputtered on to borosilicate glass and patterned using the lift-off technique. (iv) A SiO₂ passivation layer (1μm) was deposited using a PECVD. (v) A second lithography step was used to pattern SiO₂ and (vi) etched using RIE to define the SEs, CE geometry and contact pads. (vii) Remaining photoresist was dissolved with acetone. (viii) Finally, DSA were laser cut to define the channels' geometry and subsequently adhered to the chips. A top PDMS lid with inlets and outlets was placed on top to seal channel cavities.

Figure 4.5: An illustration of the microfluidic impedance platform and experimental set-up. The microfluidic impedance chip was connected via a card reader to a custom printed circuit board designed to automatically collect impedance spectra from an impedance analyzer (100 Hz-1 MHz) and store the acquired data in a portable computer. The chip was also coupled to a peristaltic pump and medium reservoirs for perfusion purposes, subjecting cells to shear stresses between 0-60 dyne/cm². The chip, pump, and medium reservoirs were maintained in an incubator chamber with controlled temperature and humidity during experimentation.

4.4 Microfluidic Impedance Platform Characterization

A. Microfluidic Platform Sensitivity

In order to optimize the sensitivity of the impedance sensor, devices were fabricated on different substrates including oxidized silicon wafers, soda lime and borosilicate glass. Impedance measurements (Fig. 4.6) of the bare (i.e. no media) devices showed that both soda lime and borosilicate glass are more sensitive than silicon wafer-based devices. Borkholder also found that silicon based substrates suffered from problematic parasitic capacitance and switched to glass substrates for the second generation of chips [107]. Soda lime and borosilicate glass have lower dielectric constants which lead to reduced substrate capacitances and higher impedances. In impedance designs, minimizing stray substrate capacitance is always desired as this parameter, contributes to the electrical noise in acquired the measurements. Capacitance values were extracted from the impedance spectra and showed values of 395 pF for silicon dioxide devices, 15 pF for soda lime glass and 14 pF for borosilicate substrates. Thus, glass based devices have the ability to show small changes in the electrical parameters that would be otherwise masked with silicon dioxide devices and, in addition, also have the additional benefit of optical transparency for cell visualization.

Figure 4.6: A Bode plot showing the magnitude of impedance versus frequency for 3 different bare (no solution) devices fabricated on silicon dioxide, soda lime glass and borosilicate glass. A potential of 10 mV was applied and measurements were acquired at room temperature. Lower dielectric constants of soda lime and borosilicate glass yield devices with minimized substrate capacitance and thus higher sensitivity.

B. Effect of Electrode Size

Different electrode geometries (200, 100, 50 μm diameters) were tested in effort to probe different sizes of subpopulation of cells. Larger electrodes will represent the overall cell population while smaller electrodes describe a more localized behavior of just a few cells. In these experiments cell culture media (27 mS/cm) was injected into the microfluidic chip and allowed to equilibrate for 15 minutes. Impedance spectra (at an applied potential of 10 mV) was acquired for four sets of electrodes and averaged. Fig. 4.7 shows that as the surface area of the electrode increases, the impedance magnitude

and phase shift decreases. In Fig. 4.7B and C, there is a larger drop in both impedance magnitude and phase shift between electrodes of 50 μm and 100 μm diameters versus 100 μm to 200 μm diameters indicating perhaps that electrodes with diameters less than 100 μm are unsuitable for this sensing application. Similarly, the frequency independent region, characterized as the portion of the Bode plot (Fig. 4. 6A) approaching a horizontal 'plateau', gets more narrow with decreasing electrode diameter. The frequency independent region characterizes the spreading resistance of the cell culture media, though in the presence of cells it would represent the cell monolayer permeability. As observed in other works, this range becomes smaller with decreasing electrode diameter [178]. For 50 μm electrodes, the frequency independent region is approximately non-existent and the behavior is that of capacitive charging as evidenced by the phase shift values of approximately -70° . Consequently, the interfacial impedance is significant such that negligible information on resistive behavior could be obtained from this type of spectrum. Initial device designs also included a smaller size electrode of 25 μm in diameter. Although the data for this electrode is not shown, you can see from the presented data that electrodes smaller than 50 μm are definitely not suitable for the desired application. However, the interfacial impedance can be minimized by increasing surface area [179] and roughness [122] through electroplating [107], [128], [144], [180] and the incorporation of non-planar features [181].

Figure 4.7: (A) Impedance spectra of cell culture media was acquired for 4 sets of electrodes (50, 100, 200 μm) and averaged. (B) Impedance magnitude and (C) phase shift as a function of electrode diameter at 1,10,100, and 1000 kHz. Impedance magnitude and phase shift increases with decreasing electrode diameter. Error bars represent +/- one standard deviation.

C. Electrical Connections

While determining the proper experimental frequency sweeping parameters, the impedance platform was examined for any possible parasitic behavior attributed by the connections to the microfluidic impedance sensor. Initial testing was performed by injecting a prepared diluted potassium chloride (KCl) solution (conductivity, $\sigma \sim 20.1$ mS/cm) into the microfluidic channels and allowing the medium to equilibrate. Impedance spectra were then acquired at an extended frequency range of 40 Hz- 10 MHz and at a potential of 10 mV for different connection configurations. The impedance

platform connections can be visualized in Fig. 4.8 and be broken down to five different components: the impedance chip (A), card reader (B), ribbon cable (C), PCB adapter (D) and (E) PCB containing the switching relays. Measurements were collected manually for two arrangements consisting of the impedance chip and card reader (A+B), as well as, impedance chip, card reader and ribbon cable (A+B+C). Automated measurements were acquired for the impedance chip and PCB containing the switching relays (A+E) as well as the impedance chip, card reader, ribbon cable, PCB adapter, and PCB containing the relays (A+B+C+D+E). A total of four different electrical connection configurations were tested.

Figure 4.8: The impedance platform connections can be broken down to five different components: the impedance chip (A), card reader (B), ribbon cable (C), PCB adapter (D) and (E) PCB containing the switching relays.

The data (Fig. 4.9 and 4.10) revealed that significant noise is presented at small frequencies (< 100 Hz) and electrical connections do contribute an observable parasitic behavior at high frequencies ($> \sim 1$ MHz). The parasitic behavior is even more evident in the phase shift plots. With each additional connection, the parasitic contribution increases as observed from measurements between configurations A+B and A+B+C, as well as,

A+E and A+B+C+D+E. This phenomena of parasitic behaviors have also been observed in the ECIS systems at frequencies above ~ 200 kHz [32], [99]. Although deducing the origin of the parasitic is difficult, it can sometimes be modeled as a capacitance in parallel within the equivalent circuit model to which the data is fitted. During testing it was also observed that the PCB with the switching relays introduces a valley in both the impedance magnitude and phase shift plots at 6 MHz. As a result, it was concluded that a frequency sweep of 100 Hz to 1 MHz is appropriate for future measurements in order to eliminate noise at small frequencies and the parasitic effects of at high frequencies.

D. Variations due to Fabrication

The impedance device was characterized to determine any variations caused during fabrication. These variations include differences among electrodes within a channel (Fig. 4.11), adjacent channels (Fig. 4.12) and other chips from the same substrate (Fig. 4.13). As in most of the experiments within this dissertation, impedance spectra were acquired at a frequency spectrum of 100Hz -1 MHz at 10 mV. Before acquiring data, cell culture medium (27 mS/cm) at room temperature was injected and allowed to equilibrate for 15 minutes before data was collected.

The Bode plots (Fig. 4.11-4.13) show that, for the most part, the impedance magnitude of the electrodes is the same and independent from their locations in the substrate. However, notable deviations come from the phase shift measurements. Variations increase between different chips (Table 4.2). This can be attributed to lack of uniformity of metal deposition during fabrication process.

Figure 4.9: Bode plot captured for all electrode sizes (50, 100, and 200 μm diameters) for three electrical configurations: the impedance chip and card reader (A +B), impedance chip, card reader and ribbon cable (A+B+C), and impedance chip and PCB containing the switching relays (A+E).

Figure 4.10: Bode plot captured for all electrode sizes (50, 100, and 200 μm diameters) for all four electrical configurations: (1) the impedance chip and card reader (A +B), (2) impedance chip, card reader and ribbon cable (A+B+C), (3) impedance chip and PCB containing the switching relays (A+E), and (4) the impedance chip, card reader, ribbon cable, PCB adapter, and PCB containing the relays (A+B+C+D+E).

Figure 4.11: Impedance magnitude and phase shift versus frequency of all electrodes within single channel of cell culture medium at room temperature. The error bars represent one standard deviation.

Figure 4.12: Impedance magnitude and phase shift versus frequency of all electrodes adjacent channels of cell culture medium at room temperature. The error bars represent one standard deviation.

Figure 4.13: Impedance magnitude and phase shift versus frequency of all electrodes from two different chips (from the same substrate) filled with cell culture medium at room temperature. The error bars represent one standard deviation.

	Range of Relative Standard Deviation%					
	200μm		100μm		50μm	
	Z (Ω)	Ø (°)	Z (Ω)	Ø (°)	Z (Ω)	Ø (°)
Same Channel	0.30-5.96	0.02-4.48	1.48-5.80	0.03-5.54	4.45-6.57	0.22-2.22
Adjacent Channels	1.19-8.38	0.25-5.07	2.95-14.03	0.45-7.99	3.96-16.78	0.39-5.52
Different Chips	7.15-14.54	0.52-8.22	3.15-14.79	0.40-7.45	4.44-12.32	0.46-3.86

Table 4.2: The range of relative standard deviation percentage of impedance magnitude and phase shift of electrodes within the same channel, adjacent channels, and different chips.

E. Solution Conductivity Effects

The impedance chips were characterized using different conductivity solutions including DI water, potassium chloride (KCl), and cell culture medium (Fig. 4.14). Table 4.3 shows the values of different conductivity solution at room temperature. As the solution conductivity (σ) increases the resistance plateau in the impedance magnitude plots shifts down and to right. This is to be expected since the solution resistance (spreading resistance for a circular plate $R_{sp}=1/(2d\sigma)$, where d is the diameter of the plate) for two electrodes in series has an inverse relationship with solution conductivity. This is also evident in the phase shifts where the plot peak shifts to higher frequencies with increasing solution conductivity. The phase shift plot shows slightly higher amplitude for higher conductivity solutions KCl D and KCl E but cell culture media has about the same amplitude as KCl A, B, and C. Despite having a higher conductivity, media has a similar double layer capacitance as the lower conductivity solution, KCl E. Lower conductivity solutions like DI water and KCl A hardly have a double layer capacitance, due to the low ion concentration in the solution. As the electrode diameter decreases, the frequency independent region becomes narrower, so much so that for the 50 μm diameter electrodes there is no notable resistance plateau. Also from these plots, it is observed that the electric double layer region shifts up for decreasing electrode area consistent with other [178]. With respect to the phase shifts plots, it minimizes the amplitude of curves closer to -90° indicative of capacitive behavior and significant interfacial impedance.

Solution	Conductivity, σ (mS/cm)
DI Water	0.006
KCl A	0.0243
KCl B	0.203
KCl C	2.16
KCl D	24.7
KCl E	59.1
KCl M	27.2
Diluted PBS	27.1
Cell Culture Media	27

Table 4.3: Solution conductivities at room temperature

Figure 4.14: Bode plots for (A) 200 μm , (B) 100 μm and (C) 50 μm electrodes in different conductivity solutions: DI water (0.006 mS/cm), KCl A (0.0243 mS/cm), KCl B (0.203 mS/cm), KCl C (0.203 mS/cm), KCl D (24.7 mS/cm), KCl E (59.1 mS/cm), and Media (27 mS/cm).

F. Flowrate Effects

The effects of flowrate on impedance measurements were characterized with the impedance platform. For these studies, chips were tested with three different solutions: potassium chloride (KCl M), PBS, and cell culture media. Both KCl M and PBS were adjusted to be of similar conductivity values as cell medium as presented in Table 4.3. Fig. 4.15 show normalized values ($|Z|_{flow}/|Z|_{static}$ and $\phi_{flow}/\phi_{static}$) of impedance magnitude and phase shift as function of frequency for the solutions mentioned above where the normalized values were calculated as the ratio of measurements under flow (17.6 dyne/cm²) to static conditions. From these plots, it is observed that fluid flow produces significant changes in both $|Z|$ and ϕ for solution KCl M but not for PBS and media. As mentioned in the previous section, this can be attributed to the lower concentrations of ions in media and PBS compared to KCl M. For KCl M solution measurements, fluid flow causes impedance and phase shift to increase. Under static conditions, ions settle near the electrode surface; as fluid flows, these ions are dispersed causing increments in impedance and phase shift. As the electrode diameter decreases the noise at lower frequencies is more prominent and, therefore, the flow effects are less pronounced due to increased interfacial impedance.

Figure 4.15: Normalized measurements ($|Z|_{\text{flow}}/|Z|_{\text{static}}$ and $\phi_{\text{flow}}/\phi_{\text{static}}$) of impedance magnitude and phase shift for (A) 200 μm , (B) 100 μm and (C) 50 μm diameter electrodes. The normalized values were ratio of measurements under flow (17.6 dyne/cm²) to static conditions for different solutions: KCl M (27.2 mS/cm), media (27 mS/cm), and PBS (27.1 mS/cm).

G. The pH Effects

pH effects were examined over time using the impedance platform. Chips were injected with cell culture media and placed in the environmental chamber. The conditions in the environmental chamber were set to be similar to an incubator with temperature set at 37°C and 85% humidity but in the absence of CO₂. Fig. 4.16 summarizes the

impedance spectra acquired over time for 200, 100, and 50 μm respectively. Impedance magnitude of media decreases over long periods of time. These changes are noticeable especially at the higher frequency values ($>10^4$ Hz). Phase shift plots also shift to higher frequencies with time, suggestive of a decrease in solution resistance. This behavior holds for all electrode sizes. This data is reiterated in Fig. 4.17 where normalized impedance as a function of time for two separate runs are portrayed. In one run, an impedance spectrum was acquired for a total of 14 hours, where the first 2.4 hours (first six initial runs) the CO_2 tank was closed and then opened and remained open until the termination of the experiment. The data shows that when CO_2 is turned off for 2.4 hours and then turned on again, impedance magnitude decreases. While, in the other run, the impedance magnitude for cell culture medium in the presence of CO_2 fluctuates slightly but remains stable. Exogenous CO_2 is used to buffer the cell culture media and minimize changes in the pH. This decrease in impedance can be indicative of pH changes in the cell culture media.

H. Bubble Effects

Another environmental condition that was characterized with the impedance platform was bubble generation. With perfused microfluidic cell cultures, it is vital to determine when bubbles form since bubbles have the ability to shear cells from the surface and disrupt the monolayer. For these experiments, aerated cell culture medium was flowed into the microchannels at 2.5 dyne/cm^2 and maintained under cell culture conditions for 13 hours. Fig. 4.18 displays a normalized impedance magnitude versus time acquired during the experiment. When the bubbles emerged the impedance magnitude spiked and caused peaks in the data. As the bubble (air pocket) replaces the

media from the surface of the electrode, the current path is blocked therefore increasing the measured impedance.

Figure 4.16: Bode plot of media measurements over time with (A) 200, (B) 100 and (C) 50 μm electrodes. Measurements were acquired at 37°C and 85% humidity.

Figure 4.17: Normalized impedance magnitude (with respect to $|Z|$ at $t=0$ hr) as a function of time for 200, 100, and 50 μm diameter where (A) CO_2 tank is turned on and off and where (B) CO_2 remains on.

Figure 4.18: (A) Normalized impedance magnitude (with respect to $|Z|$ at $t=0$ hr) as a function of time for 200, 100, and 50 μm diameter electrodes of aerated cell culture media flowing at 2.5 dyne/cm^2 . (B) Schematic of current path in the absence of bubbles and (C) the blocked current path in the presence of bubbles.

I. Sensitivity of the Monitoring Frequency

In an impedance biosensor identifying the frequency values and spectrum (i.e. the sensitivity of monitoring frequency) at which proper cell assessment is accomplished depends on the cell type under evaluation, the target of the analysis, and the sensor system itself. After the initial characterization with biologically relevant electrolyte solutions, the impedance platform was characterized with HUVECs to determine the sensitivity of the monitoring frequency. This was accomplished by acquiring complex impedance spectra (Fig. 4.19) of cell covered and cell-free electrodes and graphing the

normalized values (cell-covered to cell-free) of the impedance magnitude ($|Z|_{\text{cells}}/|Z|_{\text{cell-free}}$), phase shift ($\phi_{\text{cells}}/\phi_{\text{cell-free}}$), real ($Z'_{\text{cells}}/Z'_{\text{cell-free}}$) and imaginary impedance ($Z''_{\text{cells}}/Z''_{\text{cell-free}}$) as a function of frequency.

For these initial experiments HUVECs were obtained from PromoCell (Heidelberg, Germany, HUVEC single donor; Cat. No. C-1220) and cultured in Medium 200 (Life Technologies, Carlsbad, CA; Cat No. M-200-500) containing LSGS (Low Serum Growth Supplement, Life Technologies, Carlsbad, CA; Cat No. S-003-10) and 5% PSG (Penicillin-Streptomycin-Glutamine, Life Technologies, Carlsbad, CA; Cat No. 10378-016). HUVECs were subcultured up to four passages or less in T-25 flasks coated with a 0.2% gelatin solution and media exchanges were performed every 48 hours.

Prior to seeding HUVECs into channels, impedance chips were subjected to an AMI (acetone, methanol and isopropanol) cleaning and rinsed with DI water to remove any debris that may have remained from the fabrication process. The devices were sterilized in an isopropanol bath for at least 2 hours. They were then removed from the bath and allowed to dry inside a cell culture hood. The channels were rinsed with PBS three times and were injected with 100 $\mu\text{g/mL}$ fibronectin (Fibronectin from human plasma, Sigma-Aldrich, St. Louis, MO; Cat. No. F0895-1MG) [78]. The fibronectin was allowed to incubate for 1 hr at room temperature. Finally, the channels were prepped for cell inoculation and rinsed with fresh pre-warmed (37 °C) cell culture medium.

The control cell-free channel was infused with cell culture medium while the right channel was seeded with HUVECs at 80% confluency. After the fact, cells were cultured for an additional 48 hours to reach complete confluency and form tight junctions. During

this time, medium was replenished in both channels every 12 hours and prior to the beginning of an experiment. The chip was then transferred to an environmental chamber maintained at incubation conditions of 5% CO₂ at 37 °C and 85% relative humidity. After allowing the device to equilibrate to the environment for 15 minutes, the chip was inserted into a card reader connected to the automated data acquisition system. The impedance analyzer was calibrated to manufacturer's instructions to null the connection parasitics. Static readings were then acquired for a frequency spectrum of 100 Hz -1MHz at 10 mV.

Fig. 4.19 displays the impedance measurements for all electrode sizes (200, 100 and 50 μ m) for HUVEC-covered and cell-free electrodes and Fig. 4.20 illustrates the equivalent circuit model representing the microfluidic impedance platform and the cellular microenvironment. For the control cell-free electrodes, impedance spectra shows that at low frequencies (<10 kHz), impedance magnitude has a negative linear relationship with frequency and the values for phase shift are $\sim -85^\circ$ which characterizes the EDL capacitance (or *CPE*). At higher frequencies (>50 kHz), the impedance magnitude reaches a plateau while the phase shifts decreases, describing the resistive behavior of the cell culture medium (R_{MED}). Even though the behavior is similar for all electrode sizes, the discrepancies among electrode sizes must be noted. With decreasing electrode size, the plateau region pertaining to R_{MED} gets more narrow and sloped and the phase shift are larger. While the 200 μ m electrodes phase shift decreases to -30° , the 100 μ m increases to -50° , and, for the 50 μ m electrodes, it increases to only -70° . This is indicative of what was observed earlier, as the electrode area decreases, the capacitance at the electrode interface becomes more prominent.

When HUVECs are attached to the electrode, impedance magnitude increases at the mid frequency range (10 kHz- 400 kHz). Within this frequency range, a new plateau forms (1 kHz to 10 kHz) corresponding to the resistive behavior of the cell monolayer (R_{TER}) along with a new intermediate peak in the phase shift plot with values of -40° for 200 μm , -60° for 100 μm and -75° for 50 μm diameter electrodes. At higher frequencies (>10 kHz), the impedance magnitude decreases linearly with increasing frequency while the phase shift values grow to -70° for 200 μm , -80° for 100 μm and -80° for 50 μm diameter electrodes demonstrating the capacitive behavior of the cell membrane (C_C). Though this behavior pattern is common among all electrode sizes, it can be observed that the magnitudes do differ. As electrode diameter decreases, changes among HUVEC-covered and cell-free impedance magnitude and phase shift measurements decrease highlighting the capacitive effects due to the electrode interface impedance with decreasing electrode surface area.

Figure 4.19: Bode plots of HUVEC covered electrodes and control cell-free measurements for electrode diameters 200 μm (A), 100 μm (B) and 50 μm (C) . The left axis and solid plots pertain

to the impedance magnitude while the right axis and dashed line plots describe the phase shifts HUVECs and cell-free electrodes.

Figure 4.20: Equivalent circuit model for (A) control cell-free electrodes and (B) HUVEC-covered electrodes. (C) Illustration of the different electrical parameters on a Bode plot of a 200 μm electrode impedance spectrum of HUVECs and cell-free medium.

The sensitivity of sampled frequency values was determined by calculating normalized values of HUVEC to cell-free impedance measurements and plotting them against frequency (Fig. 4.21). The most sensitive probing frequency values will show the largest differences among HUVEC to cell-free measurements as peaks and valleys. Fig. 4.21A-C demonstrates that for normalized $|Z|$, the most sensitive frequency values are 24, 23 and 42 kHz for electrode diameters 200, 100, and 50 μm respectively. By looking at the Bode plots in Fig. 4.19 and Fig. 4.20C, it is observed that these values pertain to the transition point between R_{TER} and C_C . For normalized ϕ , the largest differences were observed at 4 and 165 kHz for 200 μm , 3 and 155 kHz for 100 μm , and 4 and 240 kHz for 50 μm electrodes where the smaller frequency characterizes the R_{TER} and the larger frequency characterizes R_{MED} .

Additionally, by plotting normalized values of real impedance (Z') (Fig. 4.21D-F), which represents the resistance of the cell monolayer [99], it is observed that the sensitive frequency values are 4.5, 3.7, and 6.5 kHz for electrode diameters 200, 100, and 50 μm respectively. These values with the exception of the 50 μm electrodes are similar to those obtained with the normalized ϕ plots. Normalized imaginary impedance (Z'') (Fig. 4.21D-F), corresponds to the capacitive behavior of the cell membranes [99] and is most significant at 57.5, 32.7 and, 30.2 kHz for 200, 100, and 50 μm electrode diameters respectively. Three observations were made from this data analysis. The first being that the most sensitive frequency values in the microfluidic impedance platform presented here are similar to those used in ECIS systems. According to Keese *et al.* [137], morphological and motility fluctuations were accurately monitored at 4 kHz, while cell membrane changes were captured more precisely measured

at 32 and 40 kHz for gold, 250 μm diameter electrodes [99]. The second observation made was that these values can be used to examine singular cell behavior parameters and improve temporal resolution. However, by probing cells across a frequency spectrum, several cell behavior parameters and how they change in conjunction with one another can be monitored. This data analysis further proves that for a comprehensive cell assessment where several parameters are of interest a spectrum versus selected few frequency values is a more suitable approach, as demonstrated in our case. The final observation was on electrode size based behavior. Though all electrode sizes do present a change between HUVEC-covered and cell-free measurements, the magnitude at which this change presents itself decreases with electrode size. Additionally, noise at low frequencies are more noticeable with smaller electrodes.

Figure 4.21: Normalized (A) impedance magnitude ($|Z|$, left axis, black), phase shift (ϕ , right axis, green), real impedance (Z' , left axis, blue), and imaginary impedance (Z'' , right axis, pink) as a function of frequency where normalized values were calculated from ratio of HUVEC

covered electrodes to control cell-free electrodes measurements for all electrode sizes (50, 100, 200 μm).

J. Effects of Electrical Parameters on Impedance Spectra

In an effort to understand impedance spectroscopy data and specifically how each electrical parameter affects the spectrum, simulated circuit fittings were performed. For this characterization portion, impedance spectra acquired from control cell-free electrodes and HUVEC covered electrodes were initially fitted to equivalent circuit models illustrated in Fig. 4.20 using a commercially available software ZView (Scribner Associates INC.). Substrate capacitances were extracted from the control cell-free spectrums. The substrate capacitances were then used and fixed for fits involving HUVEC-covered electrode measurements. The electrical parameter values extracted from this fit were used as median values for simulated fits. For the simulations, one individual electrical parameter was varied, while the other four were held constant. The values of the actual fit and simulations are all defined in Table 4.4.

The effects of each electrical parameter on the impedance spectra are plotted in Fig. 4.22 where the arrows indicate the increasing of said parameter. Even though the effects are shown for all electrode size diameters (200, 100 and 50 μm), the results discussed are primarily based on the observations obtained from the 200 μm diameter electrodes. For increasing electrical parameter $CPE-Y$ and $CPE-n$, both the $|Z|$ and ϕ decrease at lower frequency values. Whereas increasing values of R_{TER} produce an upwards and left (to lower frequencies) shift of the plateau in $|Z|$ and at the same time shifting ϕ plot to lower frequencies. Increments in C_c shift the hump in the $|Z|$ plot

toward the direction of lower frequencies and cause the ϕ to move to the right toward higher frequencies. Finally, increasing values of R_{MED} causes the plateau to elevate in the $|Z|$ plot at mid frequencies while decreasing ϕ down and slightly left to lower frequencies. Increasing R_{MED} also causes changes at higher frequencies shifting both the $|Z|$ and ϕ plot upwards. As far as electrode size-based behavior, as mentioned before it can be observed that the magnitude of the shifts decrease with decreasing electrode diameter.

diameter	200 μm					
Fit	Actual	Simulated				
	(----)	(---)	(---)	(---)	(---)	(---)
CPE-Y	6.19E-09	6.20E-11	6.2E-10	2.6E-09	4.6E-09	6.6E-09
CPE-n	0.89	0.83	0.86	0.92	0.95	0.98
R_{TER}	28056	7000	14000	28056	112000	224000
C_C	1.66E-10	1.7E-12	1.7E-11	6.7E-10	1.2E-09	1.7E-09
R_{MED}	4693	1200	2400	9400	18800	37600
C_s	1.68E-10	fixed				
R_{WIRE}	205.3	fixed				

diameter	100 μm					
Fit	Actual	Simulated				
	(----)	(---)	(---)	(---)	(---)	(---)
CPE-Y	1.2E-09	1.20E-11	1.2E-10	3.2E-09	5.2E-09	7.2E-09
CPE-n	0.91	0.85	0.88	0.94	0.97	1.00407
R_{TER}	118270	30000	60000	236000	472000	944000
C_C	7.9E-11	7.9E-13	7.9E-12	5.8E-10	1.1E-09	1.58E-09
R_{MED}	40408	10000	20000	80000	160000	320000
C_s	1.68E-10	fixed				
R_{WIRE}	205.3	fixed				

diameter	50 μm					
Fit	Actual	Simulated				
	(----)	(---)	(---)	(---)	(---)	(---)
CPE-Y	2.3E-10	2.30E-12	2.3E-11	2.2E-09	4.23E-09	6.23E-09
CPE-n	0.93	0.87	0.90	0.96	0.99416	1.02416
R_{TER}	251640	63000	126000	503000	1006000	2012000
C_C	4.2E-11	4.2E-13	4.2E-12	5.4E-10	1.04E-09	1.54E-09
R_{MED}	70483	17500	35000	140000	280000	560000
C_s	1.68E-10	fixed				
R_{WIRE}	205.3	fixed				

Table 4.4: Electrical parameter values for HUVEC-covered electrodes (*actual fit*) extracted from an equivalent circuit fit and the values used for simulations. For simulated fits, one electrical parameter was varied while the other four were held constant at the *actual fit* values.

Figure 4.22: Bode plots of HUVEC covered (black) electrodes, control cell-free (grey) electrodes and simulated impedance spectra (dashed) for varying values of electrical parameters $CPE-Y$ (A), $CPE-n$ (B), R_{TER} (C), C_C (D), and R_{MED} (E) for electrode sizes 200 (1), 100 (2) and 50 (3) μm . For simulated spectra, one electrical component was varied, while the others were held constant.

4.5 Conclusions

In this chapter, the microfluidic impedance platform's components and their design aspects were discussed in detail. It was shown that the platform incorporated

fluidic channels to mimic the arterial structure, size, and microenvironment, as well as, encompassed a control cell-free channel for the propose of monitoring environmental-conditions in the acquired data. The platform design also featured three different electrode diameters (200, 100 and 50 μm) in order to evaluate different sizes of subpopulations of endothelial cells within the microchannel. Initial design optimization experiments demonstrated that though impedance chips were fabricated from both silicon and glass-based substrates, glass substrate chips performed better due to their inherent higher impedance which minimized stray capacitances through the substrate itself. Consequently, the 50 μm diameter electrodes will be disregarded in any future analysis. Additionally, characterizations of the impedance platform with biologically relevant electrolyte solutions concluded that the 50 μm diameter electrodes (as fabricated in the chip described in this work) displayed both significant interface impedance and noise at lower frequency values making them unsuitable for the sensing application in this work. Investigations exploring parasitic contributions due to electrical connections concluded that each additional connecting component such as the ribbon cable and PCB with the switching relays do induce a parasitic component at high frequencies ($> 10^6$ Hz). As a result, the proper measuring frequency spectrum was established to 100 Hz –1 MHz in order to eliminate the parasitic effects. Variations in performance due the fabrication process were also explored and results showed that variations increase as the distance between electrode arrays and chips increase which can be attributed to the lack of uniformity of sputtering metal deposition. Likewise, the study on solution conductivity effects on impedance spectra determined that increasing solution conductivity decreases both impedance magnitude and phase shift. Analysis on flowrate effects on impedance

spectra indicated that fluid flow does causes an increase in impedance magnitude and phase shift at lower and mid frequencies ($<10^5$ Hz). However, these changes are more significant in an electrolyte solution of KCl versus PBS and cell culture medium. Additionally, observations on the effects due to pH changes and bubble effects revealed that changes in pH can be visualized as a decrease in impedance magnitude and phase shift whereas emergence of bubbles produced substantial increases in impedance magnitude.

Moreover, the microfluidic impedance platform was characterized with cultured HUVECs to study the sensitivity of monitoring frequencies. These experiments revealed that for the microfluidic impedance platform the resistance of the monolayer produces that most changes at 4 and 3 kHz for 200 and 100 μm diameter electrodes respectively whereas cell membrane capacitances produce the most changes at 57.5 and 32.7 kHz for 200 and 100 μm diameter electrodes respectively. Lastly, the effect of individual electrical parameters on impedance spectra were also examined and showed that for increasing $CPE-Y$ and $CPE-n$, both the $|Z|$ and ϕ decrease at lower frequency values. Whereas increasing values of R_{TER} produce an upwards and left (to lower frequencies) shift of the plateau in $|Z|$ and at the same time shifting ϕ plot to lower frequencies as well. Increments in C_c shift the hump in the $|Z|$ plot toward the direction of lower frequencies and cause the ϕ to move to the right toward higher frequencies. Whereas, increasing values of R_{MED} causes the plateau to elevate in the $|Z|$ plot at mid frequencies while decreasing ϕ down and slightly left to lower frequencies. Increasing R_{MED} also causes changes at higher frequencies shifting both the $|Z|$ and ϕ plot upwards.

CHAPTER 5

IN VITRO ANALYSIS OF HUVECS UNDER HYDRODYNAMIC SHEAR STRESS

5.1 *Introduction*

The effects of mechanical forces on endothelium function have been of specific interest in many investigations [1]–[6]. As blood flows through the circulatory system, endothelial cells are subjected to two mechanical forces: a normal pressure force and a tangential shear force. The vessel wall cushions the normal force, whereas the endothelial cells absorb all the shear stress [1]. As endothelial cells sense shear stress on the apical side, they transmit signals to the cytoskeleton by transmembrane mechanosensors located on the cell surface [7]. These biochemical signals act as communication avenues between the endothelium and the underlying smooth muscle cells, generating modifications in the arrangement and function of the endothelium and the vessel itself [4]. Generally, shear stress causes endothelial cells to adapt by re-orienting, aligning, and migrating in the direction of flow. These changes are often accompanied by significant modulations in the actin cytoskeleton [182] and barrier function which is regulated by cell-cell (adherent and tight junctions) [8] and cell-substrate junctions (cell adhesion molecules) [9][4]. VE-

cadherin junctions (adherent junction) have been specifically observed to be regulated by hydrodynamic shear stress [183]. Previous investigations have demonstrated that VE-cadherin complexes in static EC cultures form a thick, peripheral band [98] and in some areas form a three-dimensional lattice [184]. Once under laminar shear stress, HUVECs begin to form compact, linear cell-cell junctions which outline the periphery of the cells [183]. When the junctions are disrupted, the endothelium integrity is compromised which enables the passage of circulating foreign agents to the vascular wall and eventually leading to aforementioned pathophysiological processes.

Understanding the relationship between shear stress and the dynamic morphological responses of cells, in particular barrier function is of the utmost importance to gain a better prospective of the underlying mechanisms involved in endothelium pathology and how to treat and prevent them. *In vitro* flow models such as parallel plate flow chambers [15]–[18] and cone-plate apparatuses [19]–[23] have long been used for the purpose examining barrier function in endothelial cell cultures. These studies are complemented with measurements of secreted proteins, immunohistological stains, and gene expression levels *after* the cessation of shear stress exposure [24]–[30]. However, valuable information is lost in this approach since endothelial cells responses to shear stress vary from seconds to hours [16] and a real-time approach would be more appropriate for these investigations.

It has been shown that the behavior of cells cultured *in vitro* can be monitored in real-time via electrical impedance. Impedance systems have been used to measure a wide array of cellular properties such as permeability, changes in shape, toxicity, and cell behavior under flow [16], [20], [32], [34]–[36]. While it has been shown that

characterization of cell behavior is frequency dependent [37]–[41] many of the proposed impedance platform operate at a single frequency and others opt for several selected frequency values. Studies on frequency dependent cell behavior suggest that at lower frequencies current prefers to flow beneath the cells, through the intercellular junctions, and into the bulk medium resulting in access to information regarding barrier function [42]. At higher frequencies cell-matrix interactions and cell membrane integrity are probed since the current passes through the cell membrane and into the bulk medium [42]. Moreover, measurements acquired over a frequency spectrum can extract extensive monolayer data including interfacial and cell interactions.

On this basis, the microfluidic impedance platform introduced in the previous chapter was designed and utilized for the electrical assessment of HUVEC kinetics while undergoing fluid shear stress *in vitro*. The platform was used to capture the impedance spectra of HUVECs under static, constant shear (2.5 and 17.6 dyne/cm²), and stepped shear (23.7-35-58.1 dyne/cm²) conditions. The electrical parameters of static and sheared monolayers were extracted with an equivalent circuit model. The extracted electrical parameters were compared for shear-based behavior in different sized sub-populations of cells using the two different diameter electrodes (of 200 and 100 μm).

5.2 Material and Methods

A. Cell Culture

HUVECs were obtained from PromoCell (Heidelberg, Germany, HUVEC single donor; Cat. No. C-1220) and cultured in Medium 200 (Life Technologies, Carlsbad, CA;

Cat No. M-200-500) containing LSGS (Low Serum Growth Supplement, Life Technologies, Carlsbad, CA; Cat No. S-003-10) and 5% PSG (Penicillin-Strptomycin-Glutamine, Life Technologies, Carlsbad, CA; Cat No. 10378-016). HUVECs were subcultured to a maximum of four passages or less in T-25 flasks coated with a 0.2% gelatin solution and media exchanges were performed every 48 hours.

B. Experimental Setup

Prior to seeding HUVECs into channels, impedance chips were subjected to an AMI (acetone, methanol and isopropanol) cleaning and rinsed with DI water to remove any debris that may have remained from the fabrication process. The devices were sterilized in an isopropanol bath for at least two hours. They were then removed from the bath and allowed to dry inside the cell culture hood. The channels were rinsed with PBS three times and were injected with 100 $\mu\text{g/mL}$ fibronectin (Fibronectin from human plasma, Sigma-Aldarich, St. Louis, MO; Cat. No. F0895-1MG) [78] . The fibronectin was allowed to bond with the channel surfaces for one hour at room temperature. Following, the channels were rinsed with fresh pre-warmed (37 °C) cell culture medium.

The control cell-free (left) channel was infused with cell culture medium, the right channel was seeded with HUVECs at 80% confluency. Cells were cultured for an additional 48 hours to reach complete confluency and form tight junctions. During this time, medium was replenished in both channels every 12 hours and prior to beginning an experiment. After the 48 hours, tubing and tubing connectors were attached to the chip and connected to a peristaltic pump. Cultured cells were imaged with both an inverted and upright microscope with a 10X objective prior to being exposed to shear. The upright

microscope images were necessary to observe the cells at the electrode sites since the electrodes were not transparent. The chip and pump were transferred to an environmental chamber (Fig. 4.5) maintained at incubation conditions of 5% CO₂ at 37 °C and 85% relative humidity.

C. Automated Data Acquisition

After allowing the device to equilibrate to the environment for 15 minutes, the chip was inserted into a card reader connected to the automated data acquisition system. The impedance analyzer was calibrated to manufacturer's instructions to null the connection parasitics. Impedance spectra was collected at an applied potential of 10 mV and a frequency spectrum of 100 Hz- 1MHz. A total of 149 frequency values were sampled within this spectrum. Baseline static readings were acquired every 24 minutes for approximately one hour. For shear condition experiments, the peristaltic pump was turned on and cells were subjected to two constant shear stress conditions (2.5 and 17.6, dyne/cm²) and a stepped shear condition (23.7-35-58.1 dyne/cm²). For the stepped shear condition, cells were initially sheared at 23.7 dyne/cm² for two hours. Next, the cells were sheared at 35 dyne/cm² for an additional two hours, before the flow rate was increased so that cells were finally sheared at 58.1 dyne/cm² for the remaining 14 hours. This stepped approach was performed in order to avoid shocking the cells due to an aggressive acute flow and/or changing the extracellular matrix protein concentration used in the other cell studies.

Impedance spectra from each SE within the array were acquired approximately every 24 minutes for 14 hours; at which point, the pump was stopped and microscopy

images were taken to observe cell morphology after the application of shear stress. The entire experimentation procedure is summarized in Fig. 5.1.

D. Equivalent Circuit Analysis

Impedance measurements were interpreted with the use of equivalent circuit fits. In this work, a commercial software package, ZView (Scribner Associates INC., Southern Pines, NC), was utilized to extract the best-fit electrical parameters. This software uses a complex non-linear least-squares regression (refer to Appendix for a complete explanation of regression model) to fit the empirical data to the equivalent circuit models depicted in Fig. 4.20 for the entire frequency range (100 Hz- 1MHz) of impedance data.

Each electrical component in the circuit model is representative of a characteristic in the cell monolayer, measuring system, or environment. The model included a capacitance through the borosilicate glass substrate and passivation layer (C_s). R_{WIRE} represented the resistances in the electrical connections. R_{MED} accounted for the electrical resistance of the liquid cell culture medium. C_C measured the capacitance through the endothelial cell membranes. R_{TER} was the trans-endothelial resistance (TER) which describes the permeability between individual cells. Finally, Z_{CPE} designated the impedance of the constant phase element (CPE) which represented the interfacial impedance resulting from the electric double layer that forms at the electrode-electrolyte interface. Recalling from Chapter 3, the CPE is composed of two separate variables: Y which is a constant describing the magnitude of CPE (units are expressed in

Simens·(sec)ⁿ) and n which is a power factor ranging between 0 to 1 describing how closely the CPE resembles a capacitor ($n=1$) or resistor ($n=0$).

For each device and electrode dimension, the impedance acquired from the control electrodes were fitted to the equivalent circuit model in Fig. 4.20A and used to determine R_{MED} , Z_{CPE} , C_s and R_{WIRE} . For both electrode sizes, it was presumed that R_{WIRE} would remain constant, yet C_s would differ. This is in result of the interfacial impedance that smaller dimension electrodes experience, which may induce additional stray capacitance in the substrate and passivation layer. The values of C_s and R_{WIRE} attained from the control electrodes were fixed for subsequent model (Fig. 4.20B) fittings involving cells, while the other electrical parameters R_{MED} , Z_{CPE} , R_{TER} , and C_C were extracted. This approach was implemented because it was assumed that the resistances due to wires and electrical connections, in addition to the substrate and insulator layer capacitance were not to change with the presence of cells. The goodness of the circuit fit was determined by residual (relative errors, e_{rel}) calculations between the measured and fit parameter value for each single data point, as well as, the small weighted sum of squares due to error which is proportional to average error percentage calculated from the experimental and fitted data. Refer to the Appendix for a complete explanation on goodness of fit and respective relative errors of fits presented in this work.

For convenience in regards to the discussion of the electrical parameters for the different sized electrodes, the electrode diameter value being discussed will be added as subscript to the electrical component annotation (ex: $R_{TER,200}$).

E. Immunofluorescence Staining

HUVECs were stained within the microfluidic impedance chip for VE-cadherin (adherence junction), actin filaments and nuclei. To initiate the staining protocol, cells were fixed with 4% formaldehyde (Thermo Fisher Scientific, Waltham, MA; Cat. No. 28906) prepared in PBS. Subsequently, they were treated with a 0.05% Triton X-100 (Sigma-Aldrich, St. Louis, Mo; Cat. No. T8787) solution (prepared with the 4% formaldehyde) for two minutes and rinsed three times with PBS. This low concentration of Triton was utilized to avoid disintegration of transmembrane proteins. HUVEC monolayers were then incubated with rabbit anti-VE-Cadherin antibody (Abcam, Cambridge, MA; Cat. No. ab33168) at 1:50 ratio in 0.1% BSA (Bovine Serum Albumin, Sigma -Aldrich, St. Louis, Mo; Cat. No.) for 45 minutes at 37 °C. After this incubation period, chips were removed from the incubator and flushed with PBS for five minutes. A mixture of 1:50 secondary antibody (Goat anti-Rabbit IgG (H+L) Secondary Antibody, Alexa Fluor® 488 conjugate, Invitrogen; Cat. No. A-11034) and 1:40 of the actin stain, Rhodamine Phalloidin (Invitrogen; Cat. No. R415) in 0.1% BSA was injected into the channel cavities. Chips were then placed in the incubator once again for 45 minutes at 37 °C. After this incubation period, chips were removed from the incubator and flushed with PBS for five minutes. Finally, the nuclei of the cells were stained with 300 nM of DAPI (4',6-Diamidino-2-Phenylindole, Dihydrochloride, Invitrogen; Cat. No. D1306) in 0.1% BSA for three minutes at room temperature. After flushing cells with PBS for five minutes, cells were washed with DI water for an additional five minutes. Coverslips were mounted on the chips with FluorSave Reagent (Millipore, San Diego, CA; Cat. No. 345789) and imaged with a Nikon Confocal microscope.

F. Image Analysis

Cell-cell junction integrity was analyzed through the immunostaining of VE-cadherin of HUVEC cellular monolayers under static and shear conditions (2.5, 17.5, and 23.7-35-58.1 dyne/cm²). The expression of VE-cadherin was quantified through a modified image analysis protocol using ImageJ freeware (refer to the Appendix for protocol and image analysis examples). To initiate the analysis, the DAPI and VE-cadherin images of tested samples were processed using a Gaussian Blur filter in order to smoothen clusters of fluorescent pixels. This step enabled the ability to capture thins of expressed VE-cadherin that would go unrecognized by the software if otherwise. Once images were pixels were smoothed out, they were converted to binary images. The binary images of DAPI images were subtracted from the VE-cadherin images to eliminate any DAPI fluorescence in the VE-cadherin images. Subtracted images were inspected and if any nuclei signal remained, these were manually removed. Then, using the ellipse tool, the specified area of analysis (electrode area) was defined. The “Analyze Particles” command was selected to measure the binary images of VE-cadherin. This command basically scans the selected area until it detects the edge of VE-cadherin junctions. Using the wand tool, this command outlines the VE-cadherin junction and measures the area, grey value and integrated density value of selected pixels. Next, it fills measured object to make it undetectable until it completes processing the entire selected electrode area. The integrated density (mean fluorescence) values were summed up for each individual sample and normalized (divided) by the number cells (number of stained nuclei within the electrode surface) and electrode area (cm²).

G. Statistical Analysis

Statistical analysis was performed by one-way analysis of variance (ANOVA) to both the quantification of VE-cadherin and normalized R_{TER} . A value of $P < 0.05$ was considered statically significant. Tukey's honestly significant difference (HSD) was performed as a post hoc test to determine difference between sheared HUVEC samples.

Figure 5.1: Schematic of the entire experimental process involved when determining HUVEC kinetics under the influence of shear stress with the microfluidic impedance platform.

5.3 Results and Discussion

A. Impedance Spectra Analysis

Impedance spectra were simultaneously acquired for cell-free control and HUVEEC covered electrodes under static and shear conditions. Fig. 5.2 displays a typical averaged impedance spectrum for the 200 and 100 μm diameter electrodes containing no cells and those containing HUVECs under static conditions and after 20 min and 6 hours of fluid shear stress (17.6 dyne/cm^2). With the presence of a cell monolayer, the impedance magnitude at early frequencies (100 Hz -1 kHz) sits slightly above that of the control cell-free measurement. In the phase shift plot, the cell monolayer causes the phase shift to decrease (with respect to the cell-free measurement) at these early frequencies as well. This difference results from changes in the *CPE* (behavior of electric double layer) which can be attributed to cell attachment and thus the rearrangement of ionic charge near the electrode surface [185]. If we refer to Fig. 4.22, it can be observed that this change in the *CPE* can either be a decrease in the magnitude of the *CPE* (*CPE-Y*) and/or the power factor (*CPE-n*). Acquired measurement (Table 5.1 and 5.2) confirms that the change in *CPE* is mostly attributed to a reduction in *CPE-n*.

When cells attach impedance magnitude also increases significantly giving rise to a new resistive region (R_{TER}) at the mid frequency range of $\sim 1 - 500 \text{ kHz}$. Likewise, the phase shift plot experiences changes at this same mid frequency range. The phase shift caused by HUVECs is $\phi_{200} \sim -38^\circ$ and $\phi_{100} \sim -55^\circ$ and differs from that generated by cell culture medium alone at $\phi_{200} \sim -23^\circ$ and $\phi_{100} \sim -47^\circ$. This increase accounts for the cell membrane parameter which is capacitive in nature and usually generates phase shifts of $\phi \sim -90^\circ$. It must be noted that though the phase shift captured for HUVECs is greater than

that by cell culture medium alone for both 200 and 100 μm electrode diameters, the difference is much larger in the 200 μm diameter electrodes. This is a consequence of the higher interfacial impedance that exist in the smaller 100 μm diameter electrodes.

Similar to other studies of endothelial cells under flow, we observed that the onset of shear causes impedance to increase over the impedance of a static cell culture [16], [20], [123]. As time progressed, the impedance spectra began to decrease and fell below its static measurement. This is demonstrated further in Fig. 5.3-5.10, where the equivalent circuit parameters are plotted versus time.

B. Equivalent Circuit Analysis

i. Best fit of Circuit Parameters

Fitting the data to the circuit depicted in Fig. 4.20, R_{TER} , C_C , $CPE-Y$, $CPE-n$, and R_{MED} values were extracted. Fig. 5.2 shows a typical acquired (solid line) and fitted (dashed line) impedance spectra for control cell-free and HUVECs measurements. Fitted impedance spectra are shown to closely agree with the empirical impedance. The only visible deviation observed between fitted and measured values are in the phase shift plots, and in particular for the cell measurements. However, these deviations are not substantial since error percentages do not exceed 10% (please refer to the Appendix). for any of the calculated fitted values presented in this work as seen in Table 5.1 and 5.2

ii. Comparison of Specific Electrical Parameters

Table 5.1 and 5.2 show the extracted electrical parameters for both 200 and 100 μm electrode diameters. In these tables, values were normalized with respect to electrode

area in order to facilitate comparison among the different electrode sizes. By conventional methods, resistive electrical parameters were multiplied by the area of the electrode whereas the capacitive elements were divided by the electrode area. From these tables, changes in electrical parameters caused by both cell attachment and shear stress can be observed (discussed in the proceeding sections).

a. Cell Attachment Effects

Cell attachment on the electrode surface produces a decrease in the power factor of the constant phase element ($CPE-n$) between control cell-free and HUVEC (before shear) measurements. In this particular case, $CPE-n_{200}$ and $CPE-n_{100}$ decreases by 6% and 1% respectively. Additionally, the cell monolayer blocks the path of current resulting in an increase in the resistance of the cell culture medium (R_{MED}) from 0.501 and 0.275 $\Omega \cdot \text{cm}^2$ to 1.227 and 4.241 $\Omega \cdot \text{cm}^2$ for 200 μm and 100 μm electrodes respectively. Yet, the values for R_{MED} are still well below the values for the cell layer resistance (R_{TER}) due to the highly insulated nature of cell membranes. Previously, it was reported that R_{TER} values for confluent endothelial monolayers can range between 3 $\Omega \cdot \text{cm}^2$ to 1000 $\Omega \cdot \text{cm}^2$ [20], depending on cell type and passage, where R_{TER} decrease in later passages [41]. In previous investigations, HUVEC primary cultures have been measured at 14 ± 6 $\Omega \cdot \text{cm}^2$ [183]. For all experiments discussed in this document, HUVEC monolayers (passage 4 and 5) had initial $R_{TER,200}$ readings of 10-14 $\Omega \cdot \text{cm}^2$ and $R_{TER,100}$ of 7-10 $\Omega \cdot \text{cm}^2$.

b. Shear Stress Effects

At the early onset of shear stress (20 min after shear stress), the cell layer resistance (R_{TER}) increases and then falls below the static measurement after 6 hours of shear stress. For $R_{TER,200}$ and $R_{TER,100}$, shear stress increases cell layer resistance by 24% and 15% before it falls 37% and 17%, respectively, below their initial value at $t=0$ hour. On the other hand, cell membrane capacitance (C_C) experiences behavior opposite of R_{TER} as also seen in other impedance based endothelial cell investigations [37]. At the onset of shear stress, C_C decreases but then increases by the 6 hour mark. The onset of shear stress increases the magnitude of the constant phase element ($CPE-Y$) followed by decrease for a longer exposure time shear stress (6 hours).

c. Electrode Size Effects

Similar trends are observed for most of the electrical parameters (specifically $CPE-n$, R_{TER} , and C_C) acquired with 200 and 100 μm electrode diameters. However, two factors that fail to replicate the same behavior amongst electrode sizes are the magnitude of the constant phase element ($CPE-Y$) and the resistance of cell culture medium (R_{MED}). Fitted impedance spectra for 200 μm electrode indicate that $CPE-Y$ increases as a result of cell attachment. However, the $CPE-Y$ for 100 μm electrode decreases when the cell layer was added. In addition, at the early stages of applied shear stress, R_{MED} increases for both electrode sizes but by the 6 hour of shear stress $CPE-Y$ increases for the 200 μm electrode and decreases for the 100 μm electrode.

Another electrical parameter which was affected by the electrode dimensions is the capacitance through the substrate and passivation layer (C_S). C_S was found to be 0.535

$\mu\text{F}/\text{cm}^2$ for the 200 μm electrode, whereas C_S was found to be $2.127 \mu\text{F}/\text{cm}^2$ for the 100 μm electrode. A possible reason for this difference in C_S can be attributed to the higher interfacial impedance that exists for smaller electrodes. As current is constricted to a smaller surface area, some of the current path is blocked, thereby causing a stray capacitance in both the substrate and passivation layer of the impedance device.

Similar to C_S , derived C_c values are larger (~two folds larger) in 100 μm electrode compared to 200 μm electrode. For the 200 μm electrode, C_c is approximately $0.553 \mu\text{F}/\text{cm}^2$ which agrees with the value suggested by the literature for both the apical and basolateral membranes [20], [77], [106]. On the other hand, the 100 μm electrode measurements yielded an approximate C_c value of $0.994 \mu\text{F}/\text{cm}^2$ which is closer to the value of one layer of cell membrane.

iii. Comparison of Normalized Electrical Parameters

So far, specific electrical parameters have been examined between control cell-free and HUVECs measurements sheared at $17.6 \text{ dyne}/\text{cm}^2$. In this section, we expand the analysis to all the tested hydrodynamic shear stress levels 0, 2.5, 17.6, and 23.7-35-58.1 dyne/cm^2 . It must be noted that though all shear levels were tested, the 2.5 dyne/cm^2 shear condition resulted in sub-confluent cell layers and for the sake of simplicity, the findings are discussed in a separate section from the other shear conditions (Shear Condition: 2.5 dyne/cm^2). To compare all experimental trials at the different shear conditions, all the electrical parameter values were normalized with respect to the value ($t=0$) prior to the onset of hydrodynamic shear stress, also referred to as the baseline in

this document. This was performed for each data set collected. Average normalized parameters ($n=6$) were subsequently plotted versus time for static and constant shear conditions (Fig. 5.3-5.10) where the bars in the plots are representative of the standard error percentage of the different measurements.

Figure 5.2: An exemplary Bode plot of a measured (solid line) impedance spectrum and the corresponding impedance spectrum generated by the equivalent circuit fits (dashed line) for (A) 200 and (B) 100 μm diameter electrode. Impedance spectra is shown for control cell-free measurements (grey line), as well as, HUVEC measurements under shear conditions (17.6 dyne/cm^2) at different time points: before the onset of shear (pink line), 20 min after shear (green line), and 6 hours after shear (blue line). For raw data, refer to the Appendix.

Extracted Electrical Parameter	200 μm electrode diameter							
	Control		HUVECs					
	Cell- free	Error %	Before shear	Error %	20 min after shear	Error %	6 hr after shear	Error %
$R_{WIRE} (\Omega)$	205.3	2.98	205.3	–	205.3	–	205.3	–
$C_S (\mu\text{F}/\text{cm}^2)$	0.535	0.90	0.535	–	0.535	–	0.535	–
$CPE-Y (\mu\text{F}\cdot\text{s}^{n-1}/\text{cm}^2)$	15.80	0.49	23.27	3.53	23.84	3.06	21.48	3.29
$CPE-n$	0.951	0.05	0.896	0.49	0.901	0.89	0.902	0.44
$R_{MED} (\Omega\cdot\text{cm}^2)$	0.501	0.20	1.227	2.71	1.228	2.39	1.295	2.27
$R_{TER} (\Omega\cdot\text{cm}^2)$	–	–	11.69	1.29	14.50	0.89	7.37	1.37
$C_C (\mu\text{F}/\text{cm}^2)$	–	–	0.451	2.07	0.440	1.41	0.906	2.10

Table 5.1: An example of electrical parameters extracted from impedance spectra acquired with a 200 μm diameter electrode. The electrical parameters correspond to control cell-free measurements and HUVEC measurements under shear conditions (17.6 dyne/cm^2) at different time points: before shear ($t=0$ hr), 20 min after shear, and 6 hr after shear. Some parameters were normalized to the area of the electrode to facilitate value comparison among different electrode diameter sizes.

Extracted Electrical Parameter	100 μm electrode diameter							
	Control		HUVECs					
	Cell- free	Error %	Before shear	Error %	20 min after shear	Error %	6 hr after shear	Error %
$R_{WIRE} (\Omega)$	205.3	3.56	205.3	–	205.3	–	205.3	–
$C_S (\mu\text{F}/\text{cm}^2)$	2.127	0.38	2.127	–	2.127	–	2.127	–
$CPE-Y (\mu\text{F}\cdot\text{s}^{n-1}/\text{cm}^2)$	15.29	0.69	13.57	2.34	14.12	2.37	12.63	2.32
$CPE-n$	0.953	0.07	0.940	0.33	0.938	0.31	0.942	0.30
$R_{MED} (\Omega\cdot\text{cm}^2)$	0.275	0.38	4.241	2.55	4.612	2.77	3.487	2.37
$R_{TER} (\Omega\cdot\text{cm}^2)$	–	–	7.688	1.41	8.811	1.38	6.340	1.40
$C_C (\mu\text{F}/\text{cm}^2)$	–	–	1.751	2.89	1.503	3.17	2.258	2.73

Table 5.2: An example of electrical parameters extracted from impedance spectra acquired with a 100 μm diameter electrode. The electrical parameters correspond to control cell-free measurements and HUVEC measurements under shear conditions (17.6 dyne/cm²) at different time points: before shear ($t=0$ hr), 20 min after shear, and 6 hr after shear. Some parameters were normalized to the area of the electrode to facilitate value comparison among different electrode diameter sizes.

a. Resistance of cell monolayer, R_{TER}

The resistance of the monolayer (R_{TER}) captured with 200 μm and 100 μm electrodes are shown in Fig. 5.3A and B respectively. The onset of shear produces a brief increase in R_{TER} . For the 200 μm electrode size, R_{TER} increased by approximately 24% and 42% from the baseline value for shear stress conditions of 17.6 and 23.7-35-58.1 dyne/cm² respectively. After this initial increase, $R_{TER,200}$ for sheared HUVECs declines, eventually falling below static measurements and then stabilizing before beginning a slow ascend around $t=10$ hours. For the shear condition of 17.6 dyne/cm², $R_{TER,200}$ falls below the static measurements after 2.8 hours of shear stress and continues to decrease to a minimum (by 38% below baseline) around the sixth hour of shear exposure. The stepped shear stress condition of 23.7-35-58.1 dyne/cm² measurements decreased below the static measurements after 5.6 hours of shear stress and continues to decrease to a minimum (31% below baseline) around $t=8.4$ hours of shear exposure. After approximately ten hours of shear stress, $R_{TER,200}$ for both shear measurements begin to increase and end the experimental observation period at 28% and 5% below the baseline value for 17.6 and 23.7-35-58.1 dyne/cm² respectively.

Comparing R_{TER} measurements among electrode sizes, some similar trends do exist between the two different subpopulations in 200 μm and 100 μm electrode areas. Like in the 200 μm electrodes, the addition of shear stress also generated a jump in $R_{TER,100}$. Specifically, $R_{TER,100}$ increased by 15% and 36% from the baseline at the onset of shear stress conditions of 17.6 and 23.7-35-58.1 dyne/cm² respectively. After the initial jump in $R_{TER,100}$ due to the onset of shear stress, $R_{TER,100}$ decreased. Similar to measurements in the 200 μm electrodes, this decrease was observed at earlier time points

for the lower shear stress conditions 17.6 dyne/cm^2 . For HUVECs sheared at 17.6 dyne/cm^2 , $R_{TER,100}$ falls by approximately 41% to a minimum after 3.2 hours of shear stress and also fluctuates above and below static measurements for the remaining observation period. For HUVECs sheared at the stepped shear stress condition of $23.7\text{-}35\text{-}58.1 \text{ dyne/cm}^2$, $R_{TER,100}$ falls below the static measurement at $t= 6.8$ hours and continues to decrease by to a minimum of 50% to a minimum after 10 hours of shear stress before in begins to increase. At the end of experimental time, $R_{TER,100}$ show be the comparable for static and 17.6 dyne/cm^2 shear condition at 30% and 33% below baseline value. Whereas, $R_{TER,100}$ for the stepped shear condition $23.7\text{-}35\text{-}58.1 \text{ dyne/cm}^2$ was higher sitting only 19% below baseline value. Unlike the sheared HUVECs in both 200 and 100 μm electrodes, static recordings begin to decline after $t=0$ hour. Static $R_{TER,200}$ continue to decrease for the first two hours ($t=2 \text{ hr}$). At this point, the static $R_{TER,200}$ measurements fluctuate above and below a median value 26% below baseline value until the end of the experiment. For $R_{TER,100}$, the static recordings decreased to 59% of the baseline at 4.4 hours and is followed by an increase for two hours where it stabilizes at a median value 30% below the baseline.

Fluctuations in R_{TER} for sheared and static cellular monolayers on 200 μm electrodes are subtle but increase significantly with decreasing electrode diameter. For static cell cultures, the fluctuations can be indicative of cell migration in zig-zag movements. For sheared cell cultures, theses fluctuations are representative of cell locomotion and cell re-orientation in the direction of flow [20], [61].

Figure 5.3: Normalized plots of the cell layer resistance (R_{TER}) with respect to time collected with (A) 200 and (B) 100 μm diameter electrodes. R_{TER} was recorded for HUVECs under static, constant shear (17.6 dyne/cm^2), and a stepped shear condition (23.7-35-58.1 dyne/cm^2). For the stepped shear condition, HUVECs were initially sheared at 23.7 dyne/cm^2 for 2 hours ($t=0-2\text{ hr}$). Subsequently, HUVECs were sheared at 35 dyne/cm^2 ($t=2-4\text{ hr}$) for an additional 2 hours. Finally, they were sheared at 58.1 dyne/cm^2 for 14 hours ($t=4-18\text{ hr}$). In these plots, the changes

in shear stress for the stepped shear condition are indicated by the orange triangle markers. Error bars are representative of the standard error.

b. Cell membrane capacitance, C_C

The cell membrane capacitance (C_C) parameter has been used in the past to monitor cell attachment and cell confluency in other impedance systems. The variations observed in this parameter can therefore be indicative of how cells are detaching and re-orienting in the direction of flow. Fig. 5.4A and B portray the captured C_C with respect to time in the microfluidic impedance platform. The onset of shear decreases $C_{C,200}$ by 3% and 16% for 17.6 and 23.7-35-58.1 dyne/cm² respectively. This momentary decrease is immediately followed by a slow rise in $C_{C,200}$ for HUVECs under static and sheared states. Under static conditions, $C_{C,200}$ reaches a maximum value two-fold from the baseline value by $t=4$ hour before it sharply falls and stabilizes to 18% above the baseline around $t=6.4-10$ hours. After the tenth hour marker, the static measurement begins to increase for two hours, where $C_{C,200}$ is 47% above the baseline value and remains at this value until the end of the experiment. At 17.6 dyne/cm², $C_{C,200}$ increases after the onset of shear and reaches a peak value two times greater than the baseline value at $t=6$ hours. Similar to static conditions, this increase is accompanied by sudden decrease reaching 26% above the baseline value at $t=8.4$ hours. This stable period is short lived for two more recordings before $C_{C,200}$ began to increase again reaching another maximum 68% above the baseline at $t=10.4$ hours. At that point, $C_{C,200}$ for HUVECs sheared at 17.6 dyne/cm², decreased to above 10% above the baseline value by 12.8 hours and until the end of the trial. For the stepped sheared condition, it can be observed that at the onset of a new shear condition is accompanied by a slight dip in $C_{C,200}$. After the beginning the

shear condition of 58.1 dyne/cm², $C_{C,200}$ ascends to a maximum value 35% above the baseline value and subsequently slowly decreases to stable value 14% below the baseline value after 14 hours of shear stress. An additional rise is observed in $C_{C,200}$ under this shear condition is observed around $t=16.8$ hours and then stabilizes 4% above the baseline value. As in $C_{C,200}$, $C_{C,100}$ are lower for the stepped shear condition 23.7-35-58.1 dyne/cm² and higher for the static and 17.6 dyne/cm² shear condition.

c. Constant phase element magnitude, $CPE-Y$ and power factor, $CPE-n$

Normalized CPE parameters represent the electric double layer at the interface of the electrode and media/cells and has been correlated with cell density distribution [186]. For this parameter (Fig. 5.5 and 5.6), it is visible there is no discernible change in the parameter due to the onset of shear stress. At $t=0$ hr, $CPE-Y_{200}$ for both static and 17.6 dyne/cm² initiated a descent. Around the fourth hour, the static $CPE-Y_{200}$ jumps and increases, reaching a maximum value 14% above the baseline by $t=6.4$ hr and decreases (by 5%) for the next 2 hours. At that point, the static $CPE-Y_{200}$ begins to increase until its 9% greater than the baseline by $t=14$ hr. Similar to static measurements, HUVECs sheared at 17.6 dyne/cm² generate an increase in $CPE-Y_{200}$ at the fourth hour which is continued by a stepwise increase until reaching a maximum of 2% above the baseline value. This is followed by a decline and $CPE-Y_{200}$ for the 17.6 dyne/cm² shear condition reaches a minimum of 86% of the baseline value by the end of the experiment. For the step shear condition 23.7-35-58.1 dyne/cm², the onset of a new shear condition is proceeded by a decrease in $CPE-Y_{200}$. At $t=4$ hr, $CPE-Y_{200}$ begins to slowly descend and reaches a fluctuating minimum of 16% below the baseline value at 6.8 hours and remains

around this value for the remaining observation period. For $CPE-Y$ at 100 μm disks, similar trends are observed however the fluctuations are amplified. Like $CPE-Y_{200}$, $CPE-Y_{100}$ for static measurements increases at the fourth hour while sheared $CPE-Y_{100}$ settle below to values 6% less than the baseline recording.

For both 100 and 200 μm electrodes, $CPE-Y$ for sheared cells lies below the non-sheared cells at the later time points. $CPE-n$ values (Fig. 5.6A and B) have mirrored opposite trend to $CPE-Y$ values for both 100 and 200 μm diameter disks. For sheared monolayers, $CPE-n$ values were above the static recordings after $t=4$ hr and $t=6$ hr for 200 and 100 μm diameters electrodes respectively.

Interestingly, $CPE-Y$ values extracted from control cell-free measurements shown in Fig. 5.7 A and B indicate a difference not only at the onset of shear but also as a result of shear condition. Normalized $CPE-Y$ for shear conditions decreased at the initiation of flow while static measurements remain near the baseline value. For culture media flowing at 17.6 dyne/cm^2 , $CPE-Y_{200}$ and $CPE-Y_{100}$ decreased by 0.05% and 0.03% at the end of the experimental trial. For culture media flowing at 23.7-35-58.1 dyne/cm^2 , $CPE-Y_{200}$ and $CPE-Y_{100}$ decreased by 0.1% and 0.06%, respectively, at the end of the experimental trial. Similar to the $CPE-Y$ extracted from experiments involving cell monolayers, static values lay above the shear condition values.

$CPE-n$ values extracted from control cell-free measurements (Fig 5.8A and B) of 200 μm electrodes, mirror the opposite $CPE-Y_{200}$ trend. Yet, this is not case for $CPE-n_{100}$. Control cell-free $CPE-n_{200}$ values for sheared cell culture medium are above static measurements. For $CPE-n_{100}$, there noticeable small fluctuation in the measurements and specifically for shear conditions. Though $CPE-n_{100}$ values remain around the baseline for

all flow conditions, around the $t=8$ hr, $CPE-n$ values for 23.7-35-58.1 dyne/cm², begin to decline and decrease to 0.003% below the baseline value by the end of the experimental trial.

d. Resistance of cell culture medium, R_{MED}

In previous impedance systems, changes in the resistance of cell culture medium are indicative of temperature changes and environmental changes [41]. R_{MED} values captured with our system indicate that there are no changes in R_{MED} triggered by shear stress effects. Fig. 5.9A show that $R_{MED,200}$ values began to decrease at $t=0$ hr for static and shear conditions (23.7-35-58.1 dyne/cm²), yet remaining stable around the baseline value for HUVECs at 17.6 dyne/cm². $R_{MED,200}$ decreased to a minimum value of 90% for 23.7-35-58.1 dyne/cm² and static conditions. For $R_{MED,100}$, measurements fluctuate significantly. For these measurements, all conditions experience an initial transient jump trailed by a decrease until the forth hour. At which in time, $R_{MED,100}$ for static condition continues to decrease by 58% by the end of the experiment. At $t=4$ hr, $R_{MED,100}$ measurements begin to ascend fluctuating by 13% above and 37% below that baseline for 17.6 dyne/cm². While measurements for 23.7-35-58.1 dyne/cm² condition, fluctuate between 6% above and 31% below the baseline value. Distinct from $R_{MED,200}$ for the 23.7-35-58.1 dyne/cm² condition which lies below the 17.6 dyne/cm² measurements as so does the static measurement, $R_{MED,100}$ for the 23.7-35-58.1 dyne/cm² condition climbs above the 17.6 dyne/cm² and static measurements after 12 hours of shear stress.

For comparison, R_{MED} values (Fig. 5.10A and B) extracted from control cell-free measurements are also shown. It can be observed that $R_{MED,200}$ derived from cell

experiments agree with $R_{MED,200}$ and $R_{MED,100}$ extracted from control cell-free measurements. Though the differences between the shear conditions are smaller, the trend is the same where values for shear condition 17.6 dyne/cm² are slightly above static and 23.7-35-58.1 dyne/cm² condition. It must be noted that there is a substantial discrepancy between $R_{MED,100}$ derived from cell experiments and $R_{MED,100}$ extracted from control cell-free measurements. While $R_{MED,100}$ derived from cell experiments present fluctuations about the baseline, $R_{MED,100}$ extracted from control cell-free measurements remained constant about the baseline.

R_{MED} values (Fig. 5.10A and B) extracted from control cell-free measurements show values remain close to the baseline for all shear conditions. Around the t=8 hour and t=6 hour, static $R_{MED,200}$ and $R_{MED,100}$ measurements decrease slightly by 0.07% and 0.08% respectively. It is presumed this decrease for static measurements resulted from an increase in the cell culture media conductivity induced by evaporation. Unlike the shear conditions, static cell culture media remained stagnant increasing the possibility of exchange between the solution and incubator atmosphere.

Figure 5.4: Normalized plots of the cell membrane capacitance (C_c) with respect to time collected with (A) 200 and (B) 100 μ m diameter electrodes. C_c was recorded for HUVECs under static, constant shear (17.6 dyne/cm²), and a stepped shear condition (23.7-35-58.1 dyne/cm²). For the stepped shear condition, HUVECs were initially sheared at 23.7 dyne/cm² for 2 hours ($t=0-2$ hr). Subsequently, HUVECs were sheared at 35 dyne/cm² ($t=2-4$ hr) for an additional 2 hours. Finally, they were sheared at 58.1 dyne/cm² for 14 hours ($t=4-18$ hr). The changes in shear stress for the stepped shear condition are indicated by orange triangle markers. Error bars are representative of the standard error.

Figure 5.5: Normalized plots of the magnitude of the constant phase element ($CPE-Y$) with respect to time collected with (A) 200 and (B) 100 μm diameter electrodes. $CPE-Y$ was recorded for HUVECs under static, constant shear (17.6 dyne/cm^2), and a stepped shear condition ($23.7\text{-}35\text{-}58.1 \text{ dyne/cm}^2$). For the stepped shear condition, HUVECs were initially sheared at 23.7 dyne/cm^2 for 2 hours ($t = 0\text{-}2 \text{ hr}$). Subsequently, HUVECs were sheared at 35 dyne/cm^2 ($t = 2\text{-}4 \text{ hr}$) for an additional 2 hours. Finally, they were sheared at 58.1 dyne/cm^2 for 14 hours ($t = 4\text{-}18 \text{ hr}$). The changes in shear stress for the stepped shear condition are indicated by orange triangle markers. Error bars are representative of the standard error.

Figure 5.6: Normalized plots of the power factor of the constant phase element ($CPE-n$) with respect to time collected with (A) 200 and (B) 100 μ m diameter electrodes. $CPE-n$ was recorded for HUVECs under static, constant shear (17.6 dyne/cm²), and a stepped shear condition (23.7-35-58.1 dyne/cm²). For the stepped shear condition, HUVECs were initially sheared at 23.7 dyne/cm² for 2 hours ($t=0-2$ hr). Subsequently, HUVECs were sheared at 35 dyne/cm² ($t=2-4$ hr) for an additional 2 hours. Finally, they were sheared at 58.1 dyne/cm² for 14 hours ($t=4-18$ hr). The changes in shear stress for the stepped shear condition are indicated by orange triangle markers. Error bars are representative of the standard error.

Figure 5.7: Normalized plots of the magnitude of the *CPE* (*CPE-Y*) with respect to time collected with (A) 200 and (B) 100 μm diameter electrodes. *CPE-Y* was recorded for control cell-free electrodes under static, constant shear (17.6 dyne/cm^2), and a stepped shear condition ($23.7\text{-}35\text{-}58.1 \text{ dyne/cm}^2$). For the stepped shear condition, cell culture medium was set to stress shear of 23.7 dyne/cm^2 for 2 hours ($t=0\text{-}2 \text{ hr}$). Subsequently, cell culture medium was sheared at 35 dyne/cm^2 ($t=2\text{-}4 \text{ hr}$) for an additional 2 hours. Finally, shear conditions were altered to 58.1 dyne/cm^2 for 14 hours ($t=4\text{-}18 \text{ hr}$). The changes in shear stress for the stepped shear condition are indicated by orange triangle markers. Error bars are representative of the standard error.

Figure 5.8: Normalized plots of the power factor of the CPE ($CPE-n$) with respect to time collected with (A) 200 and (B) 100 μ m diameter electrodes. $CPE-n$ was recorded for control cell-free electrodes under static, constant shear (17.6 dyne/cm²), and a stepped shear condition (23.7-35-58.1 dyne/cm²). For the stepped shear condition, cell culture medium was set to stress shear of 23.7 dyne/cm² for 2 hours ($t=0-2$ hr). Subsequently, cell culture medium was sheared at 35 dyne/cm² ($t=2-4$ hr) for an additional 2 hours. Finally, shear conditions were altered to 58.1 dyne/cm² for 14 hours ($t=4-18$ hr). The changes in shear stress for the stepped shear condition are indicated by orange triangle markers. Error bars are representative of the standard error.

Figure 5.9: Normalized plots of the resistance of the cell culture medium (R_{MED}) with respect to time collected with (A) 200 and (B) 100 μm diameter electrodes. R_{MED} was recorded for HUVECs under static, constant shear (17.6 dyne/cm²), and a stepped shear condition (23.7-35-58.1 dyne/cm²). For the stepped shear condition, HUVECs were initially sheared at 23.7 dyne/cm² for 2 hours ($t = 0-2$ hr). Subsequently, HUVECs were sheared at 35 dyne/cm² ($t = 2-4$ hr) for an additional 2 hours. Finally, they were sheared at 58.1 dyne/cm² for 14 hours ($t = 4-18$ hr). The changes in shear stress for the stepped shear condition are indicated by orange triangle markers. Error bars are representative of the standard error.

Figure 5.10: Normalized values of the resistance of the cell culture medium (R_{MED}) with respect to time collected with (A) 200 and (B) 100 μm diameter electrodes. R_{MED} was recorded for control cell-free electrodes under static, constant shear (17.6 dyne/cm^2), and a stepped shear condition (23.7-35-58.1 dyne/cm^2). For the stepped shear condition, cell culture medium was set to stress shear of 23.7 dyne/cm^2 for 2 hours ($t=0-2 \text{ hr}$). Subsequently, cell culture medium was sheared at 35 dyne/cm^2 ($t=2-4 \text{ hr}$) for an additional 2 hours. Finally, shear conditions were altered to 58.1 dyne/cm^2 for 14 hours ($t=4-18 \text{ hr}$). The changes in shear stress for the stepped

shear condition are indicated by orange triangle markers. Error bars are representative of the standard error.

e. Shear Condition: 2.5 dyne/cm²

Cultured HUVECs were also monitored while undergoing hydrodynamic shear stress of 2.5 dyne/cm². However, at this shear condition, the fluid flow rate was low and resulted in some bubble generation within the flow system. Large bubbles in turn caused cells to shear off developing subconfluent cell monolayers. Fig. 5.11–5.13 compile the normalized parameters pertaining to the shear conditions 2.5 dyne/cm² with the other shear conditions (static, 17.6 and 23.7–35–58.1 dyne/cm²). It must be noted that unlike the other fitted data derived from the previously discussed shear conditions, error % resulting from 2.5 dyne/cm² experimental trials ranged between 15– 32 % , especially toward that the end of the experiment when monolayer was significantly subconfluent.

Like the other shear conditions, 2.5 dyne/cm² also produced an increase in the R_{TER} values (Fig. 5.11) after the onset of shear stress. However, the changes are quite small only 1% and 7% increase in R_{TER} for 200 and 100 μm electrode diameters, respectively. This initial jump in R_{TER} is superseded by a decline where R_{TER} for both electrode sizes fall below all the other shear and static conditions. Perhaps one the most interesting observations in these R_{TER} plots is an actual bubble obstruction at 5.2 hour, where a spike in the resistance of the cell layer was registered for both electrode sizes. Not only is the bubble disturbance observed in R_{TER} , but it can be observed in C_C (Fig. 5.12), R_{MED} (Fig. 5.13), $CPE-n$ (included in Appendix), and control cell-free R_{MED} (included in Appendix) but it was not identified in $CPE-Y$ (included in Appendix) parameter. For HUVECs sheared at 2.5 dyne/cm², $C_{C,200}$ increases five-fold and $C_{C,100}$

increases two and a half fold the baseline value throughout the experiment. As mentioned in the background section, subconfluent cell populations are usually accompanied by large increases in cell membrane capacitance.

C. Image Analysis

HUVECs were imaged immediately prior and after the experimental trial to document morphological changes. Fig. 5.14 portrays the bright field images acquired with the both the inverted and upright microscopes with a 10X objective. After cell inoculation and proliferation, an approximate average of 20 and 6 HUVECs adhered to the electrode surface for the 200 μm and 100 μm diameter electrodes, respectively. For static conditions, HUVECs arranged themselves in a cobblestone configuration where cells have a random orientation (Fig. 5.14A and D). Once cells were sheared, the cell borders were more clearly defined. Cells sheared at 17.6 dyne/cm^2 for 14 hours (Fig. 5.14 B and E) were elongated and rearranged so that their long axes were parallel to the direction of flow. HUVECs sheared under the stepped condition 23.7-35-58.1 dyne/cm^2 (Fig. 5.14C and F), were also converted to a stretched ellipsoidal morphology. However, cells exposed to this shear condition do not consistently align in the direction of flow like HUVECs under 17.6 dyne/cm^2 . Instead cells subjected to 23.7-35-58.1 dyne/cm^2 shear condition, have areas where cells are organized in a certain direction while neighboring areas show a different orientation. For the 200 μm electrode (Fig. 5.14C1 and C2), HUVECs at the leading edge have two different orientations, one which is perpendicular to flow and the other which is where cells are angled towards the direction of shear stress. At the trailing edge, two HUVEC groups are angled in opposite directions but both are

orient in the direction of flow. Cells at the electrode surface are also mostly angled at 45 degrees towards fluid flow, with one cell oriented perpendicular to flow at the center. For the 100 μm electrode (Fig. 5.14 F1 and F2), HUVECs at the leading edge are arranged mostly perpendicular to flow. At the trailing edge, HUVECs are oriented in the direction of flow. Cells at the electrode surface are also mostly angled at -45 degrees towards the direction flow. Immunofluorescence staining of F-actin and DAPI (Fig. 5.15-18) further portray the above described morphology where the direction F-actin fibers and nuclei mimic cell direction. According to previous investigations, endothelial cells under laminar shear stress undergo significant cytoskeleton remodeling. Shear stress activates the increase and re-distribution of F-actin fibers from cell borders to the center of the lateral cell membranes [182].

Cell-cell junctions were analyzed through immunofluorescence staining of VE-cadherin for HUVECs in the different shear conditions (Fig. 5.15-5.18). Under static condition, VE-cadherin complexes from the three dimensional lattices connected to linearized junctions. As cells were sheared at 17.6 and 23.7-35-58.1 dyne/cm^2 , VE-cadherin formed compact lines of cell-cell junctions, however, they were more linearized for the 17.6 dyne/cm^2 . The expression VE-cadherin was quantified by the mean fluorescence (also known as the integrated density value, I.D.V.) and plotted as a function of shear stress in Fig. 5.19. Different normalizations of the mean fluorescence were portrayed to identify that the trend with respect to electrode area and cell number. Results indicate that for the 200 μm electrodes, the shear condition of 23.7-35-58.1 dyne/cm^2 has the highest expression of VE-cadherin, followed by HUVECs monitored under static conditions and trailed by HUVECs sheared at 17.6 dyne/cm^2 . For the 100 μm

electrodes, the static, and then 23.7-35-58.1 dyne/cm² shear conditions have the highest expression of VE-cadherin, followed by HUVECs sheared at 17.6 dyne/cm².

i. Shear Condition: 2.5 dyne/cm²

Images (Fig. 5.20) were also acquired for HUVECs sheared at 2.5 dyne/cm². As seen previously, under static conditions, HUVECs had a cobblestone morphology (Fig. 5.20A and D). After experiencing 14 hours of hydrodynamic shear at 2.5 dyne/cm² where bubbles emerged, the HUVEC monolayer was observed to be subconfluent (Fig. 5.20-B, C, E, and F). HUVEC morphology had also transitioned to an ellipsoidal shape, where the cells were elongated in the direction of flow. Zoomed images of the sensing electrodes portray that the number of cells attached to electrode had decreased. For the 200 µm electrodes (Fig. 5.20-C.2), cells had decreased from approximately 20 to 6. In these electrodes, cells appeared to have stretched out significantly, where the 6 cells covered about 70% of the total electrode surface area. For the 100 µm electrodes (Fig. 5.20-F-2), only three partial cells covered the perimeter of the electrode. Cells on this electrode were slightly angled to a -45 °angle unlike cells on the 200 µm electrodes, which were angled parallel to the direction of flow for the most part. In both these images, it can be observed that the presence of VE-cadherin was significantly reduced and discontinuous compared to the static and the other shear conditions tested in this project.

Figure 5.11: Normalized plots of the cell layer resistance (R_{TER}) with respect to time collected with (A) 200 and (B) 100 μ m diameter electrodes. R_{TER} was recorded for HUVECs under static, constant shear (2 and 17.6 dyne/cm²), and a stepped shear condition (23.7-35-58.1 dyne/cm²). For the stepped shear condition, HUVECs were initially sheared at 23.7 dyne/cm² for 2 hours ($t=0-2$ hr). Subsequently, HUVECs were sheared at 35 dyne/cm² ($t=2-4$ hr) for an additional 2 hours. Finally, they were sheared at 58.1 dyne/cm² for 14 hours ($t=4-18$ hr). The changes in shear stress for the stepped shear condition are indicated by orange triangle markers. Error bars are representative of the standard error.

Figure 5.12: Normalized plots of the cell membrane capacitance (C_C) with respect to time collected with (A) 200 and (B) 100 μm diameter electrodes. C_C was recorded for HUVECs under static, constant shear (2 and 17.6 dyne/cm²), and a stepped shear condition (23.7-35-58.1 dyne/cm²). For the stepped shear condition, HUVECs were initially sheared at 23.7 dyne/cm² for 2 hours ($t = 0\text{-}2\text{ hr}$). Subsequently, HUVECs were sheared at 35 dyne/cm² ($t = 2\text{-}4\text{ hr}$) for an additional 2 hours. Finally, they were sheared at 58.1 dyne/cm² for 14 hours ($t = 4\text{-}18\text{ hr}$). The changes in shear stress for the stepped shear condition are indicated by orange triangle markers. Error bars are representative of the standard error.

Figure 5.13: Normalized plots of the resistance of the cell culture medium (R_{MED}) with respect to time collected with (A) 200 and (B) 100 μm diameter electrodes. R_{MED} was recorded for HUVECs under static, constant shear (2 and 17.6 dyne/cm^2), and a stepped shear condition (23.7-35-58.1 dyne/cm^2). For the stepped shear condition, HUVECs were initially sheared at 23.7 dyne/cm^2 for 2 hours ($t = 0\text{-}2\text{ hr}$). Subsequently, HUVECs were sheared at 35 dyne/cm^2 ($t = 2\text{-}4\text{ hr}$) for an additional 2 hours. Finally, they were sheared at 58.1 dyne/cm^2 for 14 hours ($t = 4\text{-}18\text{ hr}$). In these plots, the changes in shear stress for the stepped shear condition are indicated by the orange triangle markers. Error bars are representative of the standard error.

Figure 5.14: Bright field images acquired with a 10X objective of HUVECs under (A.1) and (D.1) static, and shear conditions of (B.1 and E.1) 17.6 , and (C.1 and F.1) 23.7-35-58.1 dyne/cm² for 200 and 100 µm electrode diameters. Zoomed upright microscope images of cells on electrode surfaces under (A.2) and (D.2) static , and shear conditions of (B.2 and E.2) 17.6 , and (C.2 and F.2) 23.7-35-58.1 dyne/cm² for 200 and 100 µm electrode diameters.

Figure 5.15: Fluorescent images of HUVECs on 200 μm diameter electrodes under (A) static, and shear conditions of (B) 17.6, and (C) 23.7-35-58.1 dyne/cm² where (1) is VE-cadherin, (2) is F-actin and (3) is a merged image showing VE-cadherin, F-actin, and DAPI.

Figure 5.16: Fluorescent images of HUVECs on 200 μm diameter electrodes under (A) static, and shear conditions of (B) 17.6 , and (C) 23.7-35-58.1 dyne/cm² where (1) is VE-cadherin, (2) is F-actin and (3) is a merged image showing VE-cadherin, F-actin, and DAPI.

Figure 5.17: Morphological arrangement of HUVECs on glass under (A) static conditions, as well as, for 200 μm diameter electrodes under (B) static, and shear conditions of (C) 17.6 , and (D) 23.7-35-58.1 dyne/cm² where (1) is VE-cadherin , (2) is F-actin and (3) is a merged image showing VE-cadherin, F-actin , and DAPI.

Figure 5.18: Morphological arrangement of HUVECs on glass under (A) static conditions, as well as, for 100 μm diameter electrodes under (B) static, and shear conditions of (C) 17.6, and (D) 23.7-35-58.1 dyne/cm² where (1) is VE-cadherin , (2) is F-actin and (3) is a merged image showing VE-cadherin, F-actin , and DAPI.

Figure 5.19: Quantification of VE-cadherin expression as a function of shear stress for both electrode sizes (200 and 100 μm). Error bars represent +/- one standard deviation. Expression of VE-cadherin differ statistically significant (* $p < 0.05$) between HUVECs sheared (on 200 μm electrode diameters) at 17.6 and 58.1 dyne/cm².

Figure 5.20: Quantification of VE-cadherin expression (A) and normalized resistance of HUVECs (B) as a function of shear stress for both electrode sizes (200 and 100 μm). Error bars represent $\pm$ one standard deviation. Expression of VE-cadherin and R_{TER} differ statistically significant (* $p < 0.05$) between HUVECs sheared (on 200 μm electrode diameters) at 17.6 and 58.1 dyne/cm^2 . For HUVECs on 100 μm diameter electrodes, there is a statistical difference (* $p < 0.05$) between R_{TER} for static and 17.6 dyne/cm^2 samples, as well as, there is statistical difference (# $p < 0.05$) between HUVECs sheared at 17.6 and 58.1 dyne/cm^2 .

Figure 5.21: Morphological arrangement of HUVECs under static conditions for (A) 200 μm and (D) 100 μm diameter electrodes where (1) is a wider field view and (2) are the zoomed images of cell arrangement on the electrode surface. Arrangement of HUVECs sheared at 2.5

dyne/cm² for 14 hr for (B) 200 μ m and (E) 100 μ m diameter electrodes where (1) is a wider field view and (2) are the zoomed images of cell arrangement on the electrode surface. Immunofluorescence images of HUVECs sheared at 2.5 dyne/cm² for 14 hr for (C) 200 μ m and (F) 100 μ m diameter electrodes where (1) is a wider field view and (2) are the zoomed images of cell arrangement on the electrode surface. In the immunofluorescence images, red signal is F-actin and the green signal is VE-cadherin.

5.4 *Summary and Conclusions*

In this chapter, the *in vitro* analysis of HUVECs undergoing physiologically relevant shear stress using the proposed microfluidic impedance platform was presented. HUVECs were monitored under static, constant shear conditions 2.5 and 17.6 dyne/cm², and a stepped shear condition 23.7-35-58.1 dyne/cm². Impedance was acquired for all these conditions for a 14-hour period and fitted to an equivalent circuit model which extracted the electrical monolayer properties. Normalized plots all extracted parameters were plotted as a function of time and were compared with respect to shear condition and electrode size.

Comparison of normalized electrical parameters between electrode sizes showed similar trends in the electrical parameters of cellular monolayers (R_{TER} , C_C , and CPE). R_{MED} was the parameter in which the most prominent discrepancies were observed between the two electrode dimensions. $R_{MED,100}$ fluctuations were as large as 60% of the baseline value compared to the 10% fluctuations observed for $R_{MED,200}$. This discrepancy may be attributed to the interfacial impedance that these smaller electrode experience.

The interfacial impedance can impede current flow, reducing electrode sensitivity to monitor properly the resistance of cell culture medium.

Normalized R_{TER} plots for the different shear conditions indicated that the initial onset of flow, generated a shear magnitude dependent increase in R_{TER} . As the shear level increased, the initial inflation of R_{TER} also increased. It must be noted that this shear magnitude dependent behavior was only visible in acute flow (sudden onset) as shown by the stepped shear condition 23.7-35-58.1 dyne/cm². The initial jump in R_{TER} has been observed before in bovine aortic endothelial cells [16], porcine endothelial cells [20], and HUVECs [183], [187]. However, the mechanism as to what causes this jump remains to be identified. Initially, it was postulated that the increase was caused by the fluid force pushing on the apical cell membrane, which reduced the small gap between the cell and electrode surface [16], but this has yet to be proven. The immediate onset (within 5 minutes) of shear stress has been associated with increased VE-cadherin phosphorylation which has been thought to be involved in the disassembly/assembly of adherent junctions and thus, the regulation of cell-cell-adhesion and permeability [98], [183]. VE-cadherin proteins were stained for on the impedance monitored HUVEC monolayers. Under static conditions, VE-cadherin junctions were observed as thick bands (with some three-dimensional lattice organization) lining the cell contours, as observed in the published literature [98], [184]. As endothelial cells are sheared, these junction bands form more compact lines due to VE-cadherin clustering [98], [183]. Some studies have shown that expression of VE-cadherin can differ due shear conditions [188], as well as, the length of shear exposure period [48], [98]. VE-cadherin is initially present under static conditions but decreases at intermediate times of shear exposure and increases

again after 24 to 48 hours of the onset of shear stress [48], [98]. At higher shear conditions ($> 28 \text{ dyne/cm}^2$), the VE-cadherin junction bands thicken [188]. Analysis on the mean fluorescence of VE-cadherin indicated that for the $200 \text{ }\mu\text{m}$ electrodes, the expression is greatest for the higher shear condition $23.7\text{-}35\text{-}58.1 \text{ dyne/cm}^2$, followed by the static condition, and trailed by the 17.6 dyne/cm^2 . For the $100 \text{ }\mu\text{m}$ electrodes, static samples expressed the most VE-cadherin, succeeded by the $23.7\text{-}35\text{-}58.1 \text{ dyne/cm}^2$ samples. For both electrode dimensions, HUVECs sheared at 17.6 dyne/cm^2 for 14 hours expressed the least VE-cadherin compared to the other shear conditions. Looking at the normalized R_{TER} values at the end of the experimental trials, it can be observed that the measured VE-cadherin expression trend agrees with $R_{TER,200}$ but not with $R_{TER,100}$. Though, VE-cadherin plays an important role in regulating endothelial cell morphology and permeability [98]. Endothelial cell morphology adaption is also modulated by cell-substrate junctions (integrins) [4], [189] which were not tested in this project.

The cell membrane capacitance (C_C) parameter has been used in the past to monitor cell attachment and cell confluency in other impedance systems [37]. Normalized C_C plots showed that for static, 17.6 , and $23.7\text{-}35\text{-}58.1 \text{ dyne/cm}^2$ shear condition, extracted C_C remained near the baseline value. However, for subconfluent cell layers (2.5 dyne/cm^2), C_C increased significantly (5 times the baseline) for $200 \text{ }\mu\text{m}$ electrodes and moderately (2.3 times the baseline) for $100 \text{ }\mu\text{m}$ electrodes. This result is indicative that the microfluidic impedance platform can monitor cell confluency as cells are sheared. It also shows that though there is a loss of sensitivity as electrode diameter is decreased.

According to previous work, *CPE* parameters represent the electric double layer at the interface of the electrode and media/cells and has been correlated with cell attachment [77], [108]. *CPE* parameters, specifically *CPE-Y* derived from control cell-free electrodes differentiate between static and flow conditions, showing that *CPE-Y* decrease with increasing shear level.

Finally, R_{MED} measurements were acquired and analyzed to see if they reflected any temperature changes and environmental changes [41]. The acquired R_{MED} values from HUVEC experimental trials did not show any shear dependent behavior. For the 200 μm electrodes, R_{MED} values remained near the baseline but fluctuated significantly for the 100 μm electrodes. Control cell-free R_{MED} measurements confirmed that environmental factors remained stable during experimental trials. These results also showed that the 100 μm are less sensitive and do not properly probe cell culture media. In addition, R_{MED} values extracted from cell-covered electrodes are influenced by the presence of the cells themselves. Confluent HUVEC layers for static and shear conditions 17.6 and 23.7-35-58.1 dyne/cm^2 yielded higher R_{MED} values than those captured for subconfluent monolayers (2.5 dyne/cm^2). Control cell-free R_{MED} measurements indicated that static values decreased slight during the experiment compared to the other shear conditions. This decrease in R_{MED} for static condition is a result of increased conductivity due the lack of media re-circulation and increased media evaporation.

The morphological adaptation of monitored HUVEC monolayers were captured through images taken prior and after the onset of shear conditions. Phase contrast, brightfield, and confocal images showed that cells under static conditions had a random cobblestone configuration. After HUVECs were exposed to shear conditions (2.5, 17.6,

23.7-35-58.1 dyne/cm²), cell morphology was converted from cuboidal to ellipsoidal shaped. Additionally, sheared cells portrayed more defined cell borders than those observed under static cultures. HUVECs exposed to 17.6 dyne/cm² re-orient and align parallel to the direction flow as confirmed by cytoskeleton re-modeling (F-actin fibers alignment). HUVECs sheared under the stepped condition 23.7-35-58.1 dyne/cm² do not consistently align in the direction of flow like HUVECs under 17.6 dyne/cm². Instead cells subjected to 23.7-35-58.1 dyne/cm² shear condition were angled (but not aligned) in the direction of flow, where some cells were oriented perpendicular to the flow. As reported by previous publications, remodeling in sheared endothelial cells is governed by the exposed shear level [190]–[192]. Studies have shown that endothelial cells under very high shear stresses show a perpendicular (to flow) alignment [193], [194]. One particular study investigated HUVECs at shear levels between 2 to 60 dyne/cm² for 16 hours. The findings showed that HUVECs aligned in the direction of flow for shear levels between 10 through 20 dyne/cm², which are levels that closely resemble the levels 8.4-12.5 dyne/cm² estimated in HUVECS. HUVECs exposed to shear levels outside of 10-20 dyne/cm² were observed to be misaligned and perpendicular to the direction of flow. This study concluded that HUVECs produced an inflammatory response, similar to that observed in vascular remodeling, when cells were sheared above or below the preferred range of shear stress [192].

CHAPTER 6

OBSERVATIONS AND CONCLUSIONS

Understanding the relationship between shear stress and endothelial cell behavior is important to gain a better prospective of endothelial cell biology and more specifically, the underlying mechanisms involved in endothelium pathology as well as how to treat and prevent it. In this work a microfluidic impedance platform was introduced for the real-time electrical assessment of endothelium monolayer kinetics while undergoing fluid shear stress *in vitro*. The specific objective of this project entailed: (1) the design/optimization, fabrication, and characterization an impedance chip/platform; (2) the application of the microfluidic impedance platform for the *in vitro* analysis of HUVECs under hydrodynamic shear conditions (static, 2.5, 17.6, and 23.7-35-58.1 dyne/cm²); and (3) the validation of the platform by conducting a comparison study of the acquired impedance measurements versus microscopic characterization techniques of HUVEC VE-cadherin expression (which has shown to regulate permeability). Though other impedance biosensors exist, our design sought to mimic the arterial size scale, monitor cells with a large frequency spectrum for comprehensive cell assessments, and probe different sizes of endothelial subpopulations.

6.1 Design, Fabrication, and Characterization of Microfluidic Impedance Chip/platform

The design of the microfluidic impedance platform incorporated fluidic channels to mimic the arterial structure, size, and microenvironment. It encompassed a control cell-free channel for the propose of monitoring environmental-conditions in the acquired data. The platform design also featured three different electrode diameters (200, 100 and 50 μm) in order to evaluate different sizes of subpopulations of endothelial cells within the microchannels. Initial design optimization experiments demonstrated that impedance chips should be fabricated on glass-based substrates to minimize stray capacitances through the substrate itself. Additionally, characterizations of the impedance platform with biologically-relevant electrolyte solutions demonstrated that electrodes with diameters less than 50 μm (as fabricated in the chip described in this work) displayed both significant interface impedance and noise at low frequencies thereby making them unsuitable for the sensing of adherent cell behavior. Consequently, 50 μm diameter electrodes were eliminates in any further analysis in this dissertation.

An automated data acquisition system was developed in efforts to collect data in a systematic fashion. The acquisition system was composed of several electrical components and connections including an in-house designed PCB for the automated switching between electrode measurements. Investigations exploring parasitic contributions due to electrical connections concluded that each additional connecting component (Fig. 4.8) such as the ribbon cable and PCB do induce a parasitic component at high frequencies ($> 10^6$ Hz). As a result, the proper measuring frequency spectrum was

established to 100 Hz –1 MHz in order to eliminate these parasitic effects. Measurement variations (Table 4.2) range between 0.02-17% for all electrode sizes as a result of the lack of uniformity in sputtering gold deposition. Variations increased as the distance between electrode arrays and chips increased. An additional characterization study on different conductivity KCl solutions demonstrated that the proposed microfluidic impedance platform provided the expected impedance spectra measurements where increasing solution conductivity decreases both impedance magnitude and phase shift.

Because the microfluidic impedance platform would be used to measure cells under perfusion conditions, analyses on flowrate effects (Fig.4.15) on collected impedance spectra were performed. Results indicated that fluid flow does causes an increase in impedance magnitude and phase shift at lower and mid frequencies ($<10^5$ Hz). These changes are more significant in an electrolyte solution of KCl versus PBS and cell culture medium. Electrical circuit fits (to the electrical model in Fig. 4.20A) of measurements acquired from cell culture media (i.e. control cell-free measurements) flowing at static, 17.6, and 23.7-35-58.1 dyne/cm² indicated that flow rate changes are captured in the extracted *CPE-Y* values (Fig. 5.7). However, flowrate effects go unnoticed in cell impedance measurements unless they are directly attributed to a cell behavior change.

Additionally, observations on the effects due to pH changes (Fig. 4.17) and bubble effects (Fig. 4.18) revealed that changes in pH can be visualized as a decrease in impedance magnitude and phase shift whereas emergence of bubbles produced substantial increases in impedance magnitude. Measurements captured from cellular experiments where bubbles were present indicated that a substantial bubble disturbance

could be identified in the extracted electrical parameters. For 200 μm electrodes, bubbles produce spikes in R_{TER} , R_{MED} parameters and valleys in C_C . For 100 μm electrodes, bubbles generated valleys in R_{TER} and jumps in C_C and R_{MED} . The discrepancies in trends observed between the two electrode sizes may be caused by the loss in sensitivity for smaller electrodes which do not properly probe all the electrical parameters. $R_{MED,100}$ (Fig. 5.9B) values demonstrated substantial fluctuation that were not observed from $R_{MED,200}$ (Fig. 5.9A) values or R_{MED} (Fig. 5.10) values derived from control cell-free measurements. It possible that by altering the suggested electrical circuit model (Fig. 4.20B) to eliminate R_{MED} the trend between electrodes size would be similar but this remains to be tested.

Moreover, the microfluidic impedance platform was characterized with cultured HUVECs to study the sensitivity of monitoring frequencies (Fig. 4.21). These experiments revealed that for the microfluidic impedance platform the resistance of the monolayer produced the most significant changes at 4 and 3 kHz for 200 and 100 μm diameter electrodes, respectively, whereas cell membrane capacitances produced the most significant changes at 57.5 and 32.7 kHz for 200 and 100 μm diameter electrodes respectively. Though the microfluidic platform introduced in this work was design with the purpose measuring impedance at a frequency spectrum, the platform could definitely be used to measure impedance at these selected frequencies for simple cellular analysis, like cell attachment and confluency.

6.2 *In vitro Analysis of HUVECs Under Hydrodynamic Shear Conditions*

The microfluidic impedance platform was used for the *in vitro* analysis of HUVECs undergoing physiologically relevant shear stress. HUVECs were monitored under static, and shear conditions of 2.5, 17.6, and 58.1 dyne/cm² (the latter was applied in a stepped fashion of 23.7-35-58.1 dyne/cm²). Impedance spectra were acquired for all of these conditions for a 14-hour period and fitted to an equivalent circuit model which extracted the electrical monolayer properties. Normalized plots of all extracted equivalent circuit parameters (R_{TER} , C_C , CPE , and R_{MED}) were plotted as a function of time and were compared with respect to shear condition and electrode size.

Comparison of normalized electrical parameters between electrode sizes showed similar trends were observed for the parameters R_{TER} , C_C , and CPE for the examined cell monolayers and shear conditions. The resistance of cell culture medium, R_{MED} , however did display discrepancies between the two electrode dimensions. $R_{MED,100}$ fluctuations were as large as 60% of the baseline value compared to the 10% fluctuations observed for $R_{MED,200}$. As mentioned before, this discrepancy may be attributed to the interfacial impedance that these smaller electrode experience. The interfacial impedance can impede current flow, reducing electrode sensitivity to monitor properly the resistance of cell culture medium.

The trans-endothelial resistance, R_{TER} , has been used to describe cell attachment and monolayer permeability. In this work, R_{TER} values (Fig. 5.3 and 5.11) for HUVECs under static and shear conditions were collected for 14 hours. Normalized R_{TER} plots

indicated that within 24 minutes of the onset of flow, an increase in R_{TER} was observed while HUVECs monitored under static conditions did not generate this initial increase. Data showed that as the shear level increased the initial magnitude inflation of R_{TER} also increased. It must be noted that this shear-dependent behavior was only observed in acute flow (sudden onset) as it was not present in the consecutive shear level changes applied in the stepped shear condition 23.7-35-58.1 dyne/cm². The initial jump in R_{TER} has been observed in previous publications [16], [20], [183], [187], but the mechanism as to what causes this jump remains to be identified. Initially it was postulated that the increase was caused by the fluid force pushing on the apical cell membrane, which reduced the small gap between the cell and electrode surface [16], but this has yet to be proven. Despite this, these results indicate that the proposed microfluidic impedance platform does replicate published findings and accurately probed cell behavior under shear stress.

For HUVECs under shear conditions, the early upsurge in R_{TER} was only transient. It was soon followed by a decrease and then a stabilization period. For the shear condition of 58.1 dyne/cm², the data shows that the consecutive changes in shear level delay the stabilization period. R_{TER} values for 58.1 dyne/cm² were higher than those at static and lower shears through most of the course of the experimental. R_{TER} values for HUVECs under static conditions were lower than those extracted for 58.1 dyne/cm² but higher than 17.6 dyne/cm². R_{TER} was lowest for 2.5 dyne/cm², which were found to be subconfluent. From the image analysis, VE-cadherin expression correlated with the extracted R_{TER} value.

The cell membrane capacitance (C_C) parameter has been used in the past to monitor cell attachment and cell confluency in other impedance systems [37].

Normalized C_C plots showed that for static, 17.6, and 23.7-35-58.1 dyne/cm² shear conditions extracted C_C remained near the baseline value. However, for subconfluent cell layers (2.5 dyne/cm²), C_C increased significantly (5 times the baseline) for 200 μ m electrodes and moderately (2.3 times the baseline) for 100 μ m electrodes. This result is indicative that the microfluidic impedance platform can monitor cell confluency as cells are sheared. It also shows that sensing sensitivity decreases as electrode diameter is decreased.

According to previous work, CPE parameters represent the electric double layer at the interface of the electrode and media/cells. These parameters have been shown to change with cell attachment [77], [108]. For the CPE parameters captured with our system, it was observed that CPE values did change in the presence of cells. CPE parameters, specifically $CPE-Y$ derived from control cell-free electrodes, differentiate between static and flow conditions, showing that $CPE-Y$ decreases with increasing shear level.

Finally, R_{MED} measurements were acquired and analyzed to see if they reflected any temperature changes and environmental changes [41]. The acquired R_{MED} values from HUVEC experimental trials did not show any shear-dependent behavior. Control cell-free R_{MED} measurements confirmed that environmental factors remained stable during experimental trials. Comparison of control and cell-covered electrodes R_{MED} values indicate that this parameter is influenced by the presence of the cells themselves. Confluent HUVEC layers for static and shear conditions 17.6 and 23.7-35-58.1 dyne/cm² yielded higher R_{MED} values than those captured for subconfluent monolayers (2.5 dyne/cm²). Control cell-free R_{MED} measurements indicated that static values decreased

slightly during the experiment relative to the other shear conditions. This decrease in R_{MED} for static conditions was a result of increased conductivity due the lack of media recirculation and increased media evaporation. These results conclude that R_{MED} measurements are best extracted from control electrodes than derived continuously from cell impedance spectra.

6.3 Microfluidic Impedance Platform Validation/ Image

Analysis

According to previous studies, the immediate onset (within five minutes) of shear stress has been associated with increased VE-cadherin phosphorylation which has been thought to be involved in the disassembly/assembly of adherent junctions and thus, the regulation of cell-cell-adhesion and permeability [98], [183]. VE-cadherin proteins were stained for on the impedance monitored HUVEC monolayers. Analysis on the mean fluorescence of VE-cadherin indicated that for the 200 μm electrodes, the expression is greatest for the higher shear condition 23.7-35-58.1 dyne/cm^2 , followed by the static condition, and trailed by those at 17.6 dyne/cm^2 . For the 100 μm electrodes, static samples expressed the most VE-cadherin succeeded by the 23.7-35-58.1 dyne/cm^2 samples. For both electrode dimensions, HUVECs sheared at 17.6 dyne/cm^2 for 14 hours expressed the least VE-cadherin compared to the other shear conditions. Statistical analysis indicated that there is no statistically difference between static and shear conditions (for both electrode dimensions) but there is statistically different ($p < 0.05$) between HUVEVs (200 μm electrodes) sheared at 176.6 and 58.1 dyne/cm^2 . Looking at

the normalized R_{TER} values at the end of the experimental trials, it can be observed that the measured VE-cadherin expression trend agrees with $R_{TER,200}$ but not with $R_{TER,100}$. Statistical analysis on normalized R_{TER} indicated that there is statistically difference ($p < 0.05$) between HUVEVs (200 and 100 μm electrodes) sheared at 176.6 and 58.1 dyne/cm^2 and also between static and 17.6 dyne/cm^2 for HUVECs monitored on 100 μm electrodes . Though, VE-cadherin plays an important role in regulating endothelial cell morphology and permeability [98]. Endothelial cell morphology adaption is also modulated by cell-substrate junctions (integrins) [4], [189] which were not tested in this project.

The morphological adaptation of monitored HUVEC monolayers were captured through images taken prior and after the onset of shear conditions. Phase contrast, brightfield, and confocal images showed that cells under static conditions had a random cobblestone configuration. After HUVECs were exposed to shear conditions (2.5, 17.6, 23.7-35-58.1 dyne/cm^2), cell morphology was converted from cuboidal to ellipsoidal shaped. HUVECs exposed to 17.6 dyne/cm^2 re-orient and align parallel to the direction flow as confirmed by cytoskeleton re-modeling (F-actin fibers alignment). HUVECs sheared under the stepped condition 23.7-35-58.1 dyne/cm^2 were angled (but not aligned) in the direction of flow, where some cells were oriented perpendicular to the flow. Endothelial cells have shown to have a preferred shear range. Cells sheared outside of this range were observed to be misaligned and perpendicular to the direction of flow, indicating an inflammatory response similar to that observed in vascular remodeling [192].

6.4 *Limitations of Work*

This work has presented a microfluidic impedance platform for the *in vitro* analysis of HUVECs undergoing hydrodynamic shear stress. Experimental outcomes have shown that this microfluidic impedance platform replicated and captured endothelial cell behavior. However, this study is particular limited by the inability to characterize endothelial cells in real-time with impedance and microscopy measurements simultaneously. Doing so would provide extremely valuable information. For instance, the electrical signature and parameter changes indicating cell elongation or re-orientation would be identified. In this study, proper comparison was only possible after the allotted experimental time since the same cellular monolayer cannot be stained twice (before and after shear stress). An additional limitation of this study is also the lack of a complementary western blot analysis on VE-cadherin expression. Though VE-cadherin fluorescence was measured through image analysis for the specific electrode areas, a western blot would provide a more accurate protein expression measurement. Another important limitation in this study is the small number of experimental trials and use of older cells as a result of time constraints and limited resources. To properly characterize cell behavior, it would best to use primary cells to eliminate cellular heterogeneity observed in later passages and repeat experimental trials.

6.5 *Future Work*

The initial findings in this work provide a solid foundation for future improvements and applications of the microfluidic impedance platform. Possible future

work entail the re-design of platform features, a more comprehensive characterization of electrical measurements, improvement of physiological relevance, and addressing other possible applications.

Moving forward, one of the main goals in future work should be improving the sensitivity of the microfluidic impedance platform. This may include the re-fabrication of smaller diameter electrodes with surface area enhancement processes to minimize interfacial impedance. In addition to, reducing the number of electrical connections as well as redesigning the PCB (electrode switching system in the automated data acquisition system) would be beneficial in reducing parasitic effects and increasing temporal resolution of data collected. Lastly, sensor sensitivity could be improved with a re-design of the electrode array configuration where the control cell-free electrodes and cell-covered electrodes would be encompassed in the same channel. In this re-design, the top PDMS lid would be replaced with a second impedance chip, whose electrode arrays would be used for control cell-free measurements. Cell attachment on this top electrode array would be minimized by gravity-driven cell settlement and coating the top electrode array with serum. This would in turn leave the adjacent channel to be used as a cellular control (for static condition).

Perhaps the most important task in the future will be a more comprehensive characterization of electrical impedance spectra. This would require analyzing sheared endothelial cell monolayers with impedance and microscopy measurements simultaneously. As mentioned before, this study would be valuable in identifying specific cell behaviors signatures in the acquired electrical measurements.

Looking at current biomedical research, it will be imperative to drive the microfluidic impedance platform to a more physiological relevant structure. Many scientists have been working on developing lumens within rectangular microchannels. One particular study from David Beebe's group [78] showed a simple method of manufacturing circular cross sectional lumens with ECM hydrogels in rectangular microchannels. This process could be implemented to construct circular lumen within the microfluidic impedance platform, leading to more physiological relevant results.

Finally, future endeavors could entail using the microfluidic impedance platform for applications other than endothelial cell behavior under shear stress. This platform is very diverse and could be used for any adherent cell type. Studies could be expanded to monitoring wound healing, toxicity effects, cancer metastasis, and drug permeability to name just a few.

To conclude, this dissertation presented the design, fabrication, characterization, and application of a microfluidic impedance platform for the *in vitro* analysis of HUVEC kinetics under hydrodynamic shear stress. Electrical measurements of sheared HUVECs indicated that monolayers sheared at higher shear levels (58.1 dyne/cm²) are less permeable than those sheared at a lower level of shear stress of 17.6 dyne/cm². Overall, this platform provides a non-invasive tool to monitor functional endothelial cell properties like permeability, in real-time.

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APPENDIX

A.1 Comparison of Shear Stress Approximation

In vitro fluid models for cell monolayers under fluid shear stress usually implement one of two shear stress approximations, the parallel plate Poiseuille flow or the rectangular duct estimation. The channel geometry usually dictates which approximation is appropriate. According to the parallel plate Poiseuille flow approximation, shear stress can be calculated from the following expression:

$$\tau_{PP} = \frac{6\mu Q}{WH^2} \quad (\text{A.1})$$

where μ the dynamic viscosity ($\mu=0.0072$ dyne·s/cm² for cell culture medium with 10 % serum at 37°C), Q is the fluid flowrate, H is the height ($H=2h$), and W is the width of the flat plates/channels ($W=2a$). For this mathematical model, flow must be fully developed and plates are presumed infinitely wide (there is no significant shear stress at the side walls).

However, the parallel plate Poiseuille flow approximation is not valid as the width approaches the height of the duct. In these cases, it is better to use the shear stress relationship of shear stress in a rectangular channel [86], [87]:

$$\tau_{RC}(x) = \mu \frac{Q}{q} \sum_{n=0}^{\infty} \frac{(-1)^n a \pi}{(2n+1)^2} \left(\frac{2}{\pi}\right)^3 \tanh \left[(2n+1) \frac{\pi h}{2a} \right] \quad (\text{A.1})$$

where q is:

$$q = \frac{4}{3} h a^3 - 8 a^4 \left(\frac{2}{\pi}\right)^5 \sum_{n=0}^{\infty} \frac{1}{(2n+1)^5} \tanh \left[\frac{(2n+1)\pi h}{2a} \right] \quad (\text{A.3})$$

To determine which equation is appropriate for the channel geometry of the impedance chip presented within this project, the error between both approximations was determined as a function of the width to height ratio ($W/H = a/h$), where the error was calculated by the following expression:

$$\tau \text{ Error } (\%) = \left(\frac{\tau_{RC} - \tau_{PP}}{\tau_{RC}} \right) \cdot 100 \quad (\text{A.4})$$

Fig. A.1 shows a plot of the error between both approximations was determined as a function of the width to height ratio for shear stress condition of 2.5 dyne/cm². The calculation indicates that the current channel geometry ($a/h = 5.97$) displays a 10.5% error. In order to minimize error below 1 %, the a/h should be greater than 60. As result, for the current channel geometry shear stress calculations was performed using the rectangular duct approximation. Using Eq. A.1 and A.2, the shear stress experienced by cells within the microelectrode areas were calculated and organized in Table A.1.

τ_{PP} (dyne/cm ²)	τ_{RC} (dyne/cm ²)
2.2	2.5
15.7	17.6
21.2	23.7
31.3	35.0
52.0	58.1

Table A.1: Shear stress calculations using both the parallel plate Poiseuille flow and flow within a rectangular duct approximation.

Figure A.1: Shear stress calculation of error between parallel plate Poiseuille flow and flow within a rectangular duct approximation as a function of the channel width to height ratio.

A.2 Shear Stress Gradient at Bottom Surface

Fluid flow was assumed unidirectional within a rectangular channel as shown in Fig. A.2. The coordinates were defined as follows: the x-axis was defined parallel to the width of the channel ($2a$), y-axis represented the height of channel ($2h$), and z-axis was parallel with the direction of fluid flow.

Figure A.2: Schematic of microfluidic channel and assumed coordinate convention utilized for fluid flow calculations. The vertical y-axis represented the height ($2h$) of the channel where the bottom of channel (surface of cell attachment) was assigned as $y=-h$. The horizontal x-axis represented the width ($2a$) of the channel and the center of the channel was assumed to be $x=0$. Whereas the z-axis represented the direction of fluid flow.

As a result, the reduced Navier-stokes equation can be expressed as [195]:

$$\mu \left(\frac{\partial^2 v}{\partial y^2} + \frac{\partial^2 v}{\partial x^2} \right) = \frac{\partial p}{\partial z} \quad (\text{A.5})$$

where μ the dynamic viscosity ($\mu=0.0072$ dyne·s/cm² for cell culture medium with 10 % serum at 37°C), v is fluid velocity, and p is pressure. Boundary conditions were assumed

to be: $v=0$ at $y=\pm h$ and $x=\pm a$. As a result, the infinite series solution to Eq. A.5 is derived as [86], [87]:

$$v(x, y) = -\frac{1}{\mu} \frac{\partial p}{\partial z} \left\{ \frac{a^2}{2} - \frac{x^2}{2} - \sum_{n=0}^{\infty} \frac{(-1)^n (2a^2)}{(2n+1)^3} \left(\frac{2}{\pi} \right)^3 \frac{\cosh\left[\frac{(2n+1)\pi y}{2a}\right]}{\cosh\left[\frac{(2n+1)\pi h}{2a}\right]} \cos\left[\frac{(2n+1)\pi x}{2a}\right] \right\} \quad (\text{A.6})$$

Using the relationship between fluid velocity and volumetric flow rate (Q), the equation can be rearranged to solve for the volumetric flowrate as:

$$Q = -\frac{1}{\mu} \frac{\partial p}{\partial z} \left\{ \frac{4}{3} ha^3 - 8a^4 \left(\frac{2}{\pi} \right)^5 \sum_{n=0}^{\infty} \frac{1}{(2n+1)^5} \tanh\left[\frac{(2n+1)\pi h}{2a}\right] \right\} \quad (\text{A.7})$$

where $\partial p/\partial z$ can be eliminated by reorganizing Eq. A.7:

$$\frac{\partial p}{\partial z} = -\mu \frac{V}{q}$$

where q is:

$$q = \frac{4}{3} ha^3 - 8a^4 \left(\frac{2}{\pi} \right)^5 \sum_{n=0}^{\infty} \frac{1}{(2n+1)^5} \tanh\left[\frac{(2n+1)\pi h}{2a}\right] \quad (\text{A.8})$$

Substituting this expression, shear stress is defined as:

$$\begin{aligned} \tau_{RC}(x, y) &= \mu \frac{\partial w(x, y)}{\partial y} = \mu \left(-\frac{1}{\mu} \frac{\partial p}{\partial z} \sum_{n=0}^{\infty} \frac{(-1)^n a \pi}{(2n+1)^2} \left(\frac{2}{\pi} \right)^3 \frac{\sinh\left[\frac{(2n+1)\pi y}{2a}\right]}{\cosh\left[\frac{(2n+1)\pi h}{2a}\right]} \cos\left[\frac{(2n+1)\pi x}{2a}\right] \right) = \\ &= \mu \left(-\frac{1}{\mu} \left(-\mu \frac{Q}{q} \right) \sum_{n=0}^{\infty} \frac{(-1)^n a \pi}{(2n+1)^2} \left(\frac{2}{\pi} \right)^3 \frac{\sinh\left[\frac{(2n+1)\pi y}{2a}\right]}{\cosh\left[\frac{(2n+1)\pi h}{2a}\right]} \cos\left[\frac{(2n+1)\pi x}{2a}\right] \right) = \\ &= \mu \frac{Q}{q} \sum_{n=0}^{\infty} \frac{(-1)^n a \pi}{(2n+1)^2} \left(\frac{2}{\pi} \right)^3 \frac{\sinh\left[\frac{(2n+1)\pi y}{2a}\right]}{\cosh\left[\frac{(2n+1)\pi h}{2a}\right]} \cos\left[\frac{(2n+1)\pi x}{2a}\right] \quad (\text{A.9}) \end{aligned}$$

Shear stress experienced by cells are located at $y=-h$, thus the shear stress (Fig. A.3A) was calculated as follows:

$$\tau(x) = \mu \frac{Q}{q} \sum_{n=0}^{\infty} \frac{(-1)^n a \pi}{(2n+1)^2} \left(\frac{2}{\pi} \right)^3 \tanh\left[(2n+1) \frac{\pi h}{2a}\right] \cos\left[\frac{(2n+1)\pi x}{2a}\right] \quad (\text{A.10})$$

and shear gradient (Fig. A.3B) was calculated as follows:

$$\frac{\partial \tau}{\partial y} = \mu \frac{Q}{q} \sum_{n=0}^{\infty} \frac{(-1)^n \pi}{(2n+1)} \left(\frac{2}{\pi}\right)^2 \tanh \left[(2n+1) \frac{\pi h}{2a} \right] - \sin \left[\frac{(2n+1)\pi x}{2a} \right] \quad (\text{A.11})$$

Shear stress calculations (Fig. A.3A) indicate that shear stress is uniform in the center area of the rectangular channel and decreases to approximately 0 at the side walls. The uniform center area of shear stress decreases in width with increasing shear stress value. For the largest shear stress value of 58.1 dyne/cm², the shear stress is uniform along a center distance of 0.1 cm, while for the shear stress value of 2.5 dyne/cm², the shear stress is uniform across a center distance of 0.16 cm. According to the spatial shear stress gradient, cells within 50 μm, 250 μm, 300 μm, and 400 μm from the side wall of the rectangular microchannel experience significant spatial gradients for shear stress values of 2.5, 17.5, 23.7, and 58.1 dyne/cm² respectively.

To insure that cell analyzed within the microelectrode areas experience uniform shear stress, it is crucial to make a valid attempt to center the microchannel cavities with microelectrodes. However, according to these calculations, even at the largest shear stress condition, the largest electrode diameter (200 μm) can be misaligned up to 400 μm (to the left or right) before cells experience any significant spatial gradient. As long as channels and electrodes are appropriately aligned, cells within the electrode areas should experience uniform shear across the electrode diameter.

Figure A.3: Shear stress (A) and and shear stress (B) gradient across the width of the channel.

A.3 ZView Equivalent Circuit Fitting Method

ZView (Scribner Associates INC., Southern Pines, NC) uses a complex non-linear least squares (CNLS) fitting technique to yield the equivalent circuit parameters. In this technique, parameter values are found by seeking to minimize the sum of squares (SS):

$$SS = \sum_{i=1}^n \{(Z'_{i,exp} - Z'_{i,th})^2 + (Z''_{i,exp} - Z''_{i,th})^2\} \quad (A.12)$$

where $Z'_{i,exp}$ and $Z'_{i,th}$ are the experimental and theoretical real impedance, respectively associated with the i th data point. $Z''_{i,exp}$ and $Z''_{i,th}$ are the experimental and theoretical imaginary impedance, respectively associated with the i th data point and n represents the number of data points [196].

Weighing factors (w_i) are often included in the calculation of the sum of squares in order to prevent large values from dominating the calculation. This is especially the case when impedance varies of over several orders of magnitude in the measured frequency range [196].

$$SS = \sum_{i=1}^n \{w_i (Z'_{i,exp} - Z'_{i,th})^2 + (Z''_{i,exp} - Z''_{i,th})^2\} \quad (A.13)$$

In the work, the data was weighted by ‘Calc-Modulus’ or function (theoretical) modulus weighting (FMWT) where each data point weight is normalized by its magnitude. Modulus weighing has is suitable when the real and imaginary components of impedance are comparable in magnitude.

The goodness of the circuit fit was determined by residual (relative errors, e_{rel}) calculations between the measured and fit parameter value for each single data point, as well as, the weighted sum of squares due to error which is proportional to average error percentage calculated from the experimental and fitted data. A good fit is demonstrated by value close to 0, indicative that the fit has a smaller random error and a close prediction of parameter values.

Fig. A.4 encompasses the relative errors for the impedance magnitude and phase shift captured with 200 μm diameter electrodes before shear, 20 minutes after shear, and 6 hours after shear for HUVECs (solid filled circles) and cell culture medium (asterisks) sheared at 17.6 dyne/cm^2 . Fig. A.5 encompasses the relative errors for the impedance magnitude and phase shift captured with 100 μm diameter electrodes before shear, 20 minutes after shear, and 6 hours after shear for HUVECs (solid filled circles) and cell culture medium (asterisks) sheared at 17.6 dyne/cm^2 . The percentage of relative errors of the parameters were defined as:

$$|Z|e_{rel}(\%) = \frac{|Z|_{measured} - |Z|_{fit}}{|Z|_{measured}} \times 100 \quad (\text{A.14})$$

$$\phi e_{rel}(\%) = \frac{\phi_{measured} - \phi_{fit}}{\phi_{measured}} \times 100 \quad (\text{A.15})$$

As can be observed from the plots (Fig. A.4 -5) and the respective data Tables A.2-13, the relative error for any parameter does not exceed 10 %. For impedance magnitude collected from HUVECs, the relative error fluctuates between 0-7.7 % and 0-5.5 % for 200 and 100 μm electrodes respectively. For the phase shift collected from HUVECs, the relative error fluctuates between 0-9.5 % and 0-5.6 % for 200 and 100 μm

electrodes respectively. For the impedance magnitude collected from cell culture medium, the relative error fluctuates between 0-4.5 % and 0-4.2 % for 200 and 100 μm electrodes respectively. For the phase shift collected from cell culture medium, the relative error fluctuates between 0-5 % and 0-6.3 % for 200 and 100 μm electrodes respectively.

Figure A.4: The relative errors with respect to frequency for the impedance magnitude (A) and phase shift (B) captured with 200 μm diameter electrodes before shear (red), 20 minutes after shear (green), and 6 hours after shear (blue) for HUVECs (solid filled circles) and cell culture medium (asterisks) sheared at 17.6 dyne/cm^2 .

Figure A.5: The relative errors with respect to frequency for the impedance magnitude (A) and phase shift (B) captured with 100 μm diameter electrodes before shear (red), 20 minutes after shear (green), and 6 hours after shear (blue) for HUVECs (solid filled circles) and cell culture medium (asterisks) sheared at 17.6 dyne/cm^2 .

	HUVECs at 17.6 dyne/cm² (part 1 of 7)							
	200 μm							
time	Before shear							
	FIT				MEASURED		e_{rel} (%)	
<i>f</i> (Hz)	<i>Z'</i> (Ω)	<i>Z''</i> (Ω)	 <i>Z</i> (Ω)	ϕ ($^{\circ}$)	 <i>Z</i> (Ω)	ϕ ($^{\circ}$)	 <i>Z</i> 	ϕ
102	93259	-4.53E+05	4.62E+05	-78.37	472953.8	-84.38	2.21	7.13
108	89869	-4.30E+05	4.39E+05	-78.20	443784.1	-83.35	0.98	6.18
115	86340	-4.06E+05	4.15E+05	-78.01	418213	-82.09	0.66	4.98
122	83199	-3.85E+05	3.94E+05	-77.81	397850	-82.21	0.95	5.35
130	80005	-3.64E+05	3.72E+05	-77.59	373840.9	-81.64	0.39	4.96
139	76832	-3.42E+05	3.51E+05	-77.35	352594.5	-81.35	0.50	4.91
148	74027	-3.23E+05	3.32E+05	-77.11	331135.8	-80.87	0.20	4.65
157	71530	-3.07E+05	3.15E+05	-76.87	313621.6	-80.66	0.39	4.70
167	69056	-2.90E+05	2.98E+05	-76.61	295824.1	-80.12	0.76	4.39
178	66641	-2.74E+05	2.82E+05	-76.32	278864.5	-79.92	1.02	4.50
189	64494	-2.59E+05	2.67E+05	-76.03	264431	-79.11	1.04	3.89
201	62408	-2.45E+05	2.53E+05	-75.72	248623.7	-78.71	1.80	3.79
214	60399	-2.32E+05	2.40E+05	-75.39	234813.1	-78.44	2.00	3.88
228	58480	-2.19E+05	2.27E+05	-75.04	220670.5	-77.39	2.68	3.03
243	56657	-2.07E+05	2.14E+05	-74.67	208971.2	-77.21	2.55	3.29
258	55036	-1.96E+05	2.03E+05	-74.30	197160.7	-76.41	3.17	2.75
275	53402	-1.85E+05	1.92E+05	-73.89	187227.5	-76.11	2.78	2.92
292	51948	-1.75E+05	1.83E+05	-73.48	178161.5	-75.54	2.55	2.73
311	50502	-1.66E+05	1.73E+05	-73.03	168128.5	-74.63	2.93	2.14

(Part 2 of 7)								
331	49150	-1.57E+05	1.64E+05	-72.57	158470.2	-73.68	3.51	1.52
352	47887	-1.48E+05	1.56E+05	-72.08	151076.7	-72.98	3.03	1.23
375	46658	-1.40E+05	1.48E+05	-71.56	141694.9	-72.77	4.10	1.66
399	45517	-1.32E+05	1.40E+05	-71.02	133945.6	-71.75	4.51	1.00
424	44459	-1.25E+05	1.33E+05	-70.47	127962.5	-70.57	3.95	0.14
452	43405	-1.18E+05	1.26E+05	-69.87	120725.2	-70.12	4.47	0.36
480	42466	-1.12E+05	1.20E+05	-69.28	115376.7	-69.29	4.01	0.03
511	41539	-1.06E+05	1.14E+05	-68.63	109518	-68.07	4.09	0.83
544	40662	-1.00E+05	1.08E+05	-67.96	104000.1	-67.59	4.17	0.55
579	39834	-95028	1.03E+05	-67.26	99307.39	-66.51	3.76	1.13
616	39053	-89968	98079	-66.54	94792.27	-65.40	3.47	1.74
656	38302	-85119	93340	-65.77	90380.24	-64.52	3.27	1.94
698	37599	-80606	88944	-64.99	86812.54	-63.36	2.46	2.59
742	36941	-76408	84870	-64.20	83053.91	-62.48	2.19	2.75
790	36301	-72347	80943	-63.35	79447.99	-61.42	1.88	3.15
841	35694	-68525	77264	-62.49	75630.36	-60.37	2.16	3.51
894	35130	-65004	73889	-61.61	72822.04	-59.30	1.47	3.91
952	34578	-61588	70631	-60.69	70271.32	-58.22	0.51	4.25
1010	34084	-58556	67753	-59.80	67535.25	-57.16	0.32	4.62
1080	33550	-55322	64701	-58.77	64741.07	-56.28	0.06	4.42
1150	33074	-52476	62029	-57.78	62531.23	-55.29	0.80	4.51
1220	32646	-49951	59673	-56.83	60584.18	-54.22	1.50	4.81
1300	32206	-47396	57303	-55.80	58523.05	-53.48	2.08	4.34

(Part 3 of 7)								
1380	31810	-45135	55218	-54.83	56425.96	-52.22	2.14	4.99
1470	31408	-42886	53157	-53.78	54552.02	-51.44	2.56	4.56
1560	31044	-40897	51345	-52.80	52908.6	-50.63	2.96	4.29
1670	30644	-38760	49410	-51.67	51602.69	-49.62	4.25	4.14
1770	30315	-37051	47873	-50.71	49889.23	-48.91	4.04	3.69
1890	29955	-35245	46255	-49.64	48419.58	-48.17	4.47	3.05
2010	29629	-33662	44844	-48.65	47171.39	-47.58	4.93	2.25
2140	29305	-32155	43506	-47.66	45901.55	-46.57	5.22	2.33
2270	29008	-30829	42331	-46.74	44746.28	-45.80	5.40	2.05
2420	28693	-29488	41143	-45.78	43549.32	-45.40	5.53	0.84
2570	28401	-28314	40104	-44.91	42360.52	-44.74	5.33	0.38
2740	28094	-27153	39072	-44.02	41441.26	-44.21	5.72	0.42
2910	27809	-26142	38167	-43.23	40405.35	-43.69	5.54	1.05
3100	27510	-25159	37280	-42.45	39608.58	-43.21	5.88	1.77
3300	27214	-24264	36460	-41.72	38649	-42.82	5.66	2.57
3510	26920	-23452	35702	-41.06	37771.3	-42.58	5.48	3.57
3740	26613	-22687	34971	-40.45	36917.63	-42.30	5.27	4.39
3970	26321	-22029	34323	-39.93	36115.54	-42.09	4.96	5.13
4230	26003	-21393	33672	-39.45	35346.03	-41.96	4.74	5.98
4500	25684	-20832	33071	-39.05	34487.54	-41.76	4.11	6.51
4790	25353	-20322	32492	-38.72	33723.77	-41.78	3.65	7.34
5100	25007	-19864	31936	-38.46	33035.86	-41.63	3.33	7.61
5420	24658	-19467	31416	-38.29	32270.6	-41.40	2.65	7.51

(Part 4 of 7)								
5770	24284	-19106	30898	-38.20	31590.41	-41.69	2.19	8.37
6140	23894	-18790	30397	-38.18	30927.8	-41.87	1.72	8.82
6530	23488	-18516	29909	-38.25	30182.59	-41.96	0.91	8.83
6950	23057	-18276	29421	-38.40	29457.91	-42.30	0.13	9.22
7400	22599	-18066	28932	-38.64	28857.26	-42.66	0.26	9.41
7870	22125	-17888	28452	-38.96	28197.47	-42.78	0.90	8.95
8380	21617	-17731	27959	-39.36	27529.94	-43.17	1.56	8.82
8920	21084	-17595	27461	-39.85	26919.04	-43.66	2.01	8.74
9490	20528	-17475	26959	-40.41	26170.56	-44.03	3.01	8.22
10100	19942	-17364	26442	-41.05	25521.44	-44.61	3.61	7.98
10700	19375	-17266	25952	-41.71	24907.52	-45.11	4.19	7.55
11400	18727	-17158	25399	-42.50	24229.69	-45.67	4.83	6.95
12200	18004	-17039	24788	-43.42	23583.06	-46.25	5.11	6.11
12900	17389	-16932	24271	-44.24	22926.99	-46.86	5.86	5.60
13800	16624	-16789	23627	-45.28	22278.35	-47.55	6.05	4.77
14700	15890	-16635	23005	-46.31	21619.01	-48.23	6.41	3.97
15600	15186	-16471	22404	-47.32	20955.73	-49.00	6.91	3.42
16600	14443	-16275	21760	-48.41	20323.97	-49.69	7.07	2.56
17700	13670	-16046	21079	-49.57	19651.08	-50.49	7.27	1.83
18800	12945	-15803	20428	-50.68	19032.91	-51.17	7.33	0.96
20000	12206	-15525	19749	-51.83	18393.85	-51.93	7.37	0.19
21300	11464	-15214	19049	-53.00	17760.1	-52.75	7.26	0.47
22600	10780	-14894	18386	-54.10	17139.06	-53.53	7.28	1.08

(Part 5 of 7)								
24100	10058	-14521	17664	-55.29	16506.36	-54.29	7.01	1.83
25600	9402.4	-14146	16986	-56.39	15904.42	-55.16	6.80	2.23
27300	8731.6	-13725	16267	-57.54	15312.33	-55.89	6.23	2.95
29000	8130	-13311	15597	-58.58	14711.54	-56.67	6.02	3.37
30900	7530.2	-12860	14903	-59.65	14143.28	-57.48	5.37	3.77
32900	6971.3	-12403	14228	-60.66	13558.07	-58.21	4.94	4.21
35000	6454	-11944	13576	-61.62	13001.5	-58.98	4.42	4.46
37200	5977.8	-11486	12949	-62.51	12461.23	-59.74	3.91	4.64
39600	5523.7	-11016	12323	-63.37	11933.88	-60.48	3.26	4.78
42200	5096.9	-10539	11706	-64.19	11424.64	-61.15	2.46	4.98
44900	4713.9	-10077	11125	-64.93	10926.48	-61.88	1.82	4.93
47700	4370.5	-9634.2	10579	-65.60	10442.9	-62.52	1.30	4.93
50800	4043.4	-9181.8	10033	-66.23	9961.801	-63.08	0.71	5.00
54100	3745.6	-8740.6	9509.3	-66.80	9520.154	-63.70	0.11	4.87
57500	3483.2	-8325.4	9024.7	-67.30	9077.765	-64.28	0.58	4.69
61200	3239.5	-7914.1	8551.4	-67.74	8657.654	-64.87	1.23	4.42
65100	3020.8	-7521	8105	-68.12	8254.792	-65.35	1.81	4.24
69300	2820.8	-7138.5	7675.6	-68.44	7858.772	-65.85	2.33	3.93
73800	2639.3	-6769.6	7265.9	-68.70	7471.904	-66.39	2.76	3.47
78500	2478.6	-6423.6	6885.2	-68.90	7109.294	-66.80	3.15	3.15
83500	2333.7	-6093.4	6525	-69.04	6766.7	-67.25	3.57	2.67
88900	2201.1	-5774.6	6179.9	-69.14	6431.48	-67.63	3.91	2.22
94600	2082.5	-5474.4	5857.1	-69.17	6140.572	-68.00	4.62	1.73

(Part 6 of 7)								
101000	1970.1	-5175.1	5537.4	-69.16	5822.417	-68.37	4.90	1.16
107000	1880.3	-4925.4	5272.1	-69.11	5532.748	-68.70	4.71	0.60
114000	1790.7	-4666	4997.8	-69.00	5264.278	-68.88	5.06	0.17
121000	1714	-4435.7	4755.4	-68.87	4995.181	-69.16	4.80	0.42
129000	1638.8	-4202.6	4510.8	-68.70	4745.797	-69.48	4.95	1.13
137000	1573.9	-3996.4	4295.1	-68.50	4505.824	-69.69	4.68	1.70
146000	1510.6	-3791.4	4081.2	-68.28	4276.351	-69.89	4.56	2.31
155000	1455.4	-3610.3	3892.6	-68.05	4059.212	-70.00	4.10	2.79
165000	1401.4	-3432.4	3707.5	-67.79	3860.429	-69.97	3.96	3.11
176000	1348.9	-3260.5	3528.5	-67.52	3666.405	-70.23	3.76	3.85
187000	1302.2	-3109	3370.7	-67.27	3481.737	-70.30	3.19	4.30
199000	1256.3	-2963.1	3218.5	-67.02	3305.838	-70.35	2.64	4.73
212000	1211.3	-2823.9	3072.8	-66.78	3134.99	-70.39	1.98	5.12
226000	1167.2	-2692	2934.1	-66.56	2979.367	-70.38	1.52	5.43
240000	1126.6	-2575.4	2811.1	-66.37	2829.988	-70.33	0.67	5.63
256000	1083.7	-2457.6	2685.9	-66.20	2689.157	-70.29	0.12	5.82
272000	1043.9	-2353.2	2574.3	-66.08	2554.745	-70.26	0.77	5.95
289000	1004.5	-2254.3	2468	-65.98	2428.97	-70.23	1.61	6.05
308000	963.38	-2155.9	2361.3	-65.92	2310.341	-70.11	2.21	5.97
328000	923.02	-2063.5	2260.5	-65.90	2195.999	-69.98	2.94	5.82
349000	883.49	-1976.5	2165	-65.92	2089.559	-69.89	3.61	5.68
371000	844.89	-1894.5	2074.4	-65.97	1987.327	-69.73	4.38	5.40
395000	805.76	-1813.8	1984.7	-66.05	1889.673	-69.58	5.03	5.08

(Part 7 of 7)								
420000	768.02	-1737.7	1899.8	-66.16	1800.834	-69.38	5.50	4.65
447000	730.44	-1663.1	1816.4	-66.29	1715.38	-69.22	5.89	4.24
476000	693.42	-1590.3	1734.9	-66.44	1633.95	-69.04	6.18	3.77
506000	658.46	-1521.7	1658	-66.60	1557.677	-68.85	6.44	3.27
539000	623.55	-1452.9	1581	-66.77	1485.123	-68.70	6.46	2.81
573000	591.08	-1388.2	1508.8	-66.94	1417.649	-68.50	6.43	2.29
610000	559.35	-1323.8	1437.2	-67.10	1353.604	-68.31	6.18	1.78
649000	529.5	-1262	1368.6	-67.24	1291.797	-68.14	5.95	1.32
691000	500.98	-1201.3	1301.6	-67.36	1233.144	-68.02	5.55	0.96
735000	474.6	-1143.3	1237.9	-67.46	1177.966	-67.84	5.09	0.57
782000	449.84	-1087	1176.4	-67.52	1126.743	-67.77	4.41	0.37
833000	426.37	-1031.5	1116.2	-67.54	1076.955	-67.57	3.64	0.05
886000	405.17	-979.24	1059.8	-67.52	1029.139	-67.50	2.98	0.03
943000	385.42	-928.32	1005.2	-67.45	984.049	-67.49	2.15	0.05
100000 0	368.31	-882.18	955.98	-67.34	940.909	-67.45	1.60	0.16

Table A.2: The equivalent circuit fit, measured impedance spectrum captured with 200 μm diameter electrodes and their respective relative errors for HUVECs before the onset of shear stress (17.6 dyne/cm²).

	HUVECs at 17.6 dyne/cm ² (part 1 of 7)							
	200 μ m							
time	20 min after shear							
	FIT				MEASURED		e _{rel} (%)	
<i>f</i> (Hz)	Z' (Ω)	Z'' (Ω)	 Z (Ω)	ϕ ($^{\circ}$)	 Z (Ω)	ϕ ($^{\circ}$)	 Z 	ϕ
102	96415	-4.61E+05	4.71E+05	-78.18	484763.7	-81.14	2.91	3.65
108	93513	-4.37E+05	4.47E+05	-77.92	451448.6	-81.41	0.99	4.28
115	90495	-4.13E+05	4.22E+05	-77.63	424758.2	-80.45	0.57	3.50
122	87810	-3.91E+05	4.00E+05	-77.33	399358.8	-80.08	0.28	3.43
130	85081	-3.69E+05	3.78E+05	-77.00	379782.1	-80.07	0.40	3.83
139	82371	-3.47E+05	3.56E+05	-76.63	356031.8	-79.39	0.06	3.48
148	79978	-3.27E+05	3.37E+05	-76.26	335361.3	-78.93	0.43	3.38
157	77847	-3.10E+05	3.20E+05	-75.90	315618.7	-77.99	1.24	2.68
167	75738	-2.93E+05	3.02E+05	-75.50	298277.2	-77.53	1.41	2.62
178	73680	-2.76E+05	2.86E+05	-75.06	281922.5	-77.19	1.40	2.75
189	71852	-2.61E+05	2.71E+05	-74.63	265117.5	-76.90	2.27	2.95
201	70075	-2.47E+05	2.57E+05	-74.17	252793.6	-76.03	1.60	2.44
214	68365	-2.33E+05	2.43E+05	-73.67	239937.7	-75.37	1.34	2.25
228	66732	-2.20E+05	2.30E+05	-73.14	227106.6	-75.03	1.32	2.52
243	65181	-2.08E+05	2.18E+05	-72.58	212762.7	-73.93	2.34	1.83
258	63802	-1.97E+05	2.07E+05	-72.03	202493.3	-73.81	2.13	2.41
275	62412	-1.86E+05	1.96E+05	-71.42	193328.2	-72.38	1.29	1.33
292	61176	-1.76E+05	1.86E+05	-70.81	182364.7	-71.19	2.05	0.54
311	59946	-1.66E+05	1.76E+05	-70.14	172132.4	-70.45	2.53	0.43

(Part 2 of 7)								
331	58795	-1.57E+05	1.68E+05	-69.45	162739.9	-70.26	2.94	1.15
352	57721	-1.48E+05	1.59E+05	-68.74	154071.8	-68.94	3.33	0.28
375	56674	-1.40E+05	1.51E+05	-67.98	147895.2	-67.98	2.21	0.00
399	55702	-1.33E+05	1.44E+05	-67.20	140722.8	-67.54	2.15	0.50
424	54800	-1.25E+05	1.37E+05	-66.41	133637.9	-66.66	2.46	0.38
452	53900	-1.19E+05	1.30E+05	-65.54	127762.4	-65.42	1.89	0.19
480	53098	-1.12E+05	1.24E+05	-64.70	121652.5	-63.96	2.12	1.15
511	52306	-1.06E+05	1.18E+05	-63.79	115981.4	-62.95	2.10	1.33
544	51554	-1.01E+05	1.13E+05	-62.85	110780.3	-61.81	1.97	1.67
579	50842	-95142	1.08E+05	-61.88	105978.4	-60.77	1.78	1.82
616	50171	-90116	1.03E+05	-60.89	101681.5	-59.57	1.43	2.22
656	49522	-85310	98642	-59.87	97386.14	-58.68	1.29	2.01
698	48913	-80849	94494	-58.83	93677.45	-57.47	0.87	2.37
742	48341	-76712	90674	-57.78	90011.77	-56.30	0.74	2.63
790	47782	-72722	87015	-56.69	86835.27	-55.16	0.21	2.77
841	47249	-68980	83611	-55.59	83856.77	-53.83	0.29	3.28
894	46750	-65545	80509	-54.50	80877.95	-52.78	0.46	3.25
952	46259	-62227	77538	-53.37	78208.97	-51.66	0.86	3.32
1010	45815	-59294	74932	-52.31	75587.6	-50.54	0.87	3.51
1080	45331	-56181	72189	-51.10	73209.64	-49.70	1.39	2.82
1150	44895	-53457	69808	-49.98	71255.74	-48.85	2.03	2.31
1220	44497	-51055	67725	-48.93	69212.3	-47.39	2.15	3.25
1300	44083	-48640	65644	-47.81	67332.67	-46.72	2.51	2.34

(Part 3 of 7)								
1380	43703	-46519	63828	-46.79	65727.63	-45.52	2.89	2.78
1470	43311	-44425	62044	-45.73	64064.7	-44.75	3.15	2.19
1560	42949	-42591	60487	-44.76	62441.96	-43.89	3.13	1.98
1670	42541	-40640	58833	-43.69	61064.56	-43.16	3.65	1.23
1770	42196	-39099	57526	-42.82	59715.55	-42.35	3.67	1.11
1890	41809	-37491	56157	-41.88	58503.75	-41.65	4.01	0.57
2010	41446	-36101	54965	-41.06	57244.13	-41.12	3.98	0.16
2140	41075	-34801	53835	-40.27	56094.65	-40.53	4.03	0.63
2270	40721	-33677	52843	-39.59	54939.8	-40.01	3.82	1.05
2420	40331	-32563	51836	-38.92	53850.5	-39.36	3.74	1.14
2570	39956	-31611	50949	-38.35	52956.46	-39.30	3.79	2.41
2740	39545	-30695	50060	-37.82	51898.93	-38.96	3.54	2.93
2910	39146	-29920	49271	-37.39	50987.98	-38.77	3.37	3.56
3100	38711	-29193	48484	-37.02	50156.75	-38.65	3.34	4.20
3300	38261	-28556	47743	-36.74	49268.45	-38.47	3.10	4.49
3510	37795	-28004	47040	-36.54	48401.4	-38.62	2.81	5.39
3740	37291	-27511	46341	-36.42	47466.24	-38.54	2.37	5.52
3970	36790	-27111	45700	-36.39	46720.9	-38.75	2.19	6.10
4230	36227	-26749	45033	-36.44	45921.23	-38.87	1.93	6.26
4500	35645	-26454	44389	-36.58	45081.03	-39.34	1.54	7.00
4790	35020	-26209	43741	-36.81	44219.98	-39.70	1.08	7.28
5100	34353	-26010	43089	-37.13	43306.1	-39.94	0.50	7.04
5420	33665	-25856	42448	-37.53	42441.34	-40.51	0.02	7.37

(Part 4 of 7)								
5770	32914	-25732	41779	-38.02	41620.63	-41.02	0.38	7.31
6140	32123	-25635	41098	-38.59	40705.21	-41.61	0.96	7.24
6530	31294	-25559	40406	-39.24	39873.08	-42.13	1.34	6.86
6950	30408	-25495	39682	-39.98	38992.1	-42.96	1.77	6.95
7400	29470	-25435	38928	-40.80	38042.17	-43.69	2.33	6.62
7870	28504	-25373	38161	-41.67	37120.1	-44.41	2.80	6.16
8380	27476	-25298	37349	-42.64	36232.54	-45.29	3.08	5.85
8920	26414	-25206	36511	-43.66	35189.65	-46.30	3.75	5.70
9490	25325	-25088	35648	-44.73	34242.58	-47.25	4.10	5.33
10100	24200	-24938	34750	-45.86	33223.23	-48.07	4.60	4.60
10700	23136	-24765	33891	-46.95	32255.81	-48.99	5.07	4.17
11400	21950	-24533	32919	-48.18	31226.57	-49.97	5.42	3.57
12200	20670	-24231	31850	-49.53	30218.88	-50.92	5.40	2.72
12900	19615	-23939	30949	-50.67	29239.78	-51.99	5.85	2.54
13800	18347	-23532	29839	-52.06	28188.57	-53.00	5.85	1.78
14700	17174	-23098	28783	-53.37	27180.36	-54.01	5.90	1.20
15600	16093	-22643	27779	-54.60	26179.45	-54.96	6.11	0.65
16600	14990	-22123	26723	-55.88	25181.6	-55.95	6.12	0.12
17700	13889	-21541	25630	-57.19	24200.55	-56.95	5.91	0.42
18800	12895	-20957	24606	-58.40	23225.21	-57.91	5.95	0.83
20000	11920	-20325	23563	-59.61	22271.01	-58.88	5.80	1.23
21300	10979	-19654	22512	-60.81	21323.46	-59.89	5.57	1.54
22600	10142	-19002	21539	-61.91	20380.05	-60.69	5.69	2.01

(Part 5 of 7)								
24100	9290.4	-18277	20503	-63.06	19475.65	-61.56	5.28	2.43
25600	8543.5	-17587	19552	-64.09	18629.54	-62.48	4.95	2.57
27300	7804.9	-16845	18566	-65.14	17772.95	-63.28	4.46	2.94
29000	7163.6	-16149	17666	-66.08	16952.37	-64.09	4.21	3.10
30900	6543.1	-15422	16752	-67.01	16148.18	-64.86	3.74	3.31
32900	5981.6	-14711	15881	-67.87	15376.36	-65.57	3.28	3.51
35000	5475.6	-14023	15054	-68.67	14635.39	-66.28	2.86	3.60
37200	5021.2	-13359	14272	-69.40	13928.74	-66.90	2.46	3.74
39600	4597.7	-12696	13503	-70.09	13240.97	-67.58	1.98	3.71
42200	4208.4	-12042	12757	-70.74	12580.4	-68.15	1.40	3.79
44900	3865.7	-11426	12063	-71.31	11960.28	-68.82	0.86	3.62
47700	3564.1	-10847	11418	-71.81	11358.89	-69.29	0.52	3.64
50800	3281.5	-10268	10779	-72.28	10774.78	-69.75	0.04	3.62
54100	3028.1	-9712.9	10174	-72.68	10223.89	-70.24	0.49	3.49
57500	2808	-9199.3	9618.3	-73.03	9700.177	-70.62	0.84	3.41
61200	2606.1	-8697.9	9080	-73.32	9196.485	-71.05	1.27	3.20
65100	2427.2	-8225	8575.7	-73.56	8720.808	-71.42	1.66	2.99
69300	2265.2	-7770.2	8093.7	-73.75	8250.438	-71.68	1.90	2.89
73800	2119.7	-7336.2	7636.3	-73.88	7819.924	-72.01	2.35	2.60
78500	1992.1	-6932.7	7213.2	-73.97	7414.243	-72.24	2.71	2.40
83500	1877.9	-6550.7	6814.6	-74.00	7021.916	-72.51	2.95	2.06
88900	1774.4	-6184.4	6433.9	-73.99	6649.979	-72.69	3.25	1.79
94600	1682.5	-5841.6	6079.1	-73.93	6319.314	-73.00	3.80	1.29

(Part 6 of 7)								
101000	1596	-5501.6	5728.5	-73.82	5971.257	-73.10	4.07	0.99
107000	1527.5	-5219.2	5438.1	-73.69	5664.953	-73.19	4.00	0.68
114000	1459.5	-4926.8	5138.5	-73.50	5361.215	-73.34	4.15	0.22
121000	1401.8	-4668.1	4874	-73.29	5078.434	-73.33	4.03	0.06
129000	1345.6	-4406.8	4607.7	-73.02	4805.52	-73.44	4.12	0.57
137000	1297.5	-4176.3	4373.2	-72.74	4557.915	-73.47	4.05	0.99
146000	1250.9	-3947.5	4140.9	-72.42	4318.202	-73.50	4.11	1.47
155000	1210.5	-3745.7	3936.4	-72.09	4091.236	-73.50	3.78	1.92
165000	1171.3	-3547.8	3736.2	-71.73	3879.812	-73.41	3.70	2.29
176000	1133.4	-3356.8	3543	-71.34	3677.987	-73.37	3.67	2.76
187000	1100	-3188.8	3373.1	-70.97	3486.256	-73.25	3.25	3.12
199000	1067.2	-3027.2	3209.9	-70.58	3305.82	-73.15	2.90	3.52
212000	1035.2	-2873.5	3054.3	-70.19	3132.298	-73.01	2.49	3.86
226000	1003.9	-2728.2	2907	-69.80	2971.242	-72.90	2.16	4.25
240000	975.14	-2600.2	2777	-69.44	2820.521	-72.71	1.54	4.49
256000	944.69	-2471.4	2645.8	-69.08	2676.244	-72.49	1.14	4.71
272000	916.33	-2357.9	2529.7	-68.76	2540.676	-72.31	0.43	4.91
289000	888.05	-2251	2419.9	-68.47	2413.047	-72.19	0.28	5.15
308000	858.33	-2145.4	2310.7	-68.20	2292.97	-71.90	0.77	5.15
328000	828.86	-2047.1	2208.5	-67.96	2178.371	-71.73	1.38	5.26
349000	799.67	-1955.4	2112.6	-67.76	2070.789	-71.46	2.02	5.17
371000	770.79	-1869.8	2022.5	-67.60	1969.094	-71.19	2.71	5.05
395000	741.1	-1786.4	1934	-67.47	1872.666	-70.89	3.28	4.82

(Part 7 of 7)								
420000	712.03	-1708.5	1851	-67.38	1783.258	-70.66	3.80	4.65
447000	682.61	-1633	1769.9	-67.31	1698.349	-70.40	4.21	4.38
476000	653.14	-1559.9	1691.1	-67.28	1617.702	-70.11	4.54	4.03
506000	624.85	-1491.7	1617.3	-67.27	1541.474	-69.82	4.92	3.65
539000	596.1	-1423.7	1543.5	-67.28	1470.417	-69.56	4.97	3.27
573000	568.92	-1360.3	1474.5	-67.30	1403.003	-69.31	5.10	2.89
610000	541.91	-1297.6	1406.2	-67.33	1339.177	-69.08	5.00	2.53
649000	516.08	-1237.6	1340.8	-67.36	1278.928	-68.79	4.84	2.08
691000	491.01	-1178.8	1277	-67.39	1221.213	-68.58	4.57	1.75
735000	467.47	-1122.8	1216.2	-67.40	1166.935	-68.40	4.22	1.46
782000	445.06	-1068.5	1157.4	-67.39	1116.486	-68.12	3.66	1.07
833000	423.53	-1014.9	1099.8	-67.35	1066.158	-68.01	3.16	0.98
886000	403.83	-964.48	1045.6	-67.28	1019.73	-67.92	2.54	0.94
943000	385.25	-915.3	993.07	-67.17	975.793	-67.81	1.77	0.94
1000000	368.99	-870.67	945.63	-67.03	933.436	-67.65	1.31	0.91

Table A.3: The equivalent circuit fit, measured impedance spectrum captured with 200 μm diameter electrodes and their respective relative errors for HUVECs 20 minutes after the onset of shear stress (17.6 dyne/cm^2).

	HUVECs at 17.6 dyne/cm ² (part 1 of 7)							
	200 μ m							
time	6 hr after shear							
	FIT				MEASURED		e_{rel} (%)	
f (Hz)	Z' (Ω)	Z'' (Ω)	$ Z $ (Ω)	ϕ ($^{\circ}$)	$ Z $ (Ω)	ϕ ($^{\circ}$)	$ Z $	ϕ
102	78811	-4.51E+05	4.58E+05	-80.08	475444	-83.34	3.77	3.91
108	75741	-4.28E+05	4.34E+05	-79.96	446782.9	-83.89	2.78	4.68
115	72548	-4.04E+05	4.10E+05	-79.82	418187.9	-84.08	1.89	5.07
122	69707	-3.83E+05	3.89E+05	-79.67	392943.8	-83.47	1.04	4.55
130	66820	-3.61E+05	3.67E+05	-79.51	371618.6	-83.60	1.22	4.89
139	63955	-3.40E+05	3.46E+05	-79.33	349624.7	-83.12	1.18	4.56
148	61424	-3.21E+05	3.26E+05	-79.15	329188.3	-82.96	0.84	4.59
157	59172	-3.04E+05	3.09E+05	-78.98	310454	-82.18	0.32	3.89
167	56943	-2.87E+05	2.93E+05	-78.78	294596.7	-82.08	0.66	4.02
178	54769	-2.71E+05	2.76E+05	-78.57	275962.7	-82.17	0.12	4.39
189	52838	-2.56E+05	2.62E+05	-78.35	259134	-81.50	1.01	3.86
201	50962	-2.42E+05	2.48E+05	-78.12	245422.5	-81.04	0.91	3.59
214	49158	-2.29E+05	2.34E+05	-77.88	231529.9	-80.89	1.10	3.72
228	47436	-2.16E+05	2.21E+05	-77.61	217998	-80.56	1.44	3.65
243	45802	-2.04E+05	2.09E+05	-77.33	205190.2	-80.04	1.79	3.39
258	44349	-1.93E+05	1.98E+05	-77.06	193889.4	-79.81	2.11	3.45
275	42887	-1.82E+05	1.87E+05	-76.74	184037.1	-79.38	1.62	3.33
292	41587	-1.72E+05	1.77E+05	-76.43	174197.4	-78.68	1.78	2.86
311	40296	-1.63E+05	1.68E+05	-76.09	164135	-78.32	2.14	2.85

(Part 2 of 7)								
331	39089	-1.54E+05	1.59E+05	-75.74	153717.4	-77.41	3.20	2.17
352	37963	-1.45E+05	1.50E+05	-75.36	145946.2	-76.87	2.95	1.96
375	36868	-1.37E+05	1.42E+05	-74.96	137031.4	-76.68	3.70	2.23
399	35853	-1.30E+05	1.35E+05	-74.55	130092.8	-75.53	3.45	1.29
424	34913	-1.23E+05	1.28E+05	-74.12	123143.2	-75.01	3.63	1.19
452	33977	-1.16E+05	1.21E+05	-73.65	116273.8	-74.68	3.82	1.38
480	33145	-1.10E+05	1.15E+05	-73.19	110291	-74.02	3.89	1.12
511	32325	-1.04E+05	1.09E+05	-72.68	104501.5	-73.16	3.88	0.66
544	31550	-97937	1.03E+05	-72.14	98897.93	-72.29	4.04	0.20
579	30818	-92574	97569	-71.59	93974.84	-71.60	3.82	0.02
616	30131	-87544	92585	-71.01	89215.56	-70.91	3.78	0.13
656	29470	-82724	87817	-70.39	84809.68	-69.91	3.55	0.68
698	28852	-78238	83388	-69.76	80507.77	-68.90	3.58	1.24
742	28276	-74065	79279	-69.10	76320.14	-68.19	3.88	1.34
790	27716	-70026	75312	-68.41	72862.06	-67.16	3.36	1.86
841	27186	-66225	71588	-67.68	69394.29	-66.33	3.16	2.04
894	26696	-62721	68166	-66.94	66312.36	-65.42	2.80	2.33
952	26217	-59321	64856	-66.16	63432.63	-64.44	2.24	2.66
1010	25789	-56300	61926	-65.39	60473.75	-63.25	2.40	3.39
1080	25328	-53076	58810	-64.49	57934.44	-62.20	1.51	3.69
1150	24920	-50235	56076	-63.62	55409.39	-61.44	1.20	3.55
1220	24553	-47713	53660	-62.77	53268.98	-60.44	0.73	3.85
1300	24179	-45157	51222	-61.83	50890.18	-59.30	0.65	4.27

(Part 3 of 7)								
1380	23843	-42891	49073	-60.93	49120.3	-58.39	0.10	4.36
1470	23504	-40633	46942	-59.95	47228.38	-57.24	0.61	4.74
1560	23200	-38633	45063	-59.01	45428.44	-56.20	0.80	5.01
1670	22867	-36478	43052	-57.92	43819.43	-55.25	1.75	4.83
1770	22595	-34750	41450	-56.97	42273.36	-54.48	1.95	4.57
1890	22301	-32918	39761	-55.88	40855.63	-53.43	2.68	4.60
2010	22036	-31305	38283	-54.86	39501.6	-52.60	3.08	4.30
2140	21777	-29764	36880	-53.81	38353.41	-51.60	3.84	4.28
2270	21542	-28401	35647	-52.82	37095.57	-50.78	3.90	4.03
2420	21295	-27015	34399	-51.75	36060.11	-49.91	4.61	3.69
2570	21070	-25796	33307	-50.76	34983.8	-49.20	4.79	3.16
2740	20837	-24580	32224	-49.71	34004.34	-48.38	5.24	2.76
2910	20623	-23514	31276	-48.75	33077.86	-47.86	5.45	1.84
3100	20404	-22467	30350	-47.76	32163	-47.27	5.64	1.03
3300	20190	-21505	29497	-46.81	31369.88	-46.77	5.97	0.07
3510	19982	-20621	28715	-45.90	30487.22	-46.01	5.81	0.23
3740	19770	-19778	27965	-45.01	29732.05	-45.58	5.94	1.25
3970	19571	-19043	27307	-44.22	29004.13	-45.00	5.85	1.75
4230	19360	-18320	26654	-43.42	28236.7	-44.57	5.61	2.57
4500	19152	-17671	26058	-42.70	27633.25	-44.28	5.70	3.58
4790	18939	-17068	25495	-42.03	26950.55	-43.91	5.40	4.30
5100	18722	-16514	24965	-41.41	26307.59	-43.67	5.10	5.16
5420	18507	-16022	24479	-40.88	25657.67	-43.47	4.59	5.95

(Part 4 of 7)								
5770	18280	-15562	24007	-40.41	25092.51	-43.37	4.33	6.83
6140	18047	-15148	23561	-40.01	24483.2	-43.11	3.77	7.19
6530	17807	-14777	23140	-39.69	23891.58	-42.93	3.15	7.56
6950	17555	-14440	22731	-39.44	23321.71	-42.97	2.53	8.22
7400	17290	-14136	22333	-39.27	22763.68	-42.93	1.89	8.53
7870	17017	-13871	21954	-39.18	22214.17	-42.99	1.17	8.85
8380	16726	-13631	21576	-39.18	21676.94	-43.10	0.47	9.10
8920	16420	-13420	21206	-39.26	21177.84	-43.19	0.13	9.10
9490	16102	-13235	20843	-39.42	20684.22	-43.33	0.77	9.02
10100	15764	-13070	20478	-39.66	20188.89	-43.57	1.43	8.96
10700	15435	-12935	20138	-39.96	19701.14	-43.77	2.22	8.69
11400	15056	-12801	19763	-40.37	19197.79	-44.01	2.94	8.27
12200	14629	-12672	19354	-40.90	18704.31	-44.36	3.47	7.79
12900	14260	-12573	19012	-41.40	18210.96	-44.74	4.40	7.45
13800	13796	-12459	18589	-42.09	17745.26	-45.13	4.75	6.76
14700	13341	-12353	18182	-42.80	17264.83	-45.64	5.31	6.22
15600	12898	-12252	17789	-43.53	16799.49	-46.03	5.89	5.43
16600	12420	-12141	17368	-44.35	16325.5	-46.52	6.39	4.68
17700	11912	-12017	16921	-45.25	15891.53	-47.07	6.48	3.87
18800	11425	-11890	16489	-46.14	15427.35	-47.61	6.88	3.08
20000	10916	-11746	16035	-47.10	14961.8	-48.13	7.17	2.14
21300	10394	-11583	15563	-48.10	14506.83	-48.70	7.28	1.23
22600	9900.2	-11415	15110	-49.06	14064.46	-49.37	7.43	0.62

(Part 5 of 7)								
24100	9366.2	-11213	14610	-50.13	13620.28	-49.99	7.27	0.29
25600	8868.8	-11005	14134	-51.14	13177.68	-50.64	7.26	0.97
27300	8347.5	-10765	13622	-52.21	12740.44	-51.30	6.92	1.77
29000	7868.5	-10522	13139	-53.21	12323.9	-51.96	6.61	2.41
30900	7379.6	-10249	12630	-54.25	11886.06	-52.63	6.26	3.08
32900	6913.5	-9964.7	12128	-55.25	11459.91	-53.27	5.83	3.71
35000	6472.5	-9670.5	11637	-56.21	11059.5	-53.91	5.22	4.26
37200	6058.1	-9369.9	11158	-57.12	10642.15	-54.56	4.85	4.68
39600	5655.1	-9052.7	10674	-58.01	10236.5	-55.31	4.27	4.87
42200	5269.1	-8723.2	10191	-58.87	9856.888	-55.96	3.39	5.19
44900	4916.4	-8397.8	9731	-59.65	9478.688	-56.69	2.66	5.24
47700	4595	-8078.5	9293.9	-60.37	9096.038	-57.31	2.18	5.34
50800	4284.2	-7746.6	8852.3	-61.06	8728.859	-57.96	1.41	5.35
54100	3997.1	-7417.2	8425.7	-61.68	8371.161	-58.50	0.65	5.43
57500	3740.9	-7102.4	8027.3	-62.22	8025.585	-59.04	0.02	5.40
61200	3499.9	-6786.1	7635.5	-62.72	7685.324	-59.68	0.65	5.09
65100	3281.3	-6479.9	7263.4	-63.14	7366.032	-60.30	1.39	4.72
69300	3079.3	-6178.6	6903.4	-63.51	7029.647	-60.84	1.80	4.40
73800	2894.1	-5885.1	6558.2	-63.81	6720.644	-61.41	2.42	3.92
78500	2728.7	-5607.2	6235.9	-64.05	6422.542	-61.85	2.91	3.57
83500	2578.2	-5340	5929.8	-64.23	6139.601	-62.21	3.42	3.25
88900	2439.5	-5080.2	5635.5	-64.35	5858.232	-62.78	3.80	2.49
94600	2314.5	-4834.1	5359.6	-64.42	5600.572	-63.31	4.30	1.75

(Part 6 of 7)								
101000	2195.1	-4587.4	5085.5	-64.43	5339.187	-63.62	4.75	1.27
107000	2099.1	-4380.7	4857.7	-64.40	5098.009	-63.79	4.71	0.95
114000	2002.7	-4165.2	4621.7	-64.32	4852.871	-64.42	4.76	0.16
121000	1919.7	-3973.4	4412.8	-64.21	4634.581	-64.61	4.79	0.61
129000	1837.6	-3778.7	4201.8	-64.07	4409.966	-64.97	4.72	1.40
137000	1766.4	-3606.2	4015.5	-63.90	4208.534	-64.93	4.59	1.58
146000	1696.5	-3434.4	3830.6	-63.71	4010.595	-65.28	4.49	2.40
155000	1635	-3282.5	3667.2	-63.52	3821.211	-65.53	4.03	3.06
165000	1574.5	-3133.2	3506.6	-63.32	3638.828	-65.83	3.63	3.82
176000	1515.4	-2988.7	3350.9	-63.11	3467.842	-66.01	3.37	4.39
187000	1462.4	-2861.3	3213.4	-62.93	3303.613	-66.18	2.73	4.91
199000	1410.1	-2738.6	3080.3	-62.76	3147.252	-66.25	2.13	5.28
212000	1358.4	-2621.3	2952.4	-62.61	2996.763	-66.24	1.48	5.49
226000	1307.5	-2510	2830.1	-62.49	2864.742	-66.10	1.21	5.47
240000	1260.5	-2411.5	2721	-62.40	2734.253	-66.10	0.48	5.59
256000	1210.6	-2311.5	2609.4	-62.36	2597.381	-66.33	0.46	5.99
272000	1164.3	-2222.7	2509.2	-62.35	2473.896	-66.50	1.43	6.23
289000	1118.3	-2138.2	2412.9	-62.39	2356.476	-66.68	2.39	6.44
308000	1070.2	-2053.5	2315.7	-62.47	2248.362	-66.67	2.99	6.30
328000	1023.1	-1973.6	2223	-62.60	2142.584	-66.61	3.75	6.02
349000	976.9	-1897.8	2134.4	-62.76	2044.856	-66.56	4.38	5.70
371000	931.87	-1825.7	2049.8	-62.96	1959.399	-66.24	4.61	4.96
395000	886.3	-1754.1	1965.3	-63.19	1876.425	-66.13	4.74	4.44

(Part 7 of 7)								
420000	842.44	-1686	1884.8	-63.45	1788.636	-66.07	5.38	3.96
447000	798.85	-1618.6	1805	-63.73	1699.47	-66.28	6.21	3.85
476000	756.04	-1552.2	1726.6	-64.03	1618.565	-66.35	6.67	3.50
506000	715.72	-1489.1	1652.2	-64.33	1545.185	-66.32	6.93	3.00
539000	675.58	-1425.2	1577.2	-64.64	1475.614	-66.28	6.88	2.48
573000	638.35	-1364.6	1506.5	-64.93	1410.225	-66.21	6.83	1.93
610000	602.08	-1304	1436.3	-65.22	1347.783	-66.18	6.57	1.45
649000	568.06	-1245.2	1368.7	-65.48	1292.008	-65.99	5.94	0.78
691000	535.65	-1187.2	1302.4	-65.72	1238.715	-65.95	5.14	0.35
735000	505.75	-1131.5	1239.4	-65.92	1182.139	-65.96	4.84	0.06
782000	477.76	-1077.1	1178.3	-66.08	1126.305	-66.08	4.62	0.00
833000	451.3	-1023.2	1118.3	-66.20	1075.425	-66.11	3.99	0.14
886000	427.45	-972.32	1062.1	-66.27	1030.272	-66.07	3.09	0.30
943000	405.28	-922.55	1007.6	-66.28	985.663	-66.11	2.23	0.26
1000000	386.11	-877.33	958.53	-66.25	942.39	-66.14	1.71	0.16

Table A.4: The equivalent circuit fit, measured impedance spectrum captured with 200 μm diameter electrodes and their respective relative errors for HUVECs 6 hours after the onset of shear stress (17.6 dyne/cm^2).

	Cell Culture Medium at 17.6 dyne/cm ² (part 1 of 7)							
	200 μ m							
time	Before shear							
	FIT				MEASURED		e_{rel} (%)	
f (Hz)	Z' (Ω)	Z'' (Ω)	$ Z $ (Ω)	ϕ ($^{\circ}$)	$ Z $ (Ω)	ϕ ($^{\circ}$)	$ Z $	ϕ
102	31995	-4.12E+05	4.14E+05	-85.57	421451.2	-86.40	1.83	0.97
108	30382	-3.91E+05	3.92E+05	-85.55	396198.5	-86.26	1.11	0.82
115	28709	-3.68E+05	3.69E+05	-85.54	367995	-86.47	0.28	1.08
122	27224	-3.48E+05	3.49E+05	-85.52	350515.5	-86.52	0.48	1.15
130	25719	-3.27E+05	3.28E+05	-85.51	328877.3	-86.23	0.16	0.84
139	24228	-3.07E+05	3.08E+05	-85.49	309926.9	-86.15	0.61	0.76
148	22915	-2.89E+05	2.90E+05	-85.47	292141.5	-86.22	0.67	0.87
157	21749	-2.73E+05	2.74E+05	-85.45	274628.7	-85.99	0.12	0.63
167	20597	-2.58E+05	2.59E+05	-85.43	262362.7	-86.15	1.42	0.84
178	19477	-2.43E+05	2.43E+05	-85.41	245063.7	-85.99	0.69	0.68
189	18485	-2.29E+05	2.30E+05	-85.39	230547.6	-85.77	0.29	0.44
201	17523	-2.16E+05	2.17E+05	-85.36	217381.4	-85.78	0.28	0.49
214	16600	-2.04E+05	2.04E+05	-85.34	204614.2	-85.94	0.20	0.70
228	15722	-1.92E+05	1.92E+05	-85.31	192798.9	-85.95	0.28	0.74
243	14891	-1.80E+05	1.81E+05	-85.28	181449.1	-85.24	0.29	0.05
258	14154	-1.70E+05	1.71E+05	-85.25	170009.4	-86.03	0.52	0.91
275	13415	-1.60E+05	1.61E+05	-85.22	162007.6	-86.15	0.74	1.08
292	12759	-1.51E+05	1.52E+05	-85.18	154011	-86.20	1.38	1.19
311	12110	-1.43E+05	1.43E+05	-85.14	144627	-85.66	1.10	0.60

(Part 2 of 7)								
331	11505	-1.34E+05	1.35E+05	-85.10	133855.9	-85.14	0.70	0.05
352	10942	-1.27E+05	1.27E+05	-85.06	126430.4	-85.07	0.55	0.01
375	10397	-1.19E+05	1.20E+05	-85.02	119342	-85.78	0.29	0.88
399	9893.2	-1.12E+05	1.13E+05	-84.97	112150.6	-85.11	0.60	0.17
424	9428	-1.06E+05	1.06E+05	-84.92	105537.9	-85.29	0.89	0.43
452	8966.7	-99789	1.00E+05	-84.87	99894.92	-85.00	0.30	0.16
480	8558.1	-94232	94620	-84.81	94012.35	-85.35	0.65	0.63
511	8156.8	-88774	89148	-84.75	88948.51	-85.20	0.22	0.52
544	7778.8	-83633	83994	-84.69	83501.37	-85.13	0.59	0.52
579	7424	-78807	79156	-84.62	78859	-85.00	0.38	0.45
616	7091.8	-74288	74626	-84.55	74418.1	-84.90	0.28	0.42
656	6773.9	-69964	70291	-84.47	69899.68	-84.81	0.56	0.40
698	6478.5	-65945	66263	-84.39	66000.38	-84.72	0.40	0.39
742	6204.1	-62212	62521	-84.31	62273.45	-84.51	0.40	0.24
790	5938.9	-58604	58904	-84.21	58606.07	-84.52	0.51	0.37
841	5689.5	-55211	55504	-84.12	55306.76	-84.44	0.36	0.38
894	5459.9	-52087	52372	-84.02	51974.3	-84.37	0.77	0.41
952	5237.2	-49058	49336	-83.91	49023.21	-83.93	0.64	0.03
1010	5039.6	-46369	46642	-83.80	46401.25	-84.17	0.52	0.44
1080	4828.8	-43500	43767	-83.67	43603.58	-83.89	0.37	0.26
1150	4643	-40972	41234	-83.54	41150.5	-83.72	0.20	0.22
1220	4478.1	-38729	38987	-83.40	38679.14	-83.71	0.80	0.36
1300	4310.9	-36454	36708	-83.26	36606.38	-83.47	0.28	0.25

(Part 3 of 7)								
1380	4162.7	-34437	34688	-83.11	34516.59	-83.34	0.50	0.28
1470	4014.7	-32425	32672	-82.94	32505.71	-82.97	0.51	0.04
1560	3883.5	-30639	30885	-82.78	30603.07	-82.88	0.92	0.12
1670	3741.9	-28713	28956	-82.58	28836.88	-82.73	0.41	0.19
1770	3628.1	-27165	27407	-82.39	27189.19	-82.45	0.80	0.07
1890	3507.1	-25519	25759	-82.18	25620.42	-82.26	0.54	0.11
2010	3400.2	-24066	24305	-81.96	24144.61	-81.90	0.66	0.07
2140	3297.6	-22671	22909	-81.72	22832.25	-81.85	0.34	0.16
2270	3206.5	-21432	21671	-81.49	21514.81	-81.47	0.73	0.03
2420	3113.2	-20165	20404	-81.22	20281.53	-81.30	0.60	0.10
2570	3030.6	-19042	19282	-80.96	19140.03	-80.83	0.74	0.16
2740	2947.6	-17916	18156	-80.66	18017.03	-80.64	0.77	0.02
2910	2874.2	-16918	17160	-80.36	17036.26	-80.31	0.73	0.07
3100	2801.3	-15929	16173	-80.03	16099.98	-79.91	0.45	0.14
3300	2733.5	-15008	15255	-79.68	15185.72	-79.54	0.46	0.17
3510	2670.4	-14153	14402	-79.32	14317.04	-79.21	0.59	0.13
3740	2609.3	-13323	13576	-78.92	13535.26	-78.74	0.30	0.22
3970	2555	-12588	12844	-78.53	12774.56	-78.37	0.54	0.20
4230	2500.6	-11850	12111	-78.09	12059.87	-77.95	0.42	0.17
4500	2450.6	-11173	11439	-77.63	11366.72	-77.50	0.64	0.17
4790	2403	-10529	10800	-77.14	10750.14	-76.95	0.46	0.26
5100	2358	-9920	10196	-76.63	10154.6	-76.43	0.41	0.27
5420	2316.7	-9362.8	9645.1	-76.10	9598.494	-75.88	0.49	0.29

(Part 4 of 7)								
5770	2276.7	-8822.6	9111.6	-75.53	9077.031	-75.21	0.38	0.43
6140	2239.3	-8317.1	8613.3	-74.93	8578.11	-74.76	0.41	0.22
6530	2204.2	-7845.1	8148.9	-74.31	8119.042	-74.12	0.37	0.25
6950	2170.8	-7394.8	7706.9	-73.64	7679.298	-73.30	0.36	0.47
7400	2139.1	-6968	7288.9	-72.93	7267.705	-72.63	0.29	0.42
7870	2109.7	-6573.3	6903.5	-72.21	6882.083	-71.90	0.31	0.43
8380	2081.4	-6194.1	6534.5	-71.43	6521.635	-71.07	0.20	0.50
8920	2054.9	-5839	6190	-70.61	6177.41	-70.32	0.20	0.41
9490	2030.1	-5507.2	5869.4	-69.77	5853.234	-69.48	0.28	0.41
10100	2006.5	-5192.9	5567	-68.87	5567.057	-68.48	0.00	0.57
10700	1985.8	-4918	5303.8	-68.01	5284.14	-67.65	0.37	0.53
11400	1964.4	-4633.3	5032.5	-67.02	5020.448	-66.59	0.24	0.65
12200	1942.8	-4347.3	4761.7	-65.92	4771.296	-65.61	0.20	0.47
12900	1926	-4125.7	4553.1	-64.98	4541.481	-64.51	0.26	0.71
13800	1906.8	-3873.3	4317.2	-63.79	4324.666	-63.41	0.17	0.59
14700	1889.9	-3651.4	4111.5	-62.63	4125.705	-62.33	0.34	0.49
15600	1874.8	-3454.7	3930.7	-61.51	3939.275	-61.13	0.22	0.63
16600	1859.9	-3260.9	3754.1	-60.30	3766.134	-59.94	0.32	0.60
17700	1845.4	-3072.8	3584.4	-59.01	3604.655	-58.67	0.56	0.58
18800	1832.4	-2906.5	3435.9	-57.77	3447.895	-57.43	0.35	0.59
20000	1819.9	-2745.8	3294.1	-56.46	3307.361	-56.07	0.40	0.71
21300	1807.7	-2592	3160.1	-55.11	3180.115	-54.79	0.63	0.57
22600	1796.9	-2455.8	3043	-53.81	3057.915	-53.36	0.49	0.85

(Part 5 of 7)								
24100	1785.7	-2316.9	2925.2	-52.38	2941.614	-52.03	0.56	0.68
25600	1775.8	-2194.4	2822.9	-51.02	2840.694	-50.62	0.63	0.78
27300	1765.7	-2071.8	2722.2	-49.56	2744.696	-49.25	0.82	0.62
29000	1756.7	-1963.8	2634.8	-48.19	2654.317	-47.91	0.74	0.58
30900	1747.7	-1857.2	2550.2	-46.74	2572.307	-46.49	0.86	0.54
32900	1739.1	-1758.6	2473.3	-45.32	2497.862	-45.11	0.98	0.45
35000	1731.1	-1667.4	2403.5	-43.93	2428.922	-43.78	1.05	0.34
37200	1723.5	-1583.2	2340.3	-42.57	2365.582	-42.46	1.07	0.27
39600	1716	-1502.5	2280.8	-41.20	2306.28	-41.11	1.10	0.23
42200	1708.7	-1425.7	2225.4	-39.84	2252.668	-39.81	1.21	0.08
44900	1701.8	-1355.9	2175.9	-38.55	2204.139	-38.61	1.28	0.16
47700	1695.2	-1292.4	2131.7	-37.32	2159.129	-37.43	1.27	0.28
50800	1688.6	-1230.8	2089.5	-36.09	2116.375	-36.28	1.27	0.53
54100	1682.1	-1173.6	2051	-34.90	2077.616	-35.20	1.28	0.85
57500	1675.9	-1122.2	2016.9	-33.81	2042.504	-34.13	1.25	0.95
61200	1669.6	-1073.5	1984.9	-32.74	2010.351	-33.16	1.27	1.28
65100	1663.4	-1028.9	1956	-31.74	1980.624	-32.22	1.24	1.49
69300	1657.2	-987.45	1929.1	-30.79	1952.562	-31.36	1.20	1.82
73800	1650.8	-949.19	1904.3	-29.90	1926.459	-30.58	1.15	2.22
78500	1644.5	-914.89	1881.9	-29.09	1902.413	-29.83	1.08	2.49
83500	1638.1	-883.67	1861.2	-28.35	1879.315	-29.14	0.96	2.74
88900	1631.4	-855.04	1841.8	-27.66	1858.196	-28.56	0.88	3.16
94600	1624.4	-829.55	1824	-27.05	1835.753	-27.93	0.64	3.14

(Part 6 of 7)								
101000	1616.8	-805.7	1806.5	-26.49	1819.989	-27.52	0.74	3.76
107000	1609.8	-787.15	1791.9	-26.06	1801.442	-27.12	0.53	3.92
114000	1601.7	-769.35	1776.8	-25.66	1782.958	-26.80	0.35	4.26
121000	1593.5	-754.96	1763.3	-25.35	1766.603	-26.51	0.19	4.36
129000	1584.2	-741.96	1749.4	-25.10	1750.112	-26.31	0.04	4.61
137000	1574.8	-731.98	1736.6	-24.93	1733.87	-26.17	0.16	4.73
146000	1564.2	-723.67	1723.4	-24.83	1718.242	-26.10	0.30	4.88
155000	1553.3	-717.89	1711.2	-24.80	1701.081	-26.07	0.59	4.84
165000	1541.1	-713.84	1698.4	-24.85	1686.86	-26.11	0.68	4.82
176000	1527.4	-711.7	1685	-24.98	1670.403	-26.21	0.87	4.68
187000	1513.4	-711.45	1672.2	-25.18	1654.226	-26.38	1.09	4.55
199000	1497.7	-712.86	1658.7	-25.45	1637.683	-26.62	1.28	4.40
212000	1480.5	-715.88	1644.5	-25.81	1620.189	-26.94	1.50	4.22
226000	1461.5	-720.41	1629.4	-26.24	1602.991	-27.30	1.65	3.89
240000	1442.1	-725.85	1614.4	-26.72	1585.905	-27.69	1.80	3.52
256000	1419.5	-732.78	1597.5	-27.30	1567.567	-28.19	1.91	3.15
272000	1396.5	-740.13	1580.5	-27.92	1548.597	-28.71	2.06	2.74
289000	1371.8	-748.09	1562.5	-28.61	1529.891	-29.35	2.13	2.53
308000	1343.9	-756.87	1542.3	-29.39	1509.583	-30.03	2.17	2.14
328000	1314.2	-765.7	1521	-30.23	1488.142	-30.65	2.21	1.39
349000	1283	-774.29	1498.6	-31.11	1466.606	-31.44	2.18	1.04
371000	1250.4	-782.38	1475	-32.03	1443.646	-32.24	2.17	0.64
395000	1215.1	-790.01	1449.3	-33.03	1419.444	-33.10	2.10	0.22

(Part 7 of 7)								
420000	1178.7	-796.55	1422.6	-34.05	1394.325	-34.00	2.03	0.14
447000	1140.1	-801.97	1393.9	-35.12	1369.131	-34.94	1.81	0.54
476000	1099.6	-805.94	1363.3	-36.24	1342.105	-35.89	1.58	0.98
506000	1059	-808.12	1332.1	-37.35	1314.723	-36.93	1.32	1.13
539000	1016	-808.43	1298.4	-38.51	1286.749	-37.97	0.91	1.41
573000	973.63	-806.68	1264.4	-39.64	1257.215	-39.02	0.57	1.60
610000	929.84	-802.68	1228.4	-40.80	1227.069	-40.17	0.11	1.58
649000	886.34	-796.45	1191.6	-41.94	1195.873	-41.27	0.36	1.62
691000	842.53	-787.83	1153.5	-43.08	1164.13	-42.44	0.91	1.51
735000	799.92	-777.08	1115.2	-44.17	1131.602	-43.58	1.45	1.35
782000	757.96	-764.12	1076.3	-45.23	1098.619	-44.78	2.03	1.01
833000	716.33	-748.8	1036.3	-46.27	1064.805	-45.92	2.68	0.77
886000	677.04	-731.94	997.06	-47.23	1031.917	-47.12	3.38	0.24
943000	638.91	-713.19	957.52	-48.14	997.673	-48.28	4.02	0.29
1000000	604.64	-694.16	920.57	-48.94	963.9	-49.43	4.50	0.98

Table A.5: The equivalent circuit fit, measured impedance spectrum captured with 200 μm diameter electrodes and their respective relative errors for cell culture medium before the onset of shear stress (17.6 dyne/cm²).

	Cell Culture Medium at 17.6 dyne/cm ² (part 1 of 7)							
	200 μm							
time	20 min after shear							
	FIT				MEASURED		$e_{\text{rel}} (\%)$	
f (Hz)	Z' (Ω)	Z'' (Ω)	$ Z $ (Ω)	ϕ ($^\circ$)	$ Z $ (Ω)	ϕ ($^\circ$)	$ Z $	ϕ_1
102	32161	-4.14E+05	4.15E+05	-85.56	419466.8	-86.44	1.03	1.03
108	30539	-3.92E+05	3.93E+05	-85.55	395248.2	-86.52	0.54	1.13
115	28855	-3.69E+05	3.70E+05	-85.53	372664.2	-86.20	0.63	0.77
122	27361	-3.49E+05	3.50E+05	-85.52	350114.3	-86.30	0.02	0.90
130	25846	-3.28E+05	3.29E+05	-85.50	331612.8	-86.41	0.65	1.05
139	24346	-3.08E+05	3.09E+05	-85.48	311203.3	-86.39	0.67	1.05
148	23024	-2.90E+05	2.91E+05	-85.47	292905.7	-85.98	0.59	0.60
157	21851	-2.74E+05	2.75E+05	-85.45	276679.6	-86.39	0.51	1.09
167	20692	-2.59E+05	2.60E+05	-85.43	259904.3	-86.12	0.14	0.80
178	19565	-2.43E+05	2.44E+05	-85.41	246501.4	-85.85	0.92	0.52
189	18566	-2.30E+05	2.31E+05	-85.38	231863.4	-86.00	0.51	0.72
201	17599	-2.17E+05	2.18E+05	-85.36	217915.4	-85.97	0.18	0.70
214	16670	-2.04E+05	2.05E+05	-85.33	204009.9	-86.00	0.45	0.77
228	15786	-1.92E+05	1.93E+05	-85.31	193650	-85.72	0.38	0.49
243	14950	-1.81E+05	1.82E+05	-85.28	181985	-86.26	0.23	1.14
258	14209	-1.71E+05	1.71E+05	-85.25	171991.4	-85.30	0.29	0.06
275	13464	-1.61E+05	1.61E+05	-85.21	160301.2	-85.25	0.67	0.04
292	12805	-1.52E+05	1.52E+05	-85.18	150660.2	-85.63	1.17	0.53
311	12151	-1.43E+05	1.44E+05	-85.14	142043.9	-85.68	1.05	0.63

(Part 2 of 7)								
331	11542	-1.35E+05	1.35E+05	-85.11	137274.6	-85.57	1.46	0.55
352	10976	-1.27E+05	1.28E+05	-85.06	127939.4	-86.06	0.28	1.16
375	10427	-1.20E+05	1.20E+05	-85.02	120365.9	-85.15	0.21	0.15
399	9920.2	-1.13E+05	1.13E+05	-84.97	113703.1	-85.54	0.42	0.66
424	9452	-1.06E+05	1.07E+05	-84.93	107100.5	-85.10	0.22	0.20
452	8987.7	-1.00E+05	1.01E+05	-84.87	100696.5	-85.49	0.15	0.72
480	8576.5	-94572	94961	-84.82	94826.9	-85.09	0.14	0.32
511	8172.6	-89096	89470	-84.76	89362.76	-85.21	0.12	0.53
544	7792.2	-83937	84298	-84.70	84194.73	-85.12	0.12	0.50
579	7435.1	-79094	79443	-84.63	79388.35	-85.02	0.07	0.46
616	7100.7	-74559	74897	-84.56	74625.06	-85.01	0.36	0.53
656	6780.8	-70220	70546	-84.48	70307.4	-84.85	0.34	0.43
698	6483.5	-66187	66504	-84.41	66257.44	-84.78	0.37	0.44
742	6207.3	-62441	62748	-84.32	62479.79	-84.68	0.43	0.42
790	5940.3	-58819	59119	-84.23	58966.05	-84.60	0.26	0.43
841	5689.4	-55415	55706	-84.14	55388.57	-84.38	0.57	0.28
894	5458.3	-52279	52564	-84.04	52181.89	-84.24	0.73	0.24
952	5234.2	-49239	49517	-83.93	49298.78	-84.27	0.44	0.40
1010	5035.3	-46541	46812	-83.83	46325	-84.11	1.05	0.33
1080	4823	-43662	43927	-83.70	43705.07	-83.85	0.51	0.18
1150	4636.1	-41125	41386	-83.57	41208.71	-83.74	0.43	0.21
1220	4470.1	-38873	39129	-83.44	38928.3	-83.67	0.52	0.27
1300	4301.8	-36590	36842	-83.30	36589.16	-83.54	0.69	0.29

(Part 3 of 7)								
1380	4152.6	-34566	34815	-83.15	34525.51	-83.33	0.84	0.22
1470	4003.7	-32547	32792	-82.99	32611.62	-83.10	0.55	0.13
1560	3871.6	-30755	30998	-82.83	30664.63	-82.82	1.09	0.01
1670	3729.1	-28822	29062	-82.63	28942.67	-82.77	0.41	0.17
1770	3614.5	-27268	27507	-82.45	27289.1	-82.54	0.80	0.11
1890	3492.7	-25616	25853	-82.24	25739.66	-82.32	0.44	0.10
2010	3385.1	-24157	24393	-82.02	24260.79	-82.10	0.54	0.09
2140	3281.8	-22757	22993	-81.79	22908.46	-81.77	0.37	0.02
2270	3190.1	-21514	21749	-81.57	21606.1	-81.53	0.66	0.04
2420	3096.3	-20242	20477	-81.30	20334.35	-81.34	0.70	0.04
2570	3013.1	-19115	19351	-81.04	19203.13	-80.89	0.77	0.18
2740	2929.6	-17984	18221	-80.75	18098.07	-80.76	0.68	0.01
2910	2855.7	-16982	17221	-80.46	17101.15	-80.43	0.70	0.03
3100	2782.4	-15990	16230	-80.13	16117.95	-80.02	0.70	0.14
3300	2714.1	-15066	15309	-79.79	15230.51	-79.69	0.52	0.13
3510	2650.6	-14207	14452	-79.43	14355.68	-79.30	0.67	0.17
3740	2589	-13374	13623	-79.04	13532.18	-78.90	0.67	0.19
3970	2534.4	-12636	12888	-78.66	12796.47	-78.46	0.72	0.25
4230	2479.7	-11896	12152	-78.23	12101.72	-78.12	0.42	0.14
4500	2429.3	-11217	11477	-77.78	11431.52	-77.64	0.40	0.18
4790	2381.4	-10570	10835	-77.30	10766.05	-77.23	0.64	0.10
5100	2336.1	-9958.4	10229	-76.80	10179.48	-76.69	0.49	0.14
5420	2294.6	-9399	9675.1	-76.28	9599.936	-76.08	0.78	0.27

(Part 4 of 7)								
5770	2254.3	-8856.7	9139.1	-75.72	9095.562	-75.52	0.48	0.26
6140	2216.6	-8349.3	8638.5	-75.13	8599.969	-74.82	0.45	0.41
6530	2181.4	-7875.4	8172	-74.52	8127.583	-74.22	0.55	0.40
6950	2147.7	-7423.4	7727.9	-73.86	7690.716	-73.54	0.48	0.44
7400	2115.8	-6994.9	7307.9	-73.17	7277.527	-72.84	0.42	0.45
7870	2086.2	-6598.6	6920.5	-72.46	6892.257	-72.12	0.41	0.46
8380	2057.7	-6217.9	6549.6	-71.69	6536.892	-71.36	0.19	0.46
8920	2031	-5861.4	6203.3	-70.89	6186.855	-70.53	0.27	0.50
9490	2006.1	-5528.3	5881	-70.06	5864.636	-69.60	0.28	0.66
10100	1982.3	-5212.7	5576.9	-69.18	5569.711	-68.80	0.13	0.55
10700	1961.6	-4936.7	5312.1	-68.33	5283.079	-67.89	0.55	0.65
11400	1940	-4650.8	5039.2	-67.36	5019.48	-66.95	0.39	0.60
12200	1918.3	-4363.6	4766.6	-66.27	4779.875	-65.91	0.28	0.55
12900	1901.4	-4141	4556.7	-65.34	4543.619	-64.89	0.29	0.69
13800	1882.1	-3887.6	4319.2	-64.17	4327.014	-63.78	0.18	0.60
14700	1865.1	-3664.7	4112	-63.03	4115.85	-62.72	0.09	0.49
15600	1849.9	-3467.2	3929.8	-61.92	3937.574	-61.47	0.20	0.73
16600	1834.9	-3272.6	3751.9	-60.72	3760.641	-60.28	0.23	0.74
17700	1820.3	-3083.6	3580.8	-59.45	3598.31	-59.08	0.49	0.63
18800	1807.3	-2916.5	3431.1	-58.22	3444.89	-57.79	0.40	0.74
20000	1794.6	-2755	3288	-56.92	3304.585	-56.58	0.50	0.60
21300	1782.4	-2600.5	3152.7	-55.57	3170.05	-55.21	0.55	0.66
22600	1771.5	-2463.7	3034.5	-54.28	3048.408	-53.83	0.46	0.83

(Part 5 of 7)								
24100	1760.3	-2324.1	2915.5	-52.86	2933.28	-52.48	0.61	0.73
25600	1750.3	-2200.9	2812.1	-51.51	2829.986	-51.14	0.63	0.71
27300	1740.2	-2077.7	2710.2	-50.05	2734.438	-49.76	0.89	0.59
29000	1731.2	-1969.1	2621.9	-48.68	2644.711	-48.36	0.86	0.66
30900	1722.1	-1861.9	2536.2	-47.23	2559.589	-46.98	0.91	0.55
32900	1713.6	-1762.7	2458.4	-45.81	2482.699	-45.66	0.98	0.33
35000	1705.5	-1671	2387.7	-44.41	2410.125	-44.27	0.93	0.32
37200	1697.9	-1586.3	2323.6	-43.05	2348.129	-42.90	1.04	0.37
39600	1690.4	-1504.9	2263.3	-41.68	2289.034	-41.61	1.12	0.16
42200	1683.1	-1427.7	2207.1	-40.31	2235.596	-40.32	1.27	0.03
44900	1676.2	-1357.3	2156.9	-39.00	2184.753	-39.08	1.27	0.20
47700	1669.7	-1293.3	2112	-37.76	2139.337	-37.85	1.28	0.25
50800	1663.1	-1231.1	2069.2	-36.51	2096.609	-36.72	1.31	0.55
54100	1656.6	-1173.4	2030.1	-35.31	2057.481	-35.61	1.33	0.85
57500	1650.5	-1121.5	1995.5	-34.20	2021.578	-34.55	1.29	1.01
61200	1644.3	-1072.3	1963	-33.11	1987.537	-33.55	1.23	1.31
65100	1638.2	-1027.2	1933.6	-32.09	1958.056	-32.59	1.25	1.52
69300	1632	-985.23	1906.3	-31.12	1929.98	-31.69	1.23	1.81
73800	1625.8	-946.42	1881.2	-30.21	1903.5	-30.87	1.17	2.16
78500	1619.6	-911.58	1858.5	-29.37	1878.992	-30.14	1.09	2.54
83500	1613.3	-879.82	1837.6	-28.61	1855.793	-29.43	0.98	2.79
88900	1606.7	-850.63	1818	-27.90	1834.59	-28.81	0.90	3.16
94600	1599.9	-824.57	1799.9	-27.27	1813.197	-28.27	0.73	3.57

(Part 6 of 7)								
101000	1592.6	-800.12	1782.3	-26.68	1795.615	-27.76	0.74	3.91
107000	1585.7	-781.02	1767.6	-26.22	1777.323	-27.32	0.55	4.03
114000	1577.9	-762.61	1752.5	-25.80	1758.928	-26.99	0.37	4.44
121000	1570	-747.64	1738.9	-25.46	1742.276	-26.66	0.19	4.50
129000	1561	-734.01	1725	-25.18	1726.553	-26.44	0.09	4.75
137000	1552	-723.42	1712.3	-24.99	1710.063	-26.31	0.13	5.00
146000	1541.7	-714.46	1699.2	-24.86	1694.258	-26.20	0.29	5.09
155000	1531.3	-708.07	1687.1	-24.82	1678.012	-26.15	0.54	5.09
165000	1519.6	-703.38	1674.5	-24.84	1662.609	-26.16	0.72	5.06
176000	1506.5	-700.58	1661.4	-24.94	1647.587	-26.25	0.84	4.98
187000	1493	-699.72	1648.9	-25.11	1631.576	-26.40	1.06	4.90
199000	1478.1	-700.52	1635.7	-25.36	1615.346	-26.60	1.26	4.68
212000	1461.5	-702.93	1621.8	-25.69	1597.238	-26.90	1.54	4.52
226000	1443.3	-706.86	1607.1	-26.09	1581.336	-27.26	1.63	4.27
240000	1424.8	-711.77	1592.7	-26.55	1564.368	-27.61	1.81	3.87
256000	1403.1	-718.18	1576.2	-27.11	1546.139	-28.09	1.94	3.50
272000	1381.1	-725.09	1559.9	-27.70	1527.619	-28.61	2.11	3.17
289000	1357.4	-732.66	1542.5	-28.36	1509.951	-29.22	2.16	2.95
308000	1330.6	-741.1	1523	-29.12	1489.176	-29.84	2.27	2.43
328000	1302.1	-749.67	1502.5	-29.93	1469.761	-30.51	2.23	1.88
349000	1272.1	-758.1	1480.8	-30.79	1448.082	-31.27	2.26	1.53
371000	1240.6	-766.11	1458.1	-31.70	1425.513	-32.06	2.29	1.13
395000	1206.5	-773.76	1433.3	-32.67	1401.676	-32.87	2.26	0.60

(Part 7 of 7)								
420000	1171.3	-780.43	1407.5	-33.68	1377.695	-33.76	2.16	0.25
447000	1133.9	-786.08	1379.7	-34.73	1353.094	-34.72	1.97	0.04
476000	1094.6	-790.39	1350.2	-35.83	1326.581	-35.65	1.78	0.51
506000	1055.2	-793	1319.9	-36.93	1299.652	-36.65	1.56	0.76
539000	1013.2	-793.86	1287.2	-38.08	1272.406	-37.69	1.16	1.04
573000	971.86	-792.72	1254.2	-39.20	1243.915	-38.74	0.83	1.20
610000	929	-789.43	1219.1	-40.36	1214.791	-39.85	0.35	1.27
649000	886.33	-783.96	1183.3	-41.49	1183.918	-40.98	0.05	1.24
691000	843.26	-776.15	1146.1	-42.63	1152.447	-42.13	0.55	1.18
735000	801.28	-766.24	1108.7	-43.72	1121.813	-43.27	1.17	1.04
782000	759.84	-754.13	1070.5	-44.78	1088.819	-44.43	1.68	0.79
833000	718.64	-739.67	1031.3	-45.83	1055.946	-45.60	2.33	0.50
886000	679.67	-723.65	992.79	-46.80	1024.361	-46.78	3.08	0.04
943000	641.78	-705.72	953.9	-47.72	990.491	-47.96	3.69	0.50
1000000	607.65	-687.43	917.5	-48.53	955.745	-49.07	4.00	1.11

Table A.6: The equivalent circuit fit, measured impedance spectrum captured with 200 μm diameter electrodes and their respective relative errors for cell culture medium 20 minutes after the onset of shear stress (17.6 dyne/cm^2).

	Cell Culture Medium at 17.6 dyne/cm ² (part 1 of 7)							
	200 μm							
time	6 hr after shear							
	FIT				MEASURED		$e_{\text{rel}}(\%)$	
$f(\text{Hz})$	$Z'(\Omega)$	$Z''(\Omega)$	$ Z (\Omega)$	$\phi(^{\circ})$	$ Z (\Omega)$	$\phi(^{\circ})$	$ Z $	ϕ
102	32317	-4.22E+05	4.23E+05	-85.62	424668.5	-85.56	0.29	0.07
108	30685	-4.00E+05	4.01E+05	-85.61	403724.3	-85.88	0.68	0.31
115	28991	-3.77E+05	3.78E+05	-85.60	383470.2	-86.37	1.52	0.89
122	27488	-3.56E+05	3.57E+05	-85.58	357251.1	-86.31	0.08	0.84
130	25963	-3.35E+05	3.36E+05	-85.57	337587.2	-86.16	0.47	0.68
139	24454	-3.14E+05	3.15E+05	-85.55	318683.8	-86.43	1.09	1.02
148	23125	-2.96E+05	2.97E+05	-85.53	299502	-86.26	0.86	0.84
157	21944	-2.80E+05	2.81E+05	-85.52	282385.5	-86.36	0.61	0.97
167	20779	-2.64E+05	2.65E+05	-85.50	266820.3	-86.25	0.82	0.87
178	19645	-2.48E+05	2.49E+05	-85.48	248589.7	-86.18	0.17	0.82
189	18640	-2.34E+05	2.35E+05	-85.45	235335.5	-86.24	0.07	0.91
201	17667	-2.21E+05	2.22E+05	-85.43	221524	-85.82	0.11	0.45
214	16733	-2.08E+05	2.09E+05	-85.41	208952.5	-85.96	0.02	0.64
228	15844	-1.96E+05	1.97E+05	-85.38	197157.5	-85.84	0.25	0.54
243	15003	-1.84E+05	1.85E+05	-85.35	185256.9	-86.02	0.10	0.78
258	14258	-1.74E+05	1.75E+05	-85.32	176439.5	-85.95	0.92	0.73
275	13509	-1.64E+05	1.64E+05	-85.29	164977.3	-85.46	0.30	0.20
292	12846	-1.55E+05	1.55E+05	-85.26	155286.7	-85.56	0.04	0.36
311	12189	-1.46E+05	1.46E+05	-85.22	146100.4	-85.56	0.13	0.39

(Part 2 of 7)								
331	11577	-1.37E+05	1.38E+05	-85.18	138209.6	-85.99	0.25	0.94
352	11008	-1.30E+05	1.30E+05	-85.14	129238.7	-85.76	0.60	0.72
375	10456	-1.22E+05	1.22E+05	-85.10	122907.1	-85.21	0.41	0.13
399	9946.2	-1.15E+05	1.15E+05	-85.06	115880.4	-85.59	0.43	0.63
424	9475.5	-1.08E+05	1.09E+05	-85.01	109292.5	-85.54	0.37	0.63
452	9008.8	-1.02E+05	1.02E+05	-84.96	102686.9	-85.62	0.23	0.77
480	8595.4	-96369	96751	-84.90	96491.09	-85.25	0.27	0.40
511	8189.5	-90785	91153	-84.85	90734.81	-85.24	0.46	0.46
544	7807.1	-85524	85880	-84.78	85518.55	-85.12	0.42	0.40
579	7448.2	-80586	80930	-84.72	80421.68	-85.08	0.63	0.42
616	7112.2	-75963	76295	-84.65	76076.94	-84.98	0.29	0.38
656	6790.6	-71539	71860	-84.58	71528.28	-85.03	0.46	0.53
698	6491.9	-67427	67739	-84.50	67731.98	-84.99	0.01	0.57
742	6214.4	-63608	63911	-84.42	63728.59	-84.64	0.29	0.26
790	5946.1	-59917	60211	-84.33	59981.24	-84.66	0.38	0.39
841	5694	-56446	56733	-84.24	56509.1	-84.52	0.40	0.33
894	5461.7	-53250	53530	-84.14	53307.84	-84.39	0.42	0.29
952	5236.6	-50152	50424	-84.04	50149.71	-84.28	0.55	0.29
1010	5036.8	-47401	47668	-83.94	47309.72	-84.04	0.76	0.12
1080	4823.5	-44467	44728	-83.81	44573.93	-83.96	0.35	0.18
1150	4635.7	-41882	42137	-83.68	41964.96	-83.80	0.41	0.14
1220	4469	-39587	39838	-83.56	39642.21	-83.72	0.49	0.19
1300	4300	-37260	37508	-83.42	37340.2	-83.52	0.45	0.12

(Part 3 of 7)								
1380	4150.1	-35198	35442	-83.28	35249.15	-83.46	0.55	0.22
1470	4000.5	-33140	33380	-83.12	33160.41	-83.27	0.66	0.18
1560	3867.9	-31314	31552	-82.96	31202.7	-83.12	1.12	0.19
1670	3724.7	-29345	29580	-82.77	29476.65	-82.86	0.35	0.11
1770	3609.7	-27762	27995	-82.59	27790.83	-82.65	0.73	0.07
1890	3487.4	-26079	26311	-82.38	26165.21	-82.33	0.56	0.06
2010	3379.3	-24592	24823	-82.18	24704.92	-82.19	0.48	0.02
2140	3275.6	-23166	23397	-81.95	23317.07	-81.99	0.34	0.04
2270	3183.5	-21900	22130	-81.73	21988.53	-81.74	0.64	0.02
2420	3089.3	-20604	20834	-81.47	20701.46	-81.58	0.64	0.13
2570	3005.8	-19456	19687	-81.22	19548.05	-81.06	0.71	0.20
2740	2922	-18304	18536	-80.93	18429.21	-80.96	0.58	0.04
2910	2847.7	-17284	17517	-80.64	17385.07	-80.57	0.76	0.09
3100	2774.1	-16273	16508	-80.33	16411.79	-80.29	0.59	0.05
3300	2705.6	-15332	15569	-79.99	15508.5	-79.87	0.39	0.15
3510	2641.9	-14457	14697	-79.64	14612.79	-79.63	0.58	0.01
3740	2580.1	-13609	13852	-79.27	13769.26	-79.15	0.60	0.15
3970	2525.3	-12858	13103	-78.89	12996.92	-78.71	0.82	0.23
4230	2470.3	-12104	12354	-78.47	12283.91	-78.35	0.57	0.15
4500	2419.8	-11412	11666	-78.03	11598.65	-77.86	0.58	0.22
4790	2371.7	-10754	11012	-77.56	10968.71	-77.36	0.39	0.26
5100	2326.2	-10131	10395	-77.07	10358.13	-76.78	0.36	0.38
5420	2284.6	-9561.5	9830.7	-76.56	9781.561	-76.31	0.50	0.33

(Part 4 of 7)								
5770	2244.2	-9009.4	9284.7	-76.01	9237.354	-75.79	0.51	0.30
6140	2206.4	-8492.9	8774.8	-75.44	8728.684	-75.21	0.53	0.30
6530	2171	-8010.4	8299.4	-74.84	8254.613	-74.62	0.54	0.29
6950	2137.2	-7550.3	7846.9	-74.20	7814.336	-73.89	0.42	0.41
7400	2105.2	-7114.1	7419	-73.52	7392.666	-73.17	0.36	0.47
7870	2075.5	-6710.7	7024.3	-72.81	6995.594	-72.51	0.41	0.42
8380	2047	-6323.2	6646.3	-72.06	6629.669	-71.75	0.25	0.44
8920	2020.2	-5960.3	6293.4	-71.28	6283.95	-70.97	0.15	0.44
9490	1995.1	-5621.2	5964.8	-70.46	5950.701	-70.15	0.24	0.44
10100	1971.4	-5300	5654.7	-69.60	5640.492	-69.39	0.25	0.31
10700	1950.5	-5019.1	5384.8	-68.76	5352.588	-68.33	0.60	0.63
11400	1928.9	-4728.1	5106.5	-67.81	5085.718	-67.41	0.41	0.59
12200	1907.1	-4435.8	4828.4	-66.74	4838.579	-66.46	0.21	0.42
12900	1890.2	-4209.3	4614.2	-65.82	4596.277	-65.38	0.39	0.67
13800	1870.8	-3951.4	4371.9	-64.66	4378.971	-64.37	0.16	0.45
14700	1853.8	-3724.5	4160.4	-63.54	4169.518	-63.19	0.22	0.55
15600	1838.6	-3523.5	3974.4	-62.45	3976.64	-62.09	0.06	0.57
16600	1823.5	-3325.5	3792.6	-61.26	3797.72	-60.88	0.13	0.62
17700	1808.9	-3133.1	3617.8	-60.00	3631.323	-59.63	0.37	0.62
18800	1795.8	-2963.1	3464.8	-58.78	3474.667	-58.41	0.28	0.63
20000	1783.2	-2798.8	3318.5	-57.50	3328.92	-57.11	0.31	0.67
21300	1770.9	-2641.5	3180.2	-56.16	3194.557	-55.78	0.45	0.69
22600	1760	-2502.2	3059.2	-54.88	3071.607	-54.52	0.40	0.66

(Part 5 of 7)								
24100	1748.8	-2360.2	2937.5	-53.46	2953.152	-53.13	0.53	0.62
25600	1738.8	-2234.8	2831.5	-52.12	2845.08	-51.78	0.48	0.64
27300	1728.7	-2109.4	2727.2	-50.67	2744.785	-50.31	0.64	0.70
29000	1719.6	-1998.7	2636.7	-49.29	2655.215	-48.93	0.70	0.74
30900	1710.6	-1889.7	2548.9	-47.85	2571.139	-47.51	0.86	0.71
32900	1702	-1788.6	2469	-46.42	2492.614	-46.19	0.95	0.50
35000	1694	-1695.2	2396.5	-45.02	2418.883	-44.83	0.93	0.42
37200	1686.4	-1608.9	2330.8	-43.65	2352.705	-43.47	0.93	0.43
39600	1678.9	-1526.1	2268.8	-42.27	2292.858	-42.17	1.05	0.24
42200	1671.6	-1447.3	2211.1	-40.89	2237.457	-40.87	1.18	0.05
44900	1664.7	-1375.6	2159.5	-39.57	2184.283	-39.62	1.13	0.12
47700	1658.2	-1310.3	2113.4	-38.32	2137.987	-38.40	1.15	0.22
50800	1651.6	-1246.9	2069.4	-37.05	2095.283	-37.20	1.24	0.40
54100	1645.1	-1188	2029.3	-35.84	2055.723	-36.07	1.29	0.66
57500	1639	-1135	1993.7	-34.70	2018.368	-35.01	1.22	0.87
61200	1632.8	-1084.8	1960.3	-33.60	1984.994	-33.98	1.24	1.12
65100	1626.8	-1038.7	1930.1	-32.56	1954.293	-33.01	1.24	1.37
69300	1620.7	-995.73	1902.1	-31.57	1924.661	-32.14	1.17	1.79
73800	1614.5	-955.99	1876.3	-30.63	1897.475	-31.28	1.12	2.06
78500	1608.3	-920.28	1853	-29.78	1872.719	-30.49	1.05	2.33
83500	1602.1	-887.69	1831.6	-28.99	1849.57	-29.79	0.97	2.69
88900	1595.6	-857.68	1811.5	-28.26	1827.815	-29.15	0.89	3.06
94600	1588.9	-830.84	1793	-27.61	1806.189	-28.52	0.73	3.21

(Part 6 of 7)								
101000	1581.6	-805.6	1775	-26.99	1788.754	-28.03	0.77	3.71
107000	1574.9	-785.84	1760.1	-26.52	1769.307	-27.59	0.52	3.87
114000	1567.1	-766.71	1744.6	-26.07	1752.392	-27.25	0.44	4.32
121000	1559.4	-751.09	1730.8	-25.72	1734.321	-26.89	0.20	4.34
129000	1550.5	-736.79	1716.7	-25.42	1717.34	-26.66	0.04	4.65
137000	1541.7	-725.58	1703.9	-25.20	1701.484	-26.48	0.14	4.82
146000	1531.6	-716	1690.7	-25.06	1686.499	-26.37	0.25	4.99
155000	1521.4	-709.03	1678.5	-24.99	1669.648	-26.28	0.53	4.93
165000	1509.8	-703.76	1665.8	-24.99	1655.155	-26.29	0.64	4.93
176000	1496.9	-700.38	1652.7	-25.07	1639.455	-26.37	0.81	4.90
187000	1483.7	-699	1640.2	-25.23	1623.49	-26.51	1.03	4.85
199000	1469.1	-699.27	1627	-25.45	1607.132	-26.72	1.24	4.72
212000	1452.8	-701.17	1613.2	-25.76	1589.406	-26.99	1.50	4.55
226000	1435	-704.61	1598.6	-26.15	1573.763	-27.32	1.58	4.26
240000	1416.7	-709.09	1584.3	-26.59	1556.675	-27.68	1.77	3.96
256000	1395.5	-715.07	1568	-27.13	1539.499	-28.15	1.85	3.61
272000	1373.9	-721.59	1551.8	-27.71	1520.675	-28.64	2.05	3.26
289000	1350.5	-728.81	1534.7	-28.35	1502.609	-29.23	2.14	3.01
308000	1324.2	-736.92	1515.4	-29.10	1482.693	-29.88	2.21	2.63
328000	1296.2	-745.21	1495.1	-29.90	1463.136	-30.53	2.18	2.09
349000	1266.7	-753.4	1473.8	-30.74	1441.11	-31.26	2.27	1.66
371000	1235.7	-761.23	1451.4	-31.63	1418.721	-32.04	2.30	1.27
395000	1202.1	-768.74	1426.9	-32.60	1396.253	-32.87	2.19	0.83

(Part 7 of 7)								
420000	1167.4	-775.31	1401.4	-33.59	1372.161	-33.74	2.13	0.44
447000	1130.5	-780.92	1374	-34.64	1347.779	-34.67	1.95	0.11
476000	1091.8	-785.24	1344.8	-35.73	1321.483	-35.64	1.76	0.24
506000	1052.8	-787.91	1315	-36.81	1294.895	-36.63	1.55	0.51
539000	1011.3	-788.87	1282.6	-37.96	1267.539	-37.67	1.19	0.75
573000	970.4	-787.88	1250	-39.07	1238.762	-38.71	0.91	0.95
610000	927.96	-784.78	1215.3	-40.22	1209.966	-39.80	0.44	1.05
649000	885.66	-779.53	1179.9	-41.35	1179.147	-40.91	0.06	1.09
691000	842.93	-771.96	1143	-42.48	1148.504	-42.06	0.48	1.01
735000	801.24	-762.32	1105.9	-43.57	1118.067	-43.20	1.09	0.86
782000	760.06	-750.49	1068.1	-44.64	1085.883	-44.34	1.64	0.67
833000	719.07	-736.33	1029.2	-45.68	1053.01	-45.50	2.26	0.39
886000	680.27	-720.6	990.97	-46.65	1020.153	-46.74	2.86	0.18
943000	642.51	-702.95	952.35	-47.57	985.942	-47.88	3.41	0.65
1000000	608.48	-684.93	916.17	-48.38	952.398	-49.02	3.80	1.30

Table A.7: The equivalent circuit fit, measured impedance spectrum captured with 200 μm diameter electrodes and their respective relative errors for cell culture medium 6 hours after the onset of shear stress (17.6 dyne/cm^2).

	HUVECs at 17.6 dyne/cm² (part 1 of 7)							
	100 μm							
time	Before Shear							
	FIT				MEASURED		e_{rel} (%)	
<i>f</i> (Hz)	<i>Z'</i> (Ω)	<i>Z''</i> (Ω)	 <i>Z</i> (Ω)	ϕ ($^{\circ}$)	 <i>Z</i> (Ω)	ϕ ($^{\circ}$)	 <i>Z</i> 	ϕ
102	2.54E+05	-1.67E+06	1.69E+06	-81.37	1672489	-80.05	-1.27	1.66
108	2.45E+05	-1.59E+06	1.61E+06	-81.22	1599941	-82.05	0.41	1.01
115	2.36E+05	-1.50E+06	1.52E+06	-81.04	1534945	-83.22	1.25	2.62
122	2.28E+05	-1.42E+06	1.44E+06	-80.87	1405887	-82.22	2.09	1.64
130	2.19E+05	-1.34E+06	1.35E+06	-80.67	1334331	-82.46	1.44	2.18
139	2.11E+05	-1.25E+06	1.27E+06	-80.45	1273135	-82.26	0.05	2.21
148	2.04E+05	-1.18E+06	1.20E+06	-80.23	1190987	-80.88	0.85	0.81
157	1.97E+05	-1.12E+06	1.14E+06	-80.01	1135216	-81.98	0.22	2.40
167	1.91E+05	-1.06E+06	1.08E+06	-79.77	1086338	-80.88	1.04	1.37
178	1.85E+05	-9.97E+05	1.01E+06	-79.51	1012625	-80.36	0.14	1.06
189	1.79E+05	-9.43E+05	9.60E+05	-79.25	953340.1	-80.75	0.69	1.86
201	1.74E+05	-8.91E+05	9.07E+05	-78.96	912273.5	-79.37	0.53	0.51
214	1.69E+05	-8.40E+05	8.57E+05	-78.66	841477.6	-79.55	1.86	1.12
228	1.64E+05	-7.93E+05	8.09E+05	-78.34	811545.5	-79.69	0.28	1.70
243	1.59E+05	-7.47E+05	7.64E+05	-78.00	745628.3	-80.05	2.45	2.56
258	1.55E+05	-7.07E+05	7.24E+05	-77.67	740854.3	-77.83	2.31	0.21
275	1.50E+05	-6.67E+05	6.83E+05	-77.29	678116.2	-75.57	0.78	2.27
292	1.47E+05	-6.31E+05	6.48E+05	-76.92	631232.6	-75.85	2.60	1.41
311	1.43E+05	-5.95E+05	6.12E+05	-76.51	588271.6	-75.79	4.08	0.95

(Part 2 of 7)								
331	1.39E+05	-5.62E+05	5.79E+05	-76.08	579755.2	-78.81	0.08	3.46
352	1.36E+05	-5.32E+05	5.49E+05	-75.64	529523.3	-76.55	3.62	1.18
375	1.33E+05	-5.02E+05	5.19E+05	-75.17	521772.7	-75.23	0.53	0.08
399	1.30E+05	-4.74E+05	4.92E+05	-74.69	490356.7	-76.22	0.26	2.02
424	1.27E+05	-4.49E+05	4.66E+05	-74.19	458336.5	-75.77	1.76	2.08
452	1.24E+05	-4.24E+05	4.41E+05	-73.65	441928.2	-73.87	0.12	0.30
480	1.22E+05	-4.01E+05	4.19E+05	-73.12	418601.6	-72.74	0.17	0.51
511	1.19E+05	-3.79E+05	3.98E+05	-72.54	388439.2	-71.59	2.38	1.33
544	1.17E+05	-3.59E+05	3.77E+05	-71.94	372414.5	-71.27	1.32	0.95
579	1.15E+05	-3.39E+05	3.58E+05	-71.33	352422.2	-70.66	1.67	0.95
616	1.13E+05	-3.21E+05	3.41E+05	-70.69	335923.2	-70.40	1.37	0.41
656	1.11E+05	-3.04E+05	3.24E+05	-70.03	321161.8	-69.12	0.75	1.31
698	1.09E+05	-2.88E+05	3.08E+05	-69.35	304636.5	-68.85	1.07	0.73
742	1.07E+05	-2.73E+05	2.93E+05	-68.67	291763.8	-67.69	0.56	1.45
790	1.05E+05	-2.59E+05	2.79E+05	-67.95	277458.2	-67.15	0.71	1.19
841	1.03E+05	-2.46E+05	2.66E+05	-67.22	265024.5	-66.42	0.51	1.21
894	1.01E+05	-2.33E+05	2.54E+05	-66.50	252069.1	-65.35	0.94	1.75
952	99790	-2.21E+05	2.43E+05	-65.74	242512.4	-64.22	0.16	2.37
1010	98264	-2.11E+05	2.33E+05	-65.03	232186.7	-64.94	0.24	0.13
1080	96574	-2.00E+05	2.22E+05	-64.21	226688.4	-64.67	2.09	0.72
1150	95022	-1.90E+05	2.13E+05	-63.44	215339.9	-62.74	1.30	1.13
1220	93584	-1.82E+05	2.04E+05	-62.73	205163.3	-62.29	0.46	0.70
1300	92056	-1.73E+05	1.96E+05	-61.96	195751.2	-60.30	0.05	2.75

(Part 3 of 7)								
1380	90630	-1.65E+05	1.88E+05	-61.26	189810.7	-61.22	0.70	0.06
1470	89126	-1.58E+05	1.81E+05	-60.53	186635.7	-59.35	2.92	1.99
1560	87709	-1.51E+05	1.75E+05	-59.87	175922.1	-59.59	0.67	0.47
1670	86076	-1.44E+05	1.68E+05	-59.15	172917.4	-59.05	2.94	0.17
1770	84668	-1.38E+05	1.62E+05	-58.56	166392.2	-58.40	2.45	0.26
1890	83058	-1.33E+05	1.56E+05	-57.93	160203.7	-58.06	2.34	0.22
2010	81518	-1.27E+05	1.51E+05	-57.40	154506.9	-57.24	2.08	0.28
2140	79915	-1.23E+05	1.46E+05	-56.90	149212.6	-56.98	1.93	0.14
2270	78370	-1.18E+05	1.42E+05	-56.48	143743.1	-57.41	1.27	1.62
2420	76646	-1.14E+05	1.37E+05	-56.09	140509.7	-56.90	2.22	1.41
2570	74976	-1.10E+05	1.33E+05	-55.79	135766.4	-56.49	1.79	1.25
2740	73138	-1.07E+05	1.29E+05	-55.53	132503.9	-56.99	2.48	2.57
2910	71353	-1.03E+05	1.26E+05	-55.36	126906.8	-56.24	1.09	1.58
3100	69412	-1.00E+05	1.22E+05	-55.25	123911.6	-56.80	1.72	2.73
3300	67426	-97100	1.18E+05	-55.22	119448	-56.68	1.04	2.57
3510	65398	-94352	1.15E+05	-55.27	116331.3	-56.80	1.32	2.68
3740	63243	-91685	1.11E+05	-55.40	110936.9	-56.45	0.40	1.86
3970	61153	-89303	1.08E+05	-55.60	107949.6	-57.15	0.26	2.71
4230	58868	-86884	1.05E+05	-55.88	104461.7	-57.65	0.47	3.07
4500	56581	-84616	1.02E+05	-56.23	101547.1	-57.88	0.24	2.85
4790	54222	-82399	98639	-56.65	97163.5	-58.66	1.52	3.41
5100	51809	-80226	95501	-57.15	93859.8	-58.73	1.75	2.70
5420	49436	-78152	92476	-57.68	91196.45	-59.18	1.40	2.53

(Part 4 of 7)								
5770	46975	-76041	89381	-58.29	87515.27	-59.74	2.13	2.43
6140	44521	-73953	86321	-58.95	84309.2	-60.23	2.39	2.12
6530	42095	-71883	83302	-59.65	80947.74	-60.61	2.91	1.59
6950	39657	-69779	80261	-60.39	77936.78	-61.15	2.98	1.24
7400	37237	-67646	77217	-61.17	75071.41	-61.60	2.86	0.70
7870	34907	-65535	74252	-61.96	71809.41	-62.34	3.40	0.61
8380	32592	-63365	71255	-62.78	69243.95	-63.02	2.90	0.38
8920	30361	-61191	68309	-63.61	66597.88	-63.57	2.57	0.07
9490	28230	-59022	65426	-64.44	63687.91	-64.41	2.73	0.05
10100	26178	-56834	62573	-65.27	60890.55	-64.91	2.76	0.56
10700	24365	-54806	59978	-66.03	58322.64	-65.67	2.84	0.55
11400	22476	-52586	57188	-66.86	55613.59	-66.23	2.83	0.95
12200	20579	-50226	54278	-67.72	53282.8	-66.98	1.87	1.10
12900	19115	-48304	51949	-68.41	50749.79	-67.69	2.36	1.06
13800	17463	-46013	49216	-69.22	48483.74	-68.32	1.51	1.32
14700	16032	-43907	46743	-69.94	46185.82	-68.91	1.21	1.50
15600	14785	-41968	44496	-70.59	43967.43	-69.50	1.20	1.57
16600	13581	-39990	42233	-71.24	41875.96	-70.18	0.85	1.52
17700	12441	-38005	39989	-71.87	39784.84	-70.71	0.51	1.64
18800	11460	-36198	37968	-72.43	37847.24	-71.36	0.32	1.51
20000	10538	-34405	35983	-72.97	35986.43	-71.90	0.01	1.49
21300	9684.1	-32648	34054	-73.48	34181.33	-72.53	0.37	1.31
22600	8951.8	-31059	32323	-73.92	32449.82	-73.03	0.39	1.22

(Part 5 of 7)								
24100	8229.6	-29407	30536	-74.37	30755.64	-73.60	0.71	1.04
25600	7613.9	-27922	28941	-74.75	29206.99	-74.11	0.91	0.86
27300	7019.3	-26413	27330	-75.12	27696.55	-74.57	1.32	0.73
29000	6512.7	-25063	25895	-75.43	26234.12	-75.02	1.29	0.56
30900	6029.5	-23713	24468	-75.73	24862.48	-75.53	1.59	0.27
32900	5596.3	-22448	23135	-76.00	23547.36	-75.98	1.75	0.03
35000	5207.5	-21264	21893	-76.24	22291.4	-76.39	1.79	0.20
37200	4857.8	-20159	20736	-76.45	21102.46	-76.79	1.74	0.44
39600	4529.8	-19085	19615	-76.65	19963.04	-77.16	1.74	0.67
42200	4224.3	-18053	18540	-76.83	18876.79	-77.56	1.78	0.95
44900	3950.3	-17101	17552	-76.99	17872.51	-78.03	1.79	1.32
47700	3703.2	-16224	16641	-77.14	16892.59	-78.29	1.49	1.47
50800	3464.4	-15361	15747	-77.29	15969.73	-78.64	1.39	1.71
54100	3242.3	-14546	14903	-77.43	15104.72	-78.96	1.34	1.93
57500	3041	-13800	14131	-77.57	14269.45	-79.25	0.97	2.12
61200	2847.6	-13078	13385	-77.72	13489.67	-79.51	0.78	2.26
65100	2667	-12402	12685	-77.86	12752.63	-79.80	0.53	2.43
69300	2494.4	-11753	12015	-78.02	12001.91	-79.98	0.11	2.45
73800	2330	-11136	11377	-78.18	11337.55	-80.23	0.35	2.55
78500	2176.9	-10561	10783	-78.35	10700.04	-80.40	0.78	2.55
83500	2031.6	-10015	10219	-78.53	10102.59	-80.69	1.15	2.67
88900	1891.7	-9489	9675.7	-78.73	9537.906	-80.87	1.44	2.65
94600	1760.2	-8993	9163.6	-78.93	9051.647	-81.08	1.24	2.66

(Part 6 of 7)								
101000	1629.2	-8496.6	8651.4	-79.15	8504.551	-81.26	1.73	2.61
107000	1520.1	-8079.8	8221.5	-79.35	8034.007	-81.33	2.33	2.44
114000	1407	-7643.3	7771.7	-79.57	7545.121	-81.69	3.00	2.60
121000	1307.1	-7252.1	7368.9	-79.78	7152.695	-81.80	3.02	2.46
129000	1206.5	-6851.5	6956.9	-80.01	6743.756	-81.76	3.16	2.14
137000	1118.2	-6492.8	6588.4	-80.23	6362.502	-81.92	3.55	2.07
146000	1031.4	-6131.4	6217.5	-80.45	5995.631	-81.99	3.70	1.87
155000	955.82	-5807.7	5885.9	-80.65	5636.289	-82.18	4.43	1.86
165000	882.76	-5485.5	5556	-80.86	5343.787	-82.00	3.97	1.39
176000	813.51	-5169.3	5233	-81.06	5040.053	-82.17	3.83	1.36
187000	753.92	-4887.1	4944.9	-81.23	4755.873	-82.24	3.97	1.23
199000	697.99	-4611.8	4664.4	-81.39	4484.262	-82.28	4.02	1.08
212000	646.2	-4346.1	4393.8	-81.54	4229.001	-82.22	3.90	0.82
226000	598.77	-4091.6	4135.1	-81.67	3984.981	-82.27	3.77	0.72
240000	558.39	-3864.8	3904.9	-81.78	3762.341	-82.26	3.79	0.58
256000	519.22	-3634.1	3671	-81.87	3549.391	-82.26	3.43	0.48
272000	486.08	-3429	3463.2	-81.93	3346.852	-82.24	3.48	0.37
289000	456.18	-3234.7	3266.7	-81.97	3159.546	-82.34	3.39	0.44
308000	427.99	-3041.7	3071.6	-81.99	2981.981	-82.33	3.01	0.42
328000	403.07	-2861.7	2889.9	-81.98	2813.715	-82.23	2.71	0.30
349000	381.09	-2694	2720.8	-81.95	2654.76	-82.16	2.49	0.26
371000	361.73	-2538	2563.7	-81.89	2505.539	-82.07	2.32	0.22
395000	343.99	-2387.1	2411.7	-81.80	2361.436	-82.00	2.13	0.24

(Part 7 of 7)								
420000	328.51	-2247.7	2271.5	-81.69	2229.074	-81.94	1.90	0.31
447000	314.51	-2114.2	2137.4	-81.54	2106.329	-81.90	1.48	0.43
476000	301.95	-1987.3	2010.1	-81.36	1987.309	-81.78	1.15	0.51
506000	291.09	-1871	1893.5	-81.16	1876.646	-81.67	0.90	0.62
539000	281.12	-1757.8	1780.2	-80.91	1772.761	-81.59	0.42	0.83
573000	272.55	-1654.6	1676.9	-80.65	1673.485	-81.48	0.20	1.03
610000	264.77	-1555.2	1577.6	-80.34	1580.517	-81.36	0.18	1.25
649000	257.94	-1462.5	1485.1	-80.00	1492.977	-81.21	0.53	1.50
691000	251.81	-1374.3	1397.1	-79.62	1410.363	-81.09	0.94	1.81
735000	246.47	-1292.5	1315.8	-79.20	1332.827	-80.97	1.28	2.18
782000	241.72	-1215.3	1239.1	-78.75	1258.798	-80.75	1.56	2.48
833000	237.44	-1141.3	1165.7	-78.25	1189.735	-80.72	2.02	3.06
886000	233.74	-1073.3	1098.4	-77.71	1124.325	-80.52	2.31	3.48
943000	230.43	-1008.7	1034.7	-77.13	1063.412	-80.36	2.70	4.02
1000000	227.66	-951.38	978.24	-76.54	1005.122	-80.19	2.67	4.54

Table A.8: The equivalent circuit fit, measured impedance spectrum captured with 100 μm diameter electrodes and their respective relative errors for HUVECs before the onset of shear stress (17.6 dyne/cm²).

	HUVECs at 17.6 dyne/cm² (part 1 of 7)							
	100 μm							
time	20 min after shear							
	FIT				MEASURED		e_{rel}(%)	
<i>f</i>(Hz)	<i>Z'</i> (Ω)	<i>Z''</i> (Ω)	 <i>Z</i> (Ω)	ϕ ($^{\circ}$)	 <i>Z</i> (Ω)	ϕ ($^{\circ}$)	 <i>Z</i> 	ϕ
102	2.05E+05	-1.73E+06	1.74E+06	-83.21	1648649	-88.02	5.46	5.46
108	1.98E+05	-1.64E+06	1.65E+06	-83.10	1585385	-86.21	3.92	3.61
115	1.90E+05	-1.54E+06	1.55E+06	-82.97	1570127	-83.24	1.09	0.33
122	1.83E+05	-1.46E+06	1.47E+06	-82.83	1457864	-83.82	0.76	1.17
130	1.76E+05	-1.37E+06	1.38E+06	-82.68	1388856	-82.36	0.36	0.39
139	1.69E+05	-1.29E+06	1.30E+06	-82.52	1310840	-82.19	0.87	0.40
148	1.63E+05	-1.21E+06	1.23E+06	-82.35	1231222	-82.55	0.50	0.24
157	1.58E+05	-1.15E+06	1.16E+06	-82.18	1165727	-84.20	0.57	2.39
167	1.52E+05	-1.08E+06	1.09E+06	-82.00	1081088	-82.10	1.19	0.12
178	1.47E+05	-1.02E+06	1.03E+06	-81.80	1031205	-82.16	0.07	0.44
189	1.42E+05	-9.64E+05	9.74E+05	-81.60	974733	-82.33	0.05	0.89
201	1.38E+05	-9.09E+05	9.20E+05	-81.38	904275.7	-81.64	1.72	0.32
214	1.34E+05	-8.57E+05	8.68E+05	-81.15	854113.1	-82.31	1.58	1.41
228	1.29E+05	-8.08E+05	8.18E+05	-80.90	824415.7	-81.27	0.79	0.46
243	1.25E+05	-7.61E+05	7.71E+05	-80.64	761953.6	-82.55	1.17	2.32
258	1.22E+05	-7.19E+05	7.29E+05	-80.37	737618.7	-81.03	1.14	0.81
275	1.18E+05	-6.77E+05	6.87E+05	-80.08	679632.5	-79.20	1.13	1.12
292	1.15E+05	-6.40E+05	6.50E+05	-79.79	639421.2	-79.06	1.70	0.92
311	1.12E+05	-6.03E+05	6.14E+05	-79.46	591954.9	-78.38	3.65	1.39

(Part 2 of 7)								
331	1.09E+05	-5.69E+05	5.79E+05	-79.13	583654.9	-80.15	0.72	1.27
352	1.07E+05	-5.37E+05	5.48E+05	-78.78	541232.3	-81.16	1.19	2.94
375	1.04E+05	-5.06E+05	5.17E+05	-78.40	512534.1	-78.03	0.86	0.47
399	1.02E+05	-4.78E+05	4.89E+05	-78.01	481354.7	-78.09	1.50	0.11
424	99263	-4.52E+05	4.62E+05	-77.60	465257.8	-77.26	0.61	0.45
452	97004	-4.26E+05	4.36E+05	-77.16	434288.8	-76.76	0.51	0.52
480	94990	-4.03E+05	4.14E+05	-76.72	400012.2	-76.80	3.39	0.10
511	93000	-3.80E+05	3.91E+05	-76.24	387567.3	-76.23	0.91	0.02
544	91111	-3.59E+05	3.70E+05	-75.74	363828.5	-75.68	1.69	0.09
579	89324	-3.39E+05	3.50E+05	-75.22	342056.9	-74.55	2.38	0.90
616	87634	-3.20E+05	3.32E+05	-74.68	326132.9	-74.21	1.71	0.63
656	86001	-3.02E+05	3.14E+05	-74.11	310964.8	-72.98	1.00	1.55
698	84466	-2.86E+05	2.98E+05	-73.52	295827.9	-73.15	0.65	0.51
742	83022	-2.70E+05	2.83E+05	-72.92	279166.5	-72.06	1.24	1.19
790	81606	-2.55E+05	2.68E+05	-72.28	265264.3	-71.64	1.06	0.88
841	80253	-2.41E+05	2.54E+05	-71.61	252734.6	-70.58	0.67	1.47
894	78985	-2.29E+05	2.42E+05	-70.95	239697.1	-69.79	0.94	1.66
952	77732	-2.16E+05	2.30E+05	-70.24	229626.5	-69.72	0.11	0.74
1010	76594	-2.05E+05	2.19E+05	-69.55	216430.3	-68.97	1.29	0.84
1080	75350	-1.94E+05	2.08E+05	-68.76	206604	-68.24	0.65	0.76
1150	74222	-1.84E+05	1.98E+05	-67.99	198757.4	-67.31	0.35	1.01
1220	73190	-1.75E+05	1.89E+05	-67.26	189793.6	-66.12	0.24	1.72
1300	72109	-1.66E+05	1.81E+05	-66.46	180308	-65.41	0.15	1.61

(Part 3 of 7)								
1380	71113	-1.58E+05	1.73E+05	-65.71	174977.3	-64.89	1.22	1.25
1470	70079	-1.50E+05	1.65E+05	-64.90	166706.4	-63.73	0.90	1.83
1560	69119	-1.43E+05	1.58E+05	-64.14	160057.2	-62.97	0.99	1.86
1670	68029	-1.35E+05	1.51E+05	-63.28	153369.1	-62.35	1.36	1.48
1770	67104	-1.29E+05	1.46E+05	-62.55	148506.5	-61.83	1.99	1.16
1890	66060	-1.23E+05	1.40E+05	-61.74	142269.3	-61.24	1.95	0.82
2010	65076	-1.17E+05	1.34E+05	-60.99	137049.2	-60.56	2.07	0.72
2140	64065	-1.12E+05	1.29E+05	-60.26	132490.8	-60.27	2.51	0.01
2270	63100	-1.08E+05	1.25E+05	-59.61	126815.3	-59.65	1.65	0.08
2420	62035	-1.03E+05	1.20E+05	-58.93	123101.9	-59.20	2.37	0.45
2570	61011	-98899	1.16E+05	-58.33	118451	-58.58	1.90	0.43
2740	59892	-94893	1.12E+05	-57.74	115132	-58.32	2.54	1.00
2910	58808	-91394	1.09E+05	-57.24	110820.7	-57.73	1.93	0.85
3100	57632	-87979	1.05E+05	-56.77	106996.6	-57.53	1.70	1.31
3300	56427	-84846	1.02E+05	-56.37	103731.3	-57.58	1.77	2.09
3510	55194	-81974	98823	-56.05	99732.39	-57.12	0.91	1.89
3740	53874	-79227	95809	-55.78	97076.84	-57.09	1.31	2.28
3970	52584	-76820	93093	-55.61	93923.63	-57.20	0.88	2.77
4230	51159	-74430	90316	-55.50	90755.64	-57.05	0.48	2.72
4500	49713	-72248	87699	-55.47	88056.83	-56.99	0.41	2.67
4790	48198	-70177	85134	-55.52	85099.7	-57.13	0.04	2.82
5100	46621	-68214	82623	-55.65	82391.3	-57.27	0.28	2.82
5420	45039	-66402	80236	-55.85	79568.11	-57.52	0.84	2.89

(Part 4 of 7)								
5770	43363	-64620	77821	-56.14	76889.98	-57.66	1.21	2.64
6140	41654	-62914	75453	-56.49	74358.93	-58.03	1.47	2.65
6530	39921	-61272	73130	-56.91	71794.36	-58.31	1.86	2.39
6950	38136	-59646	70796	-57.41	69349.27	-58.58	2.09	1.99
7400	36315	-58034	68460	-57.96	66952.17	-59.12	2.25	1.96
7870	34514	-56464	66177	-58.56	64557.9	-59.58	2.51	1.70
8380	32674	-54867	63859	-59.23	62190.89	-59.86	2.68	1.06
8920	30851	-53276	61564	-59.93	59803.68	-60.44	2.94	0.85
9490	29061	-51690	59299	-60.65	57512.01	-61.10	3.11	0.73
10100	27289	-50083	57035	-61.42	55353.3	-61.70	3.04	0.46
10700	25682	-48584	54954	-62.14	53161.6	-62.27	3.37	0.22
11400	23965	-46926	52691	-62.95	51133.56	-62.81	3.05	0.22
12200	22193	-45140	50301	-63.82	48976.88	-63.49	2.70	0.51
12900	20793	-43666	48364	-64.54	46979.08	-64.17	2.95	0.58
13800	19176	-41882	46063	-65.40	45057.29	-64.84	2.23	0.86
14700	17742	-40215	43955	-66.19	43083.64	-65.41	2.02	1.20
15600	16468	-38656	42017	-66.93	41203.83	-66.08	1.97	1.28
16600	15214	-37040	40043	-67.67	39350.24	-66.77	1.76	1.35
17700	14005	-35394	38065	-68.41	37542.29	-67.43	1.39	1.45
18800	12948	-33874	36264	-69.08	35867.22	-68.01	1.11	1.57
20000	11941	-32345	34478	-69.74	34221.98	-68.70	0.75	1.51
21300	10994	-30826	32728	-70.37	32572.06	-69.32	0.48	1.52
22600	10172	-29436	31144	-70.94	31045.14	-69.90	0.32	1.49

(Part 5 of 7)								
24100	9353.9	-27973	29496	-71.51	29532.89	-70.54	0.12	1.38
25600	8649.6	-26645	28014	-72.02	28071.89	-71.15	0.21	1.22
27300	7964.2	-25283	26507	-72.52	26716.55	-71.75	0.78	1.06
29000	7376.5	-24052	25158	-72.95	25347.42	-72.35	0.75	0.84
30900	6813.4	-22813	23809	-73.37	24064.75	-72.85	1.06	0.71
32900	6306.9	-21642	22542	-73.75	22833.53	-73.43	1.28	0.45
35000	5851.5	-20539	21356	-74.10	21670.98	-73.97	1.45	0.17
37200	5441.8	-19503	20248	-74.41	20543.14	-74.39	1.44	0.03
39600	5058	-18492	19171	-74.70	19474.45	-74.88	1.56	0.24
42200	4701.4	-17515	18135	-74.98	18460.19	-75.35	1.76	0.50
44900	4382.9	-16612	17180	-75.22	17490.54	-75.94	1.78	0.95
47700	4096.9	-15776	16299	-75.44	16572.78	-76.36	1.65	1.21
50800	3822.4	-14951	15431	-75.66	15672.17	-76.69	1.54	1.35
54100	3568.8	-14170	14612	-75.86	14833.94	-77.07	1.50	1.56
57500	3340.7	-13454	13863	-76.06	14049.52	-77.42	1.33	1.76
61200	3123.1	-12760	13137	-76.25	13275.09	-77.83	1.04	2.03
65100	2921.4	-12109	12456	-76.44	12545.24	-78.14	0.71	2.18
69300	2730	-11485	11805	-76.63	11850.67	-78.42	0.39	2.28
73800	2548.8	-10889	11184	-76.83	11198.85	-78.75	0.13	2.44
78500	2381	-10335	10606	-77.03	10574.79	-79.02	0.30	2.52
83500	2222.5	-9808.8	10057	-77.23	9994.877	-79.29	0.62	2.60
88900	2070.3	-9301.2	9528.9	-77.45	9440.028	-79.50	0.94	2.57
94600	1927.5	-8822.7	9030.8	-77.68	8936.561	-79.99	1.05	2.89

(Part 6 of 7)								
101000	1785.5	-8343.6	8532.5	-77.92	8429.013	-80.12	1.23	2.74
107000	1667.2	-7941.1	8114.2	-78.14	7968.029	-80.26	1.83	2.64
114000	1544.5	-7519.4	7676.4	-78.39	7502.594	-80.62	2.32	2.76
121000	1435.8	-7141.1	7284	-78.63	7086.208	-80.58	2.79	2.42
129000	1326.2	-6753.4	6882.4	-78.89	6690.206	-80.80	2.87	2.37
137000	1229.9	-6405.8	6522.8	-79.13	6309.914	-80.93	3.37	2.23
146000	1134.8	-6055.2	6160.6	-79.39	5958.058	-81.14	3.40	2.17
155000	1051.6	-5740.7	5836.2	-79.62	5622.521	-81.31	3.80	2.08
165000	971	-5427.1	5513.3	-79.86	5310.507	-81.36	3.82	1.85
176000	894.32	-5119	5196.5	-80.09	5012.081	-81.46	3.68	1.68
187000	828.09	-4843.4	4913.7	-80.30	4733.052	-81.56	3.82	1.54
199000	765.72	-4574.2	4637.9	-80.50	4462.297	-81.63	3.94	1.39
212000	707.77	-4313.9	4371.6	-80.68	4204.839	-81.63	3.97	1.16
226000	654.52	-4064.2	4116.5	-80.85	3967.714	-81.70	3.75	1.04
240000	609.05	-3841.3	3889.3	-80.99	3744.644	-81.73	3.86	0.91
256000	564.81	-3614.2	3658.1	-81.12	3532.435	-81.77	3.56	0.80
272000	527.28	-3412.1	3452.6	-81.22	3334.611	-81.78	3.54	0.69
289000	493.35	-3220.3	3257.9	-81.29	3144.432	-81.90	3.61	0.74
308000	461.28	-3029.6	3064.5	-81.34	2966.476	-81.87	3.30	0.64
328000	432.87	-2851.5	2884.2	-81.37	2799.735	-81.84	3.02	0.58
349000	407.76	-2685.5	2716.2	-81.37	2642.655	-81.77	2.78	0.49
371000	385.61	-2530.8	2560	-81.34	2494.41	-81.73	2.63	0.49
395000	365.29	-2381	2408.9	-81.28	2352.336	-81.66	2.40	0.47

(Part 7 of 7)								
420000	347.53	-2242.6	2269.4	-81.19	2220.689	-81.59	2.19	0.49
447000	331.44	-2109.9	2135.8	-81.07	2097.3	-81.55	1.84	0.59
476000	316.99	-1983.7	2008.9	-80.92	1979.186	-81.47	1.50	0.67
506000	304.49	-1868.1	1892.7	-80.74	1868.967	-81.36	1.27	0.76
539000	293	-1755.4	1779.6	-80.52	1764.662	-81.27	0.85	0.91
573000	283.12	-1652.5	1676.6	-80.28	1667.256	-81.18	0.56	1.11
610000	274.14	-1553.5	1577.5	-79.99	1574.94	-81.02	0.16	1.27
649000	266.25	-1461.1	1485.1	-79.67	1487.096	-80.96	0.13	1.59
691000	259.17	-1373.1	1397.3	-79.31	1404.673	-80.81	0.52	1.86
735000	253	-1291.5	1316.1	-78.92	1327.293	-80.71	0.84	2.22
782000	247.5	-1214.5	1239.4	-78.48	1255.613	-80.59	1.29	2.62
833000	242.54	-1140.6	1166.1	-78.00	1186.694	-80.44	1.74	3.04
886000	238.26	-1072.7	1098.9	-77.48	1121.01	-80.27	1.97	3.48
943000	234.43	-1008.2	1035.1	-76.91	1059.159	-80.13	2.27	4.01
1000000	231.23	-950.98	978.69	-76.33	1001.997	-79.97	2.33	4.55

Table A.9: The equivalent circuit fit, measured impedance spectrum captured with 100 μm diameter electrodes and their respective relative errors for HUVECs 20 minutes after the application of shear stress (17.6 dyne/cm²).

	HUVECs at 17.6 dyne/cm ² (part 1 of 7)							
	100 μ m							
time	6 hr after shear							
	FIT				MEASURED		$e_{rel}(\%)$	
f (Hz)	Z' (Ω)	Z'' (Ω)	$ Z $ (Ω)	ϕ ($^{\circ}$)	$ Z $ (Ω)	ϕ ($^{\circ}$)	$ Z $	ϕ
102	2.13E+05	-1.75E+06	1.76E+06	-83.07	1811458	-82.15	2.60	1.12
108	2.05E+05	-1.66E+06	1.67E+06	-82.95	1687921	-82.60	0.94	0.42
115	1.97E+05	-1.56E+06	1.58E+06	-82.81	1590869	-83.67	0.93	1.03
122	1.90E+05	-1.48E+06	1.49E+06	-82.68	1518624	-84.34	1.82	1.97
130	1.83E+05	-1.39E+06	1.40E+06	-82.53	1405250	-82.91	0.04	0.47
139	1.75E+05	-1.31E+06	1.32E+06	-82.36	1330064	-83.41	0.82	1.26
148	1.69E+05	-1.23E+06	1.24E+06	-82.19	1251383	-83.85	0.60	1.99
157	1.63E+05	-1.17E+06	1.18E+06	-82.02	1181277	-83.34	0.37	1.58
167	1.58E+05	-1.10E+06	1.11E+06	-81.83	1115612	-83.23	0.42	1.68
178	1.52E+05	-1.04E+06	1.05E+06	-81.63	1041165	-83.10	0.52	1.77
189	1.48E+05	-9.79E+05	9.90E+05	-81.43	995837.2	-81.14	0.62	0.35
201	1.43E+05	-9.23E+05	9.34E+05	-81.21	943592.4	-82.02	0.97	0.99
214	1.38E+05	-8.71E+05	8.81E+05	-80.97	882365.5	-81.17	0.10	0.25
228	1.34E+05	-8.20E+05	8.31E+05	-80.72	835094.5	-81.21	0.48	0.61
243	1.30E+05	-7.73E+05	7.83E+05	-80.45	776574.7	-80.63	0.88	0.23
258	1.26E+05	-7.30E+05	7.41E+05	-80.18	738446.6	-80.94	0.36	0.93
275	1.23E+05	-6.88E+05	6.99E+05	-79.89	698149.6	-81.04	0.08	1.43
292	1.19E+05	-6.50E+05	6.61E+05	-79.59	653317.1	-79.75	1.20	0.20
311	1.16E+05	-6.13E+05	6.24E+05	-79.26	625117.5	-79.41	0.19	0.18

(Part 2 of 7)								
331	1.13E+05	-5.78E+05	5.89E+05	-78.92	575773.2	-80.10	2.35	1.47
352	1.10E+05	-5.46E+05	5.57E+05	-78.57	548058.1	-79.13	1.65	0.71
375	1.08E+05	-5.15E+05	5.26E+05	-78.19	522967.1	-78.61	0.56	0.54
399	1.05E+05	-4.86E+05	4.97E+05	-77.79	487755.7	-78.60	1.92	1.03
424	1.03E+05	-4.59E+05	4.71E+05	-77.39	461793.6	-78.84	1.90	1.85
452	1.00E+05	-4.33E+05	4.44E+05	-76.94	440291.5	-77.36	0.91	0.55
480	98322	-4.09E+05	4.21E+05	-76.50	415158.8	-76.74	1.42	0.32
511	96247	-3.86E+05	3.98E+05	-76.02	393219.2	-76.66	1.28	0.83
544	94277	-3.65E+05	3.77E+05	-75.51	369347.9	-74.92	2.03	0.79
579	92411	-3.45E+05	3.57E+05	-74.99	351965.6	-74.31	1.37	0.92
616	90647	-3.26E+05	3.38E+05	-74.45	331584.3	-74.48	1.95	0.04
656	88941	-3.08E+05	3.20E+05	-73.87	314532.3	-73.48	1.79	0.54
698	87335	-2.91E+05	3.04E+05	-73.28	299947.1	-72.25	1.22	1.42
742	85824	-2.75E+05	2.88E+05	-72.68	283122.7	-71.82	1.81	1.19
790	84340	-2.60E+05	2.73E+05	-72.04	269396.2	-71.21	1.52	1.16
841	82922	-2.46E+05	2.60E+05	-71.38	256481.5	-71.04	1.24	0.48
894	81591	-2.33E+05	2.47E+05	-70.71	244196.9	-69.95	1.15	1.08
952	80273	-2.21E+05	2.35E+05	-70.01	231197.2	-68.45	1.55	2.27
1010	79076	-2.10E+05	2.24E+05	-69.33	222886.9	-68.83	0.49	0.72
1080	77764	-1.98E+05	2.13E+05	-68.54	211943.6	-67.58	0.27	1.41
1150	76574	-1.87E+05	2.03E+05	-67.78	201523.8	-66.67	0.48	1.66
1220	75482	-1.78E+05	1.94E+05	-67.06	192401.4	-66.29	0.65	1.17
1300	74336	-1.69E+05	1.85E+05	-66.28	184785.5	-65.17	0.01	1.70

(Part 3 of 7)								
1380	73279	-1.61E+05	1.77E+05	-65.53	176628	-64.79	0.17	1.15
1470	72179	-1.53E+05	1.69E+05	-64.75	170484.9	-63.97	0.77	1.21
1560	71156	-1.46E+05	1.62E+05	-64.01	162759.1	-63.10	0.25	1.44
1670	69992	-1.38E+05	1.55E+05	-63.17	157162.4	-62.37	1.34	1.27
1770	69001	-1.32E+05	1.49E+05	-62.46	151254.6	-61.72	1.35	1.20
1890	67881	-1.26E+05	1.43E+05	-61.68	145434.5	-61.07	1.62	1.00
2010	66823	-1.20E+05	1.38E+05	-60.97	140175.2	-60.39	1.77	0.95
2140	65734	-1.15E+05	1.33E+05	-60.27	135156.9	-60.18	1.92	0.16
2270	64694	-1.10E+05	1.28E+05	-59.65	130670.2	-59.46	2.02	0.32
2420	63542	-1.06E+05	1.23E+05	-59.01	126125.4	-58.92	2.15	0.16
2570	62435	-1.02E+05	1.19E+05	-58.46	122027.1	-58.58	2.20	0.21
2740	61222	-97655	1.15E+05	-57.92	117850.1	-58.24	2.20	0.56
2910	60047	-94115	1.12E+05	-57.46	113859.6	-57.94	1.95	0.83
3100	58771	-90655	1.08E+05	-57.05	110264.3	-57.80	2.02	1.31
3300	57465	-87478	1.05E+05	-56.70	106611.3	-57.72	1.83	1.76
3510	56127	-84561	1.01E+05	-56.43	103032.9	-57.79	1.50	2.37
3740	54697	-81766	98374	-56.22	100146.6	-57.64	1.77	2.47
3970	53301	-79310	95557	-56.10	96648.98	-57.54	1.13	2.50
4230	51761	-76865	92668	-56.04	93500.27	-57.66	0.89	2.80
4500	50202	-74625	89940	-56.07	90231.85	-57.70	0.32	2.83
4790	48572	-72490	87259	-56.18	87295.41	-57.87	0.04	2.93
5100	46881	-70456	84628	-56.36	84445.69	-58.09	0.22	2.98
5420	45190	-68570	82122	-56.61	81830.27	-58.34	0.36	2.95

(Part 4 of 7)								
5770	43405	-66705	79583	-56.95	78737.85	-58.71	1.07	3.00
6140	41590	-64910	77091	-57.35	75983.13	-59.07	1.46	2.91
6530	39759	-63175	74645	-57.82	73167.69	-59.40	2.02	2.67
6950	37881	-61448	72186	-58.35	70763.52	-59.76	2.01	2.36
7400	35974	-59729	69726	-58.94	68043.05	-60.12	2.47	1.96
7870	34098	-58051	67324	-59.57	65500.59	-60.70	2.78	1.85
8380	32190	-56340	64888	-60.26	63019.13	-61.14	2.97	1.44
8920	30309	-54634	62478	-60.98	60650.04	-61.75	3.01	1.25
9490	28472	-52934	60105	-61.73	58318.46	-62.28	3.06	0.89
10100	26662	-51213	57738	-62.50	56011.67	-62.88	3.08	0.61
10700	25030	-49610	55566	-63.23	53692.59	-63.46	3.49	0.37
11400	23294	-47841	53211	-64.04	51488.56	-64.07	3.35	0.05
12200	21512	-45942	50729	-64.91	49313.06	-64.73	2.87	0.28
12900	20111	-44379	48723	-65.62	47137.55	-65.32	3.36	0.46
13800	18501	-42494	46347	-66.47	45157.18	-65.90	2.63	0.88
14700	17079	-40739	44174	-67.26	43140.35	-66.52	2.40	1.10
15600	15822	-39104	42183	-67.97	41207.67	-67.09	2.37	1.31
16600	14590	-37415	40159	-68.70	39331.1	-67.70	2.10	1.48
17700	13406	-35701	38135	-69.42	37556.47	-68.41	1.54	1.48
18800	12375	-34122	36297	-70.07	35770.39	-69.00	1.47	1.54
20000	11395	-32539	34477	-70.70	34084.41	-69.53	1.15	1.68
21300	10478	-30972	32697	-71.31	32481.82	-70.13	0.66	1.69
22600	9685.4	-29542	31089	-71.85	30878	-70.76	0.68	1.53

(Part 5 of 7)								
24100	8897.7	-28041	29418	-72.40	29397.1	-71.26	0.07	1.59
25600	8222.1	-26681	27919	-72.87	27918.62	-71.88	0.00	1.38
27300	7566.5	-25289	26396	-73.34	26540.22	-72.43	0.54	1.26
29000	7006	-24034	25035	-73.75	25189.08	-72.91	0.61	1.15
30900	6470.5	-22773	23674	-74.14	23931.88	-73.47	1.08	0.91
32900	5990.2	-21583	22399	-74.49	22678.06	-73.98	1.23	0.69
35000	5559.7	-20465	21207	-74.80	21511.94	-74.42	1.42	0.52
37200	5173.6	-19416	20093	-75.08	20386.9	-74.94	1.44	0.19
39600	4812.8	-18393	19013	-75.34	19334.44	-75.37	1.66	0.04
42200	4478.7	-17407	17974	-75.57	18318.3	-75.83	1.88	0.33
44900	4181.1	-16496	17018	-75.78	17352.88	-76.35	1.93	0.75
47700	3914.8	-15654	16136	-75.96	16432.81	-76.74	1.81	1.01
50800	3659.7	-14825	15270	-76.13	15543.8	-77.00	1.76	1.12
54100	3424.7	-14041	14453	-76.29	14704.57	-77.39	1.71	1.42
57500	3213.8	-13324	13706	-76.44	13921.41	-77.76	1.55	1.69
61200	3012.9	-12629	12984	-76.58	13163.02	-78.07	1.36	1.90
65100	2827	-11979	12308	-76.72	12469.27	-78.39	1.29	2.13
69300	2650.5	-11356	11662	-76.86	11748.88	-78.63	0.74	2.25
73800	2483.3	-10764	11047	-77.01	11095.84	-78.94	0.44	2.44
78500	2328.4	-10214	10476	-77.16	10484.4	-79.23	0.08	2.61
83500	2181.6	-9692.2	9934.7	-77.32	9898.811	-79.46	0.36	2.70
88900	2040.3	-9190.1	9413.9	-77.48	9355.083	-79.67	0.63	2.75
94600	1907.1	-8717.6	8923.8	-77.66	8867.563	-79.83	0.63	2.71

(Part 6 of 7)								
101000	1774.1	-8245.3	8434	-77.86	8351.015	-80.28	0.99	3.02
107000	1662.6	-7849.1	8023.3	-78.04	7884.667	-80.36	1.76	2.89
114000	1546.3	-7434.5	7593.7	-78.25	7443.967	-80.63	2.01	2.95
121000	1442.7	-7063	7208.8	-78.46	7032.948	-80.77	2.50	2.86
129000	1337.5	-6682.5	6815	-78.68	6633.588	-80.92	2.73	2.77
137000	1244.4	-6341.5	6462.4	-78.90	6256.83	-81.04	3.29	2.64
146000	1151.9	-5997.5	6107.1	-79.13	5905.856	-81.15	3.41	2.49
155000	1070.4	-5689	5788.8	-79.34	5570.996	-81.31	3.91	2.42
165000	990.93	-5381.3	5471.8	-79.57	5263.734	-81.28	3.95	2.11
176000	914.82	-5078.8	5160.5	-79.79	4972.418	-81.46	3.78	2.05
187000	848.68	-4808.1	4882.4	-79.99	4690.067	-81.51	4.10	1.86
199000	786.03	-4543.4	4610.9	-80.19	4423.861	-81.56	4.23	1.68
212000	727.49	-4287.2	4348.5	-80.37	4170.793	-81.57	4.26	1.47
226000	673.42	-4041.2	4096.9	-80.54	3934.451	-81.64	4.13	1.35
240000	627.01	-3821.4	3872.5	-80.68	3714.014	-81.65	4.27	1.19
256000	581.67	-3597.3	3644	-80.82	3503.933	-81.65	4.00	1.02
272000	543.05	-3397.5	3440.6	-80.92	3306.249	-81.66	4.06	0.91
289000	508	-3207.8	3247.8	-81.00	3120.656	-81.76	4.07	0.93
308000	474.76	-3019.1	3056.2	-81.06	2944.282	-81.76	3.80	0.85
328000	445.22	-2842.6	2877.2	-81.10	2780.415	-81.66	3.48	0.69
349000	419.05	-2677.9	2710.5	-81.11	2621.202	-81.60	3.41	0.60
371000	395.88	-2524.4	2555.3	-81.09	2475.581	-81.53	3.22	0.55
395000	374.59	-2375.7	2405	-81.04	2336.264	-81.46	2.94	0.52

(Part 7 of 7)								
420000	355.95	-2238.1	2266.2	-80.96	2205.066	-81.38	2.77	0.51
447000	339.02	-2106.1	2133.2	-80.86	2081.975	-81.38	2.46	0.64
476000	323.81	-1980.6	2006.9	-80.72	1965.138	-81.23	2.13	0.64
506000	310.62	-1865.4	1891.1	-80.55	1856.101	-81.15	1.89	0.75
539000	298.48	-1753.1	1778.4	-80.34	1754.193	-81.02	1.38	0.84
573000	288.02	-1650.7	1675.6	-80.10	1656.639	-80.89	1.14	0.98
610000	278.51	-1551.9	1576.7	-79.83	1565.309	-80.74	0.73	1.14
649000	270.15	-1459.8	1484.5	-79.52	1478.198	-80.62	0.43	1.36
691000	262.64	-1372	1396.9	-79.16	1397.149	-80.51	0.02	1.67
735000	256.09	-1290.6	1315.8	-78.78	1320.382	-80.39	0.35	2.01
782000	250.25	-1213.7	1239.2	-78.35	1249.097	-80.27	0.79	2.40
833000	244.98	-1139.9	1166	-77.87	1180.803	-80.00	1.25	2.66
886000	240.43	-1072.2	1098.8	-77.36	1115.964	-79.90	1.54	3.18
943000	236.35	-1007.8	1035.1	-76.80	1054.557	-79.75	1.85	3.70
1000000	232.94	-950.62	978.74	-76.23	998.633	-79.58	1.99	4.21

Table A.10: The equivalent circuit fit, measured impedance spectrum captured with 100 μm diameter electrodes and their respective relative errors for HUVECs 6 hours after the application of shear stress (17.6 dyne/cm^2).

	Cell Culture Medium at 17.6 dyne/cm² (part 1 of 7)							
Electrode	100 μm							
time	Before Shear							
Data Set	FIT				MEASURED		e_{rel} (%)	
<i>f</i> (Hz)	<i>Z'</i> (Ω)	<i>Z''</i> (Ω)	 <i>Z</i> (Ω)	ϕ ($^{\circ}$)	 <i>Z</i> (Ω)	ϕ ($^{\circ}$)	 <i>Z</i> 	ϕ
102	94270	-1.49E+06	1.49E+06	-86.38	1477636	-87.92	0.92	1.75
108	89336	-1.41E+06	1.41E+06	-86.37	1403441	-87.58	0.57	1.37
115	84221	-1.33E+06	1.33E+06	-86.37	1292748	-87.11	2.80	0.86
122	79684	-1.25E+06	1.26E+06	-86.36	1261622	-85.82	0.49	0.63
130	75088	-1.18E+06	1.18E+06	-86.36	1159497	-86.74	1.87	0.44
139	70539	-1.11E+06	1.11E+06	-86.35	1112277	-86.61	0.42	0.30
148	66536	-1.04E+06	1.04E+06	-86.34	1025485	-86.92	1.70	0.67
157	62985	-9.83E+05	9.85E+05	-86.34	975844.7	-86.59	0.97	0.30
167	59480	-9.27E+05	9.29E+05	-86.33	924979.2	-85.62	0.39	0.83
178	56074	-8.72E+05	8.73E+05	-86.32	861996.4	-85.88	1.32	0.52
189	53057	-8.23E+05	8.25E+05	-86.31	819395.2	-86.38	0.62	0.08
201	50138	-7.76E+05	7.77E+05	-86.30	778850.9	-86.44	0.22	0.16
214	47339	-7.30E+05	7.32E+05	-86.29	736515.4	-85.74	0.65	0.65
228	44675	-6.87E+05	6.89E+05	-86.28	697527.4	-87.40	1.29	1.28
243	42157	-6.46E+05	6.48E+05	-86.27	626072.4	-85.73	3.44	0.63
258	39928	-6.10E+05	6.11E+05	-86.26	618220.6	-87.91	1.10	1.88
275	37690	-5.74E+05	5.75E+05	-86.24	597272.3	-85.87	3.72	0.43
292	35709	-5.42E+05	5.43E+05	-86.23	559101.5	-83.35	2.91	3.45
311	33748	-5.10E+05	5.11E+05	-86.21	519586	-84.20	1.66	2.39

(Part 2 of 7)								
331	31922	-4.80E+05	4.81E+05	-86.20	470790.1	-88.22	2.22	2.29
352	30225	-4.53E+05	4.54E+05	-86.18	439510.8	-86.27	3.21	0.11
375	28582	-4.26E+05	4.27E+05	-86.16	433073.8	-86.13	1.43	0.04
399	27065	-4.01E+05	4.02E+05	-86.14	405464.6	-86.29	0.81	0.17
424	25665	-3.79E+05	3.79E+05	-86.12	381813.5	-87.08	0.64	1.10
452	24279	-3.56E+05	3.57E+05	-86.10	355923.7	-86.40	0.23	0.34
480	23051	-3.36E+05	3.37E+05	-86.08	335434.7	-86.05	0.39	0.03
511	21846	-3.16E+05	3.17E+05	-86.05	315314.4	-85.81	0.57	0.28
544	20712	-2.98E+05	2.99E+05	-86.02	299054.2	-85.84	0.15	0.22
579	19649	-2.81E+05	2.81E+05	-85.99	281264.8	-86.14	0.01	0.17
616	18654	-2.64E+05	2.65E+05	-85.96	263805.9	-85.45	0.45	0.60
656	17702	-2.49E+05	2.49E+05	-85.93	248154.3	-85.68	0.53	0.29
698	16819	-2.34E+05	2.35E+05	-85.90	232952.5	-85.72	0.89	0.21
742	15999	-2.21E+05	2.22E+05	-85.86	220636.3	-85.73	0.45	0.15
790	15207	-2.08E+05	2.09E+05	-85.82	208880.4	-85.35	0.10	0.56
841	14463	-1.96E+05	1.97E+05	-85.78	195499.2	-85.72	0.52	0.07
894	13778	-1.85E+05	1.85E+05	-85.74	184447.1	-85.56	0.47	0.21
952	13115	-1.74E+05	1.74E+05	-85.69	171551.1	-85.61	1.70	0.09
1010	12526	-1.64E+05	1.65E+05	-85.64	164002.5	-85.04	0.50	0.71
1080	11899	-1.54E+05	1.55E+05	-85.59	154220.1	-85.67	0.22	0.10
1150	11346	-1.45E+05	1.46E+05	-85.53	145808.5	-85.44	0.20	0.10
1220	10856	-1.37E+05	1.37E+05	-85.47	138008.3	-84.86	0.38	0.72
1300	10360	-1.29E+05	1.29E+05	-85.41	129320.4	-85.29	0.03	0.14

(Part 3 of 7)								
1380	9920.1	-1.22E+05	1.22E+05	-85.34	120743.2	-85.51	1.17	0.19
1470	9481.5	-1.15E+05	1.15E+05	-85.27	114527.2	-85.11	0.40	0.19
1560	9092.7	-1.08E+05	1.09E+05	-85.20	108142	-84.99	0.43	0.24
1670	8673.3	-1.01E+05	1.02E+05	-85.11	101835.2	-84.90	0.09	0.25
1770	8336.5	-95858	96220	-85.03	95907.08	-84.50	0.33	0.62
1890	7978.6	-90003	90356	-84.93	90380.73	-84.51	0.03	0.51
2010	7662.6	-84834	85180	-84.84	85751.52	-84.88	0.67	0.05
2140	7359.6	-79878	80216	-84.74	80400.01	-84.67	0.23	0.08
2270	7090.6	-75478	75811	-84.63	75556.56	-84.38	0.34	0.30
2420	6815.5	-70979	71306	-84.52	70884.32	-84.22	0.59	0.35
2570	6571.9	-66996	67317	-84.40	66869.2	-84.04	0.67	0.42
2740	6327.4	-62999	63316	-84.27	63461.27	-84.13	0.23	0.16
2910	6110.9	-59461	59774	-84.13	59851.96	-83.78	0.13	0.42
3100	5896.5	-55957	56267	-83.99	56097.45	-83.94	0.30	0.05
3300	5697	-52698	53005	-83.83	53077.72	-83.59	0.14	0.29
3510	5511.4	-49668	49973	-83.67	50110.29	-83.68	0.27	0.01
3740	5331.6	-46733	47036	-83.49	47130.56	-83.32	0.20	0.21
3970	5172.1	-44133	44435	-83.32	44512.14	-83.06	0.17	0.30
4230	5012.3	-41527	41829	-83.12	41970.91	-83.05	0.34	0.08
4500	4865.4	-39135	39436	-82.91	39400.98	-82.72	0.09	0.24
4790	4725.7	-36860	37162	-82.69	37148.12	-82.66	0.04	0.04
5100	4593.5	-34710	35013	-82.46	35097.39	-82.41	0.24	0.06
5420	4472.5	-32744	33048	-82.22	33181.87	-82.29	0.40	0.09

(Part 4 of 7)								
5770	4355.1	-30840	31146	-81.96	31280.34	-81.81	0.43	0.18
6140	4245.2	-29058	29367	-81.69	29386.66	-81.69	0.07	0.00
6530	4142.4	-27395	27707	-81.40	27714.35	-81.34	0.03	0.08
6950	4044.3	-25810	26125	-81.09	26122.45	-81.11	0.01	0.01
7400	3951.2	-24308	24627	-80.77	24652.03	-80.74	0.10	0.04
7870	3864.9	-22919	23243	-80.43	23290.61	-80.46	0.20	0.04
8380	3781.9	-21586	21915	-80.06	21970.15	-80.15	0.25	0.11
8920	3704	-20338	20672	-79.68	20749.62	-79.67	0.37	0.02
9490	3631.1	-19172	19513	-79.28	19563.7	-79.52	0.26	0.31
10100	3561.8	-18068	18416	-78.85	18430.18	-78.96	0.08	0.15
10700	3501.1	-17104	17458	-78.43	17428.59	-78.63	0.17	0.26
11400	3438	-16105	16468	-77.95	16453.9	-78.17	0.09	0.28
12200	3374.3	-15102	15474	-77.41	15558.39	-77.65	0.54	0.31
12900	3324.8	-14325	14705	-76.93	14677.14	-77.13	0.19	0.26
13800	3268.1	-13440	13832	-76.33	13876.35	-76.66	0.32	0.43
14700	3218	-12663	13065	-75.74	13109.77	-76.01	0.34	0.36
15600	3173.4	-11974	12387	-75.16	12399.45	-75.45	0.10	0.39
16600	3129	-11295	11721	-74.52	11725.43	-74.85	0.04	0.45
17700	3085.7	-10637	11075	-73.82	11081.74	-74.24	0.06	0.55
18800	3047	-10055	10506	-73.14	10512.09	-73.52	0.06	0.52
20000	3009.2	-9492.4	9958	-72.41	9942.058	-72.78	0.16	0.50
21300	2972.7	-8954.3	9434.9	-71.64	9427.279	-72.04	0.08	0.56
22600	2939.9	-8478	8973.3	-70.88	8938.816	-71.28	0.39	0.56

(Part 5 of 7)								
24100	2906	-7992.1	8504.1	-70.02	8476.838	-70.39	0.32	0.53
25600	2875.6	-7563.3	8091.5	-69.18	8055.052	-69.58	0.45	0.57
27300	2844.6	-7134.4	7680.6	-68.26	7658.829	-68.71	0.28	0.65
29000	2816.7	-6756.1	7319.8	-67.37	7288.46	-67.78	0.43	0.60
30900	2788.5	-6383	6965.5	-66.40	6937.342	-66.81	0.41	0.61
32900	2761.6	-6037.4	6639.1	-65.42	6610.246	-65.83	0.44	0.63
35000	2736	-5717.7	6338.6	-64.43	6311.262	-64.84	0.43	0.64
37200	2711.6	-5422.3	6062.5	-63.43	6024.788	-63.83	0.63	0.63
39600	2687.1	-5138.4	5798.6	-62.39	5763.581	-62.76	0.61	0.58
42200	2662.9	-4868.3	5549	-61.32	5516.039	-61.72	0.60	0.64
44900	2639.6	-4622.2	5322.8	-60.27	5293.565	-60.70	0.55	0.70
47700	2617.3	-4397.6	5117.5	-59.24	5081.108	-59.67	0.72	0.71
50800	2594.2	-4179.2	4918.9	-58.17	4886.7	-58.54	0.66	0.63
54100	2571.3	-3975.8	4734.8	-57.11	4704.259	-57.46	0.65	0.61
57500	2549	-3792.3	4569.3	-56.09	4536.851	-56.42	0.72	0.58
61200	2526	-3617.4	4412.1	-55.07	4381.364	-55.40	0.70	0.60
65100	2502.9	-3456.5	4267.5	-54.09	4238.868	-54.42	0.68	0.61
69300	2479.1	-3305.4	4131.8	-53.13	4099.968	-53.44	0.78	0.57
73800	2454.5	-3164.6	4004.9	-52.20	3976.718	-52.54	0.71	0.64
78500	2429.6	-3036.9	3889.2	-51.34	3859.272	-51.67	0.78	0.65
83500	2403.8	-2919	3781.4	-50.53	3752.306	-50.85	0.78	0.64
88900	2376.5	-2808.8	3679.3	-49.77	3651.121	-50.09	0.77	0.65
94600	2348.2	-2708.3	3584.5	-49.07	3554.429	-49.33	0.85	0.51

(Part 6 of 7)								
101000	2316.8	-2611.4	3491	-48.42	3471.847	-48.81	0.55	0.79
107000	2287.6	-2533.2	3413.2	-47.92	3388.233	-48.30	0.74	0.79
114000	2253.8	-2454.4	3332.2	-47.44	3302.458	-47.98	0.90	1.12
121000	2220.1	-2386.7	3259.7	-47.07	3233.668	-47.39	0.81	0.67
129000	2181.8	-2320.4	3185	-46.76	3159.159	-47.16	0.82	0.85
137000	2143.6	-2263.5	3117.4	-46.56	3090.692	-46.99	0.86	0.92
146000	2100.6	-2208.6	3048.1	-46.44	3022.494	-46.90	0.85	0.99
155000	2057.9	-2161.4	2984.4	-46.41	2954.914	-46.89	1.00	1.04
165000	2010.6	-2116	2918.9	-46.46	2884.011	-46.83	1.21	0.78
176000	1958.9	-2072.8	2851.9	-46.62	2828.714	-47.06	0.82	0.94
187000	1907.7	-2035	2789.3	-46.85	2765.441	-47.31	0.86	0.97
199000	1852.5	-1998.4	2725	-47.17	2701.128	-47.61	0.88	0.91
212000	1793.7	-1963	2659.1	-47.58	2632.954	-48.01	0.99	0.89
226000	1731.8	-1928.3	2591.8	-48.07	2570.164	-48.48	0.84	0.83
240000	1671.4	-1896.3	2527.7	-48.61	2505.141	-49.00	0.90	0.80
256000	1604.5	-1861.9	2457.8	-49.25	2439.691	-49.62	0.74	0.74
272000	1540	-1829.2	2391.1	-49.91	2372.531	-50.29	0.78	0.77
289000	1474.3	-1795.6	2323.3	-50.61	2306.729	-51.09	0.72	0.93
308000	1404.4	-1759.2	2251	-51.40	2236.977	-51.79	0.63	0.75
328000	1334.9	-1721.7	2178.6	-52.21	2170.714	-52.62	0.36	0.78
349000	1266.4	-1682.9	2106.2	-53.04	2100.49	-53.44	0.27	0.76
371000	1199.4	-1643	2034.2	-53.87	2031.519	-54.32	0.13	0.82
395000	1131.7	-1600.3	1960	-54.73	1961.438	-55.18	0.07	0.81

(Part 7 of 7)								
420000	1066.8	-1556.6	1887.1	-55.58	1891.702	-56.08	0.24	0.90
447000	1002.8	-1510.5	1813.1	-56.42	1822.426	-57.01	0.51	1.03
476000	940.4	-1462.5	1738.8	-57.26	1752.441	-57.94	0.78	1.17
506000	882.21	-1414.5	1667.1	-58.05	1684.213	-58.81	1.02	1.30
539000	824.87	-1363.8	1593.9	-58.83	1616.568	-59.72	1.40	1.49
573000	772.28	-1313.9	1524.1	-59.55	1549.724	-60.63	1.65	1.77
610000	721.6	-1262.4	1454.1	-60.25	1484.894	-61.52	2.07	2.06
649000	674.59	-1211.1	1386.3	-60.88	1418.707	-62.34	2.28	2.35
691000	630.27	-1159.3	1319.5	-61.47	1355.793	-63.21	2.68	2.75
735000	589.81	-1108.6	1255.8	-61.99	1294.671	-64.04	3.00	3.21
782000	552.3	-1058.4	1193.8	-62.44	1234.476	-64.80	3.30	3.64
833000	517.18	-1008	1132.9	-62.84	1177.125	-65.64	3.76	4.26
886000	485.79	-959.8	1075.7	-63.15	1120.269	-66.28	3.98	4.72
943000	456.83	-912.3	1020.3	-63.40	1066.191	-66.97	4.30	5.33
1000000	431.98	-868.83	970.29	-63.56	1014.737	-67.66	4.38	6.06

Table A.11: The equivalent circuit fit, measured impedance spectrum captured with 100 μm diameter electrodes and their respective relative errors for cell culture medium before the onset of shear stress (17.6 dyne/cm²).

	Cell Culture Medium at 17.6 dyne/cm ² (part 1 of 7)							
	100 μ m							
time	20 min after shear							
	FIT				MEASURED		e_{rel} (%)	
f (Hz)	Z' (Ω)	Z'' (Ω)	$ Z $ (Ω)	ϕ ($^{\circ}$)	$ Z $ (Ω)	ϕ ($^{\circ}$)	$ Z $	ϕ
102	94047	-1.51E+06	1.51E+06	-86.43	1554853	-85.17	2.94	1.47
108	89120	-1.43E+06	1.43E+06	-86.42	1456434	-86.63	1.91	0.23
115	84012	-1.34E+06	1.34E+06	-86.42	1348255	-86.73	0.25	0.36
122	79481	-1.27E+06	1.27E+06	-86.41	1265050	-86.93	0.44	0.59
130	74891	-1.19E+06	1.20E+06	-86.41	1184053	-86.00	0.96	0.48
139	70349	-1.12E+06	1.12E+06	-86.40	1115847	-86.68	0.45	0.32
148	66352	-1.05E+06	1.06E+06	-86.40	1055034	-86.73	0.03	0.38
157	62806	-9.95E+05	9.97E+05	-86.39	1004351	-86.42	0.73	0.03
167	59307	-9.38E+05	9.40E+05	-86.38	924378.9	-86.69	1.64	0.36
178	55906	-8.82E+05	8.84E+05	-86.37	886390.1	-87.29	0.30	1.05
189	52895	-8.33E+05	8.34E+05	-86.37	830843.6	-85.96	0.40	0.47
201	49980	-7.85E+05	7.86E+05	-86.36	785391.6	-86.30	0.11	0.07
214	47185	-7.39E+05	7.40E+05	-86.35	746209.9	-85.93	0.79	0.48
228	44527	-6.95E+05	6.97E+05	-86.34	688577.1	-87.10	1.15	0.88
243	42013	-6.54E+05	6.55E+05	-86.32	664174.1	-86.08	1.36	0.28
258	39787	-6.17E+05	6.18E+05	-86.31	607494.2	-88.29	1.81	2.24
275	37554	-5.80E+05	5.82E+05	-86.30	585337.3	-88.38	0.62	2.36
292	35577	-5.48E+05	5.49E+05	-86.29	565631.6	-86.97	2.92	0.79
311	33619	-5.16E+05	5.17E+05	-86.27	531317	-87.04	2.73	0.89

(Part 2 of 7)								
331	31797	-4.86E+05	4.87E+05	-86.26	470089.5	-84.76	3.55	1.77
352	30103	-4.58E+05	4.59E+05	-86.24	460267.5	-85.11	0.32	1.32
375	28463	-4.31E+05	4.32E+05	-86.22	423754.9	-87.77	1.88	1.77
399	26950	-4.06E+05	4.07E+05	-86.20	400130.1	-85.65	1.65	0.64
424	25553	-3.83E+05	3.84E+05	-86.18	374725.9	-86.17	2.38	0.01
452	24169	-3.60E+05	3.61E+05	-86.16	358503.7	-85.66	0.63	0.58
480	22944	-3.40E+05	3.41E+05	-86.14	336922.4	-86.67	1.07	0.61
511	21742	-3.20E+05	3.21E+05	-86.11	320312	-86.45	0.11	0.39
544	20611	-3.01E+05	3.02E+05	-86.09	301241.8	-86.50	0.23	0.47
579	19549	-2.84E+05	2.84E+05	-86.06	282303.2	-86.15	0.73	0.10
616	18557	-2.67E+05	2.68E+05	-86.03	266487.6	-85.76	0.54	0.31
656	17607	-2.52E+05	2.52E+05	-86.00	252889.5	-86.07	0.27	0.09
698	16726	-2.37E+05	2.38E+05	-85.96	236848.1	-85.60	0.32	0.43
742	15908	-2.23E+05	2.24E+05	-85.93	222259.3	-85.70	0.81	0.26
790	15118	-2.10E+05	2.11E+05	-85.89	210530.5	-85.38	0.20	0.59
841	14376	-1.98E+05	1.99E+05	-85.85	198647.4	-85.79	0.00	0.08
894	13693	-1.87E+05	1.87E+05	-85.81	187227.8	-85.59	0.05	0.25
952	13031	-1.76E+05	1.76E+05	-85.76	174772.5	-85.76	0.90	0.00
1010	12444	-1.66E+05	1.67E+05	-85.72	166860.3	-85.29	0.15	0.50
1080	11818	-1.56E+05	1.56E+05	-85.66	157368.8	-85.45	0.73	0.24
1150	11267	-1.47E+05	1.47E+05	-85.61	146911	-85.27	0.12	0.40
1220	10779	-1.39E+05	1.39E+05	-85.55	138663.1	-85.52	0.21	0.04
1300	10284	-1.30E+05	1.31E+05	-85.49	131039.3	-85.42	0.23	0.08

(Part 3 of 7)								
1380	9845	-1.23E+05	1.23E+05	-85.43	122744.7	-85.08	0.58	0.41
1470	9407.7	-1.16E+05	1.16E+05	-85.36	115642.4	-85.19	0.47	0.19
1560	9019.9	-1.09E+05	1.10E+05	-85.29	109251.5	-85.13	0.46	0.19
1670	8601.8	-1.02E+05	1.03E+05	-85.20	102483.3	-84.98	0.31	0.26
1770	8266	-96872	97224	-85.12	96523.55	-84.91	0.73	0.25
1890	7909.1	-90952	91295	-85.03	91212.48	-84.95	0.09	0.09
2010	7594.1	-85726	86062	-84.94	86165.51	-84.88	0.12	0.07
2140	7292	-80714	81043	-84.84	81197.47	-84.71	0.19	0.15
2270	7023.8	-76266	76589	-84.74	76710.95	-84.40	0.16	0.40
2420	6749.6	-71717	72034	-84.62	72050.66	-84.56	0.02	0.07
2570	6506.8	-67690	68002	-84.51	67991.66	-84.35	0.02	0.19
2740	6263.1	-63650	63957	-84.38	63868.31	-84.23	0.14	0.18
2910	6047.3	-60073	60376	-84.25	60240.96	-84.03	0.22	0.26
3100	5833.6	-56531	56831	-84.11	56761.8	-83.94	0.12	0.20
3300	5634.8	-53236	53534	-83.96	53525.86	-83.84	0.02	0.14
3510	5449.8	-50174	50469	-83.80	50520.19	-83.72	0.10	0.10
3740	5270.6	-47207	47500	-83.63	47597.15	-83.54	0.20	0.10
3970	5111.8	-44578	44870	-83.46	44795.49	-83.36	0.17	0.12
4230	4952.5	-41945	42236	-83.27	42206.43	-83.23	0.07	0.04
4500	4806.2	-39527	39818	-83.07	39828.36	-83.06	0.03	0.01
4790	4667	-37228	37520	-82.86	37565.68	-82.77	0.12	0.10
5100	4535.3	-35055	35347	-82.63	35405.11	-82.69	0.16	0.08
5420	4414.7	-33068	33362	-82.40	33471.83	-82.31	0.33	0.10

(Part 4 of 7)								
5770	4297.8	-31143	31438	-82.14	31461.56	-82.15	0.07	0.01
6140	4188.3	-29343	29640	-81.88	29678.62	-81.88	0.13	0.00
6530	4086	-27663	27963	-81.60	27976.49	-81.60	0.05	0.00
6950	3988.3	-26060	26364	-81.30	26423.2	-81.29	0.22	0.01
7400	3895.5	-24542	24850	-80.98	24888.29	-81.01	0.15	0.04
7870	3809.7	-23139	23451	-80.65	23468.81	-80.75	0.08	0.12
8380	3727	-21792	22109	-80.30	22125.15	-80.39	0.07	0.12
8920	3649.5	-20531	20853	-79.92	20874.99	-80.04	0.11	0.15
9490	3576.9	-19353	19681	-79.53	19714.31	-79.66	0.17	0.16
10100	3508	-18238	18572	-79.11	18610.21	-79.33	0.21	0.27
10700	3447.5	-17263	17604	-78.71	17548.53	-78.88	0.32	0.22
11400	3384.7	-16254	16602	-78.24	16580.7	-78.48	0.13	0.31
12200	3321.4	-15240	15598	-77.71	15668.33	-78.00	0.45	0.37
12900	3272.2	-14455	14821	-77.25	14771.89	-77.45	0.33	0.26
13800	3215.8	-13561	13937	-76.66	13988.9	-76.97	0.37	0.40
14700	3166	-12776	13162	-76.08	13232.43	-76.38	0.53	0.39
15600	3121.6	-12080	12477	-75.51	12487.49	-75.77	0.08	0.34
16600	3077.5	-11394	11802	-74.89	11809.71	-75.19	0.07	0.41
17700	3034.4	-10729	11150	-74.21	11157.2	-74.58	0.06	0.51
18800	2996	-10140	10574	-73.54	10558.33	-73.91	0.15	0.50
20000	2958.5	-9572.1	10019	-72.83	10019.21	-73.17	0.00	0.48
21300	2922.2	-9028.3	9489.5	-72.07	9479.065	-72.44	0.11	0.52
22600	2889.7	-8546.9	9022.2	-71.32	8991.538	-71.67	0.34	0.50

(Part 5 of 7)								
24100	2856.1	-8055.8	8547.1	-70.48	8522.529	-70.86	0.29	0.54
25600	2826	-7622.3	8129.3	-69.66	8096.85	-70.10	0.40	0.64
27300	2795.3	-7188.7	7713.1	-68.75	7691.595	-69.18	0.28	0.62
29000	2767.6	-6806.2	7347.4	-67.87	7318.176	-68.27	0.40	0.58
30900	2739.7	-6429	6988.4	-66.92	6962.855	-67.36	0.37	0.65
32900	2713.2	-6079.4	6657.4	-65.95	6625.301	-66.39	0.48	0.66
35000	2687.9	-5756	6352.7	-64.97	6325.539	-65.40	0.43	0.66
37200	2663.8	-5457.1	6072.6	-63.98	6037.31	-64.38	0.58	0.63
39600	2639.8	-5169.8	5804.7	-62.95	5770.778	-63.37	0.59	0.67
42200	2615.9	-4896.4	5551.4	-61.89	5523.259	-62.32	0.51	0.69
44900	2593.1	-4647.1	5321.6	-60.84	5295.995	-61.32	0.48	0.78
47700	2571.1	-4419.6	5113.1	-59.81	5080.966	-60.21	0.63	0.66
50800	2548.6	-4198.3	4911.3	-58.74	4881.943	-59.13	0.60	0.66
54100	2526.1	-3992.1	4724.2	-57.68	4696.641	-58.12	0.59	0.76
57500	2504.4	-3806	4556	-56.65	4525.351	-57.03	0.68	0.67
61200	2482	-3628.5	4396.2	-55.63	4367.521	-56.01	0.66	0.69
65100	2459.6	-3465.1	4249.3	-54.63	4220.389	-55.03	0.69	0.73
69300	2436.4	-3311.6	4111.3	-53.66	4080.755	-54.02	0.75	0.66
73800	2412.6	-3168.4	3982.4	-52.71	3955.847	-53.10	0.67	0.73
78500	2388.6	-3038.5	3864.9	-51.83	3837.161	-52.21	0.72	0.73
83500	2363.6	-2918.4	3755.5	-51.00	3727.793	-51.37	0.74	0.73
88900	2337.3	-2806	3652	-50.21	3625.586	-50.61	0.73	0.80
94600	2310.1	-2703.5	3556	-49.49	3531.289	-49.98	0.70	0.98

(Part 6 of 7)								
101000	2279.9	-2604.5	3461.4	-48.80	3442.018	-49.23	0.56	0.87
107000	2251.8	-2524.5	3382.9	-48.27	3358.453	-48.67	0.73	0.82
114000	2219.4	-2443.9	3301.3	-47.76	3279.265	-48.24	0.67	1.01
121000	2187	-2374.6	3228.3	-47.36	3201.485	-47.80	0.84	0.94
129000	2150.3	-2306.6	3153.4	-47.01	3127.465	-47.52	0.83	1.06
137000	2113.6	-2248.3	3085.8	-46.77	3058.042	-47.25	0.91	1.02
146000	2072.4	-2192	3016.6	-46.61	2991.107	-47.14	0.85	1.13
155000	2031.3	-2143.6	2953.2	-46.54	2923.011	-47.07	1.03	1.12
165000	1985.9	-2097.1	2888.2	-46.56	2860.542	-47.04	0.97	1.01
176000	1936.2	-2052.9	2822	-46.68	2796.785	-47.19	0.90	1.08
187000	1887	-2014.4	2760.2	-46.87	2734.967	-47.39	0.92	1.10
199000	1833.8	-1977.3	2696.8	-47.16	2671.636	-47.68	0.94	1.11
212000	1777.2	-1941.4	2632	-47.53	2606.089	-48.04	0.99	1.06
226000	1717.3	-1906.6	2566	-47.99	2542.537	-48.51	0.92	1.07
240000	1658.9	-1874.5	2503.2	-48.49	2477.978	-48.97	1.02	0.98
256000	1594.1	-1840.4	2434.7	-49.10	2413.941	-49.58	0.86	0.96
272000	1531.5	-1808	2369.5	-49.73	2349.115	-50.20	0.87	0.93
289000	1467.6	-1775	2303.1	-50.42	2283.646	-50.99	0.85	1.12
308000	1399.4	-1739.3	2232.4	-51.18	2215.586	-51.74	0.76	1.08
328000	1331.5	-1702.6	2161.5	-51.97	2150.659	-52.48	0.50	0.96
349000	1264.5	-1664.9	2090.6	-52.78	2082.318	-53.28	0.40	0.94
371000	1198.7	-1626	2020.1	-53.60	2014.249	-54.14	0.29	1.00
395000	1132.2	-1584.4	1947.3	-54.45	1945.464	-54.94	0.09	0.89

(Part 7 of 7)								
420000	1068.2	-1541.9	1875.8	-55.29	1876.086	-55.89	0.02	1.08
447000	1004.9	-1497	1803.1	-56.13	1807.83	-56.81	0.26	1.20
476000	943.19	-1450.2	1730	-56.96	1740.109	-57.72	0.58	1.31
506000	885.48	-1403.4	1659.4	-57.75	1671.646	-58.60	0.73	1.45
539000	828.5	-1353.8	1587.2	-58.53	1606.391	-59.54	1.19	1.69
573000	776.14	-1305	1518.3	-59.26	1539.235	-60.42	1.36	1.92
610000	725.58	-1254.4	1449.2	-59.95	1475.342	-61.28	1.77	2.17
649000	678.6	-1204.1	1382.1	-60.60	1411.158	-62.13	2.06	2.48
691000	634.25	-1153.1	1316.1	-61.19	1348.744	-62.98	2.42	2.85
735000	593.7	-1103.3	1252.9	-61.71	1287.713	-63.82	2.70	3.30
782000	556.05	-1053.7	1191.4	-62.18	1229.03	-64.59	3.06	3.73
833000	520.74	-1003.9	1131	-62.58	1171.931	-65.33	3.49	4.20
886000	489.16	-956.33	1074.2	-62.91	1115.822	-66.11	3.73	4.84
943000	459.99	-909.32	1019	-63.17	1063.593	-66.80	4.19	5.43
1000000	434.93	-866.26	969.31	-63.34	1009.882	-67.47	4.02	6.13

Table A.12: The equivalent circuit fit, measured impedance spectrum captured with 100 μm diameter electrodes and their respective relative errors for cell culture medium 6 hours after the onset of shear stress (17.6 dyne/cm^2).

	Cell Culture Medium at 17.6 dyne/cm ² (part 1 of 7)							
	100 μ m							
time	6 hr after shear							
	FIT				MEASURED		$e_{rel}(\%)$	
f (Hz)	Z' (Ω)	Z'' (Ω)	$ Z $ (Ω)	ϕ ($^{\circ}$)	$ Z $ (Ω)	ϕ ($^{\circ}$)	$ Z $	ϕ
102	94094	-1.55E+06	1.55E+06	-86.52	1541930	-88.28	0.48	1.99
108	89159	-1.46E+06	1.47E+06	-86.51	1471230	-86.93	0.32	0.48
115	84043	-1.38E+06	1.38E+06	-86.51	1351488	-85.87	2.15	0.74
122	79505	-1.30E+06	1.30E+06	-86.51	1322450	-85.85	1.38	0.77
130	74909	-1.22E+06	1.23E+06	-86.50	1235621	-86.85	0.71	0.41
139	70361	-1.15E+06	1.15E+06	-86.49	1161358	-85.24	0.95	1.47
148	66359	-1.08E+06	1.08E+06	-86.49	1097404	-86.67	1.32	0.21
157	62808	-1.02E+06	1.02E+06	-86.48	1041693	-86.22	1.78	0.30
167	59306	-9.62E+05	9.64E+05	-86.47	961593.1	-86.25	0.26	0.25
178	55901	-9.05E+05	9.07E+05	-86.47	922452.8	-87.32	1.71	0.98
189	52886	-8.54E+05	8.56E+05	-86.46	861298	-86.75	0.63	0.34
201	49968	-8.05E+05	8.07E+05	-86.45	799594.7	-85.72	0.88	0.85
214	47171	-7.58E+05	7.59E+05	-86.44	759992.5	-86.46	0.08	0.02
228	44511	-7.13E+05	7.15E+05	-86.43	708531.4	-85.93	0.84	0.58
243	41995	-6.71E+05	6.72E+05	-86.42	671206.4	-86.78	0.12	0.41
258	39768	-6.33E+05	6.34E+05	-86.41	635370.5	-85.51	0.16	1.04
275	37533	-5.95E+05	5.97E+05	-86.39	595499.7	-84.87	0.18	1.79
292	35555	-5.62E+05	5.63E+05	-86.38	547702.3	-85.72	2.81	0.77
311	33596	-5.29E+05	5.30E+05	-86.37	521525.4	-86.00	1.62	0.43

(Part 2 of 7)								
331	31773	-4.98E+05	4.99E+05	-86.35	505205.1	-87.33	1.20	1.12
352	30079	-4.69E+05	4.70E+05	-86.33	468110.5	-87.52	0.50	1.35
375	28438	-4.42E+05	4.43E+05	-86.32	437262.6	-85.24	1.23	1.26
399	26924	-4.16E+05	4.17E+05	-86.30	421662.5	-85.66	1.11	0.74
424	25527	-3.92E+05	3.93E+05	-86.28	395930.5	-85.64	0.66	0.75
452	24143	-3.69E+05	3.70E+05	-86.26	371663.7	-85.97	0.49	0.34
480	22918	-3.48E+05	3.49E+05	-86.24	346778.3	-85.84	0.66	0.46
511	21716	-3.28E+05	3.29E+05	-86.21	327425.9	-86.44	0.37	0.26
544	20585	-3.09E+05	3.09E+05	-86.19	309564.3	-86.56	0.04	0.43
579	19524	-2.91E+05	2.91E+05	-86.16	290501.2	-86.20	0.32	0.04
616	18531	-2.74E+05	2.75E+05	-86.13	273649.8	-86.00	0.34	0.15
656	17582	-2.58E+05	2.58E+05	-86.10	257069.5	-86.18	0.53	0.10
698	16701	-2.43E+05	2.43E+05	-86.07	241875.4	-85.79	0.66	0.32
742	15883	-2.29E+05	2.30E+05	-86.03	226580.7	-86.03	1.31	0.01
790	15094	-2.16E+05	2.16E+05	-86.00	214371.2	-85.71	0.82	0.33
841	14352	-2.03E+05	2.04E+05	-85.96	203076.8	-85.70	0.21	0.30
894	13669	-1.91E+05	1.92E+05	-85.92	192016.5	-85.72	0.07	0.23
952	13008	-1.80E+05	1.81E+05	-85.87	178695.8	-85.65	1.08	0.26
1010	12421	-1.70E+05	1.71E+05	-85.83	169569.1	-85.14	0.64	0.81
1080	11796	-1.60E+05	1.60E+05	-85.77	160915.3	-85.55	0.57	0.26
1150	11246	-1.50E+05	1.51E+05	-85.72	149542.9	-85.65	0.72	0.08
1220	10757	-1.42E+05	1.42E+05	-85.67	142288.7	-85.32	0.01	0.41
1300	10263	-1.33E+05	1.34E+05	-85.60	133944.7	-85.33	0.05	0.32

(Part 3 of 7)								
1380	9824.6	-1.26E+05	1.26E+05	-85.54	125875.1	-85.10	0.42	0.52
1470	9387.7	-1.19E+05	1.19E+05	-85.47	118291	-85.23	0.57	0.29
1560	9000.4	-1.12E+05	1.12E+05	-85.41	112319.4	-85.19	0.04	0.26
1670	8582.7	-1.05E+05	1.05E+05	-85.32	105326.9	-85.00	0.08	0.38
1770	8247.4	-99182	99524	-85.25	99335.3	-85.22	0.19	0.04
1890	7891	-93114	93448	-85.16	93656.05	-85.01	0.22	0.17
2010	7576.4	-87759	88085	-85.07	87903.94	-84.79	0.21	0.33
2140	7274.7	-82623	82943	-84.97	83016.13	-84.76	0.09	0.25
2270	7007	-78065	78379	-84.87	78324.95	-84.65	0.07	0.26
2420	6733.1	-73404	73713	-84.76	73841.63	-84.55	0.17	0.25
2570	6490.7	-69278	69582	-84.65	69611.24	-84.38	0.04	0.32
2740	6247.4	-65139	65438	-84.52	65437.53	-84.32	0.00	0.24
2910	6032	-61475	61770	-84.40	61658.2	-84.15	0.18	0.30
3100	5818.7	-57847	58139	-84.26	58207.73	-84.08	0.12	0.21
3300	5620.2	-54472	54761	-84.11	54756.88	-83.91	0.01	0.24
3510	5435.7	-51335	51622	-83.96	51623.8	-83.87	0.00	0.10
3740	5256.8	-48297	48582	-83.79	48717.05	-83.72	0.28	0.08
3970	5098.3	-45604	45888	-83.62	45830.75	-83.59	0.12	0.04
4230	4939.4	-42908	43191	-83.43	43313.7	-83.35	0.28	0.10
4500	4793.3	-40432	40715	-83.24	40720.3	-83.34	0.01	0.12
4790	4654.4	-38078	38361	-83.03	38410.79	-83.04	0.13	0.01
5100	4523	-35853	36137	-82.81	36244.13	-82.79	0.30	0.03
5420	4402.7	-33819	34104	-82.58	34143.12	-82.45	0.11	0.16

(Part 4 of 7)								
5770	4286.1	-31848	32135	-82.34	32140.2	-82.31	0.02	0.03
6140	4176.9	-30005	30294	-82.08	30271.44	-82.07	0.07	0.01
6530	4074.8	-28285	28577	-81.80	28621.71	-81.88	0.16	0.10
6950	3977.4	-26645	26940	-81.51	26954.01	-81.60	0.05	0.11
7400	3884.9	-25091	25390	-81.20	25399.78	-81.25	0.04	0.06
7870	3799.2	-23655	23958	-80.88	23976.22	-80.86	0.08	0.02
8380	3716.8	-22276	22584	-80.53	22618.06	-80.61	0.15	0.10
8920	3639.5	-20986	21299	-80.16	21343.68	-80.40	0.21	0.30
9490	3567.1	-19780	20099	-79.78	20120.65	-79.90	0.11	0.16
10100	3498.4	-18639	18964	-79.37	19011.98	-79.50	0.25	0.17
10700	3438.1	-17642	17973	-78.97	17939.21	-79.16	0.19	0.23
11400	3375.5	-16609	16948	-78.51	16902.95	-78.70	0.27	0.24
12200	3312.4	-15572	15920	-77.99	15976.23	-78.27	0.35	0.36
12900	3263.3	-14769	15125	-77.54	15072.24	-77.68	0.35	0.18
13800	3207.1	-13854	14221	-76.97	14236.86	-77.34	0.11	0.48
14700	3157.4	-13050	13427	-76.40	13459.19	-76.76	0.24	0.48
15600	3113.1	-12339	12725	-75.84	12699.43	-76.14	0.20	0.40
16600	3069.2	-11637	12035	-75.23	12023.93	-75.56	0.09	0.44
17700	3026.3	-10957	11367	-74.56	11373.86	-74.94	0.06	0.51
18800	2988	-10355	10777	-73.90	10755.86	-74.25	0.20	0.46
20000	2950.6	-9773.7	10209	-73.20	10174.5	-73.55	0.34	0.47
21300	2914.5	-9217.5	9667.3	-72.45	9655.557	-72.81	0.12	0.49
22600	2882.1	-8725	9188.7	-71.72	9146.701	-72.11	0.46	0.54

(Part 5 of 7)								
24100	2848.6	-8222.8	8702.2	-70.89	8669.974	-71.29	0.37	0.56
25600	2818.5	-7779.4	8274.3	-70.08	8235.923	-70.41	0.47	0.47
27300	2787.9	-7336	7847.9	-69.19	7812.369	-69.66	0.45	0.68
29000	2760.4	-6944.8	7473.3	-68.32	7432.701	-68.73	0.55	0.59
30900	2732.6	-6558.9	7105.4	-67.38	7063.264	-67.81	0.60	0.63
32900	2706.1	-6201.4	6766.1	-66.43	6727.828	-66.84	0.57	0.62
35000	2680.9	-5870.6	6453.8	-65.46	6411.794	-65.88	0.66	0.65
37200	2656.9	-5564.9	6166.6	-64.48	6122.105	-64.85	0.73	0.58
39600	2632.9	-5270.9	5891.9	-63.46	5849.674	-63.80	0.72	0.54
42200	2609.1	-4991.3	5632.1	-62.40	5595.797	-62.79	0.65	0.61
44900	2586.3	-4736.2	5396.4	-61.36	5360.679	-61.82	0.67	0.75
47700	2564.5	-4503.4	5182.4	-60.34	5143.198	-60.71	0.76	0.61
50800	2542	-4277	4975.4	-59.28	4939.694	-59.60	0.72	0.55
54100	2519.6	-4065.9	4783.3	-58.21	4749.783	-58.57	0.71	0.60
57500	2497.9	-3875.4	4610.6	-57.20	4575.812	-57.52	0.76	0.57
61200	2475.5	-3693.7	4446.5	-56.17	4412.734	-56.51	0.77	0.59
65100	2453.1	-3526.3	4295.6	-55.18	4264.198	-55.51	0.74	0.60
69300	2430	-3369	4153.9	-54.20	4118.783	-54.50	0.85	0.55
73800	2406.2	-3222.3	4021.6	-53.25	3986.593	-53.56	0.88	0.58
78500	2382.2	-3089.1	3901	-52.36	3869.182	-52.67	0.82	0.58
83500	2357.3	-2965.9	3788.6	-51.52	3757.44	-51.81	0.83	0.56
88900	2331	-2850.6	3682.3	-50.73	3653.224	-51.04	0.80	0.61
94600	2303.7	-2745.3	3583.9	-50.00	3555.019	-50.29	0.81	0.57

(Part 6 of 7)								
101000	2273.5	-2643.7	3486.8	-49.31	3466.714	-49.69	0.58	0.78
107000	2245.5	-2561.4	3406.3	-48.76	3379.169	-49.12	0.80	0.72
114000	2213	-2478.5	3322.7	-48.24	3296.9	-48.65	0.78	0.85
121000	2180.7	-2407.1	3248	-47.83	3221.02	-48.25	0.84	0.88
129000	2143.9	-2337	3171.4	-47.47	3146.404	-47.90	0.79	0.89
137000	2107.2	-2276.8	3102.3	-47.22	3075.276	-47.64	0.88	0.90
146000	2066	-2218.6	3031.6	-47.04	3006.759	-47.50	0.83	0.98
155000	2024.9	-2168.5	2966.9	-46.96	2939.396	-47.41	0.94	0.96
165000	1979.5	-2120.3	2900.7	-46.97	2875.006	-47.42	0.89	0.96
176000	1929.8	-2074.5	2833.4	-47.07	2810.438	-47.53	0.82	0.96
187000	1880.6	-2034.6	2770.5	-47.25	2745.902	-47.70	0.90	0.94
199000	1827.4	-1996	2706.2	-47.52	2681.363	-48.00	0.93	1.00
212000	1770.8	-1958.8	2640.6	-47.89	2613.507	-48.35	1.04	0.96
226000	1711	-1922.6	2573.7	-48.33	2550.549	-48.79	0.91	0.94
240000	1652.6	-1889.4	2510.1	-48.83	2487.936	-49.25	0.89	0.86
256000	1587.8	-1854	2441	-49.42	2421.966	-49.86	0.79	0.88
272000	1525.3	-1820.5	2375.1	-50.04	2355.227	-50.46	0.84	0.82
289000	1461.5	-1786.5	2308.2	-50.71	2290.618	-51.17	0.77	0.89
308000	1393.5	-1749.8	2236.8	-51.47	2223.1	-51.94	0.62	0.91
328000	1325.7	-1712.1	2165.4	-52.25	2156.261	-52.72	0.42	0.89
349000	1258.8	-1673.5	2094.1	-53.05	2087.427	-53.52	0.32	0.89
371000	1193.3	-1633.8	2023.1	-53.86	2018.73	-54.36	0.22	0.92
395000	1126.9	-1591.3	1949.9	-54.70	1949.664	-55.21	0.01	0.93

(Part 7 of 7)								
420000	1063.2	-1548.1	1878	-55.52	1879.59	-56.12	0.08	1.07
447000	1000.1	-1502.6	1805	-56.35	1811.393	-57.01	0.35	1.16
476000	938.65	-1455.1	1731.6	-57.18	1743.264	-57.91	0.67	1.27
506000	881.19	-1407.7	1660.7	-57.95	1675.049	-58.83	0.86	1.48
539000	824.47	-1357.6	1588.3	-58.73	1608.022	-59.73	1.23	1.67
573000	772.37	-1308.3	1519.2	-59.44	1542.174	-60.61	1.49	1.92
610000	722.08	-1257.3	1449.9	-60.13	1477.208	-61.44	1.85	2.13
649000	675.35	-1206.6	1382.7	-60.76	1412.864	-62.29	2.13	2.45
691000	631.25	-1155.3	1316.5	-61.35	1350.158	-63.14	2.49	2.84
735000	590.94	-1105.1	1253.2	-61.87	1290.398	-63.93	2.88	3.24
782000	553.52	-1055.3	1191.6	-62.32	1229.161	-64.69	3.06	3.66
833000	518.44	-1005.3	1131.1	-62.72	1173.483	-65.53	3.61	4.29
886000	487.06	-957.49	1074.2	-63.04	1117.062	-66.26	3.84	4.86
943000	458.09	-910.31	1019.1	-63.29	1063.211	-66.97	4.15	5.50
1000000	433.21	-867.1	969.29	-63.45	1011.296	-67.57	4.15	6.09

Table A.13: The equivalent circuit fit, measured impedance spectrum captured with 100 μm diameter electrodes and their respective relative errors for cell culture medium 20 minutes after the onset of shear stress (17.6 dyne/cm²).

A.4 Volumetric Flowrate Measurement Error

Volumetric flowrate was acquired by measuring fluid volume accumulation for a fixed time period at each pump (Ismatec, Model # EW-78017-10) setting. The deviations existing in the calculated volumetric fluid flow rate are summarized in Fig. A.6. It is demonstrated that volumetric flow rates present small deviations ranging ± 0.005 - 0.068 , where deviations increase with increasing flow rate/ pump setting.

Figure A.6: Pump curve acquired from volumetric flow rates measured, where the deviation in the measured flow rates are demonstrated. Error bars are presentative $\pm$ one standard deviation.

A.5 Protocol for the Quantification of VE-Cadherin Expression

The expression of VE-cadherin was quantified through a modified image analysis protocol [198] using ImageJ freeware.

1. Open DAPI image and VE-cadherin image

Figure A.7: DAPI image (left) and VE-cadherin (right) image.

2. Select the DAPI image
3. Click on Process → Filters → Gaussian Blur → Sigma **2.0**. This smoothens out a cluster of fluorescent pixels. Click Process → Binary → Make Binary

Figure A.8: Smoothen DAPI image (left) and smoothen and binary DAPI (right) image.

4. Select VE-cadherin image

5. Click on Process → Filters → Gaussian Blur → Sigma **1.0**. This smoothens out a cluster of fluorescent pixels. Click Process → Binary → Make Binary.

This helped the algorithm capture those thin lines of VE-cad.

Figure A.9: Smoothen VE-cadherin image (left) and smoothen and binary VE-cadherin (right) image.

6. Select Process → Image Calculator.

- Image 1: VE-cadherin Binary
- Operation: Subtract
- Image 2: DAPI Binary

7. Then manually select and erase the remaining nuclei signal (green arrow) from *subtracted binary* image.

Figure A.10: Resulting VE-cadherin image after DAPI image is subtracted. Arrow indicates the reimaging DAPI signal removed manually.

8. Define the specified area of analysis, in our case electrode area. Draw an ellipse.

Select Edit → Selection → Specify

- W, h: 650, 650 for 200 μm electrodes and 320, 320 for 100 μm electrodes
- X, Y position: 194, 194 for 200 μm electrodes and 358, 368

9. Select Analyze → Analyze Particles

- Size: 0-infinity

- Circularity: 0-1(0...polygon, 1 circle)
- Show: Overlay
- Checked Display Results and Add to manage

10. Close modified thresholded image and open original image and click measure

Figure A.11: Selection of VE-cadherin fluorescence for a 200 μm (left) and 100 μm (right) electrode diameter.

Figure A.12: Example: of a measurement on a 200 μm diameter electrode.

A.6 Shear Condition: 2.5 dyne/cm²

Figure A.13: Normalized plots of the magnitude of the constant phase element ($CPE-Y$) with respect to time collected with (A) 200 and (B) 100 μm diameter electrodes. R_{TER} was recorded for HUVECs under static, constant shear (2.5 and 17.6 dyne/cm²), and a stepped shear condition (23.7-35-58.1 dyne/cm²). For the stepped shear condition, HUVECs were initially sheared at 23.7 dyne/cm² for 2 hours ($t=0-2$ hr). Subsequently, HUVECs were sheared at 35 dyne/cm² ($t=2-4$ hr) for an additional 2 hours. Finally, they were sheared at 58.1 dyne/cm² for 14 hours ($t=4-18$ hr). The changes in shear stress for the stepped shear condition are indicated by the orange triangle markers.

Figure A.14: Normalized plots of the power factor of the constant phase element ($CPE-n$) with respect to time collected with (A) 200 and (B) 100 μm diameter electrodes. R_{TER} was recorded for HUVECs under static, constant shear (2.5 and 17.6 dyne/cm^2), and a stepped shear condition (23.7-35-58.1 dyne/cm^2). For the stepped shear condition, HUVECs were initially sheared at 23.7 dyne/cm^2 for 2 hours ($t=0-2\text{ hr}$). Subsequently, HUVECs were sheared at 35 dyne/cm^2 ($t=2-4\text{ hr}$) for an additional 2 hours. Finally, they were sheared at 58.1 dyne/cm^2 for 14 hours ($t=4-18\text{ hr}$). The changes in shear stress for the stepped shear condition are indicated by the orange triangle markers.

Figure A.15: Normalized plots of resistance of cell culture medium (R_{MED}) extracted from control cell-free measurements from (A) 200 and (B) 100 μm diameter electrodes. R_{TER} was recorded for HUVECs under static, constant shear (2.5 and 17.6 dyne/cm^2), and a stepped shear condition (23.7-35-58.1 dyne/cm^2). For the stepped shear condition, HUVECs were initially sheared at 23.7 dyne/cm^2 for 2 hours ($t=0-2\text{ hr}$). Subsequently, HUVECs were sheared at 35 dyne/cm^2 ($t=2-4\text{ hr}$) for an additional 2 hours. Finally, they were sheared at 58.1 dyne/cm^2 for 14 hours ($t=4-18\text{ hr}$). The changes in shear stress for the stepped shear condition are indicated by the orange triangle markers.

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Experience

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Graduate Research Assistant, University of Louisville,
Integrated Microfluidic Systems Laboratory.

- Development of electrical impedance sensing microfluidic platforms for characterization of endothelium layers under shear stress.
- Experimental study of AC electrokinetic and optical manipulation of particles in fluid suspensions

- 05/ 2013-11/2013 **Research Intern**, Harvard-MIT/HST, Department of Medicine, BWH, Harvard Medical School, Cambridge, MA
BAMM Labs
- Development and testing of a microfluidic chip for viable sperm cell isolation.
 - Toxicity investigation of carbon nanotubes and graphene on sperm cells
 - Inexpensive fluorescent imaging system for candida detection within microfluidic chips
- 08/ 2009-2010 **Research Scientist**, University of Louisville,
Micro/Nano Technology Center.
- Mentoring cleanroom clients in the operation and performance of photolithography equipment and methods, deep reactive ion etching systems, and material deposition systems such as sputtering and evaporation.
 - Developing and updating standard operating procedures for micro-fabrication systems in the cleanroom facility at the University of Louisville.
 - Design, fabrication and characterization of micro-electro mechanical systems and nano-structured interfaces and devices.
- 07/ 2009 **Visiting Research Assistant**, Thomas Jefferson University, Philadelphia, PA,
Kimmel Cancer Center.
- Culture, characterization, optical imaging of MCF7 breast cancer cells.
 - Experimental study of antibody functionalization methods on carbon nanotube films.
- 08/ 2008- 2010 **Graduate Research Assistant**, University of Louisville,
Small Systems Laboratory.
- Design and development of carbon nanotube biosensors for capturing circulating breast cancer cells in blood.
 - Design, fabrication and characterization of nanotube cantilevers for bioassay of prostate specific antigen.
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- 08/ 2007- 2008 **Undergraduate Research Assistant**, FSU-FAMU College of Engineering,
- Observation of animal sedation and dissection.
 - Developing and testing probes used in blood brain barrier function studies.

Skills

- Knowledgeable in multi-disciplinary computational design and simulation software (ChemCAD, ANSYS, CoventorWare, Maple, MATHCAD, FLUENT, COMSOL, MATLAB)
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Scholarly Activities

Book Chapters:

1. V. Velasco, E. Wickstrom and B. Panchapakesan, "Applications of nanoparticles for molecular targeting and selective destruction of breast cancer cells," Nanoparticle Synthesis, Characterization and Applications (Edited by R.V. Chaughule and R.S. Ramanujan) American Scientific Publishers (2010).

Journal Publications:

1. S Lu, S Ahir, **V. Velasco**, B King, P. Xu, E Terentjev and B Panchapakesan, "Photomechanical actuation of carbon nanotubes: mechanisms and applications in micro and nanodevices," *Journal of Micro and Nanomechatronics*, Vol.5, 29-41, (2009).
2. B Panchapakesan, R Caprara, **V Velasco**, J Loomis, B King, P Xu, T Burkhead, P Sethu, LJ Stallons, WG McGregor. "Micro- and nanotechnology approaches for capturing circulating tumor cells," *Cancer Nanotechnology*, Vol. 1, 3, (2010).
3. **V Velasco**, AH Work, Jr., and SJ Williams, "Electrokinetic concentration and patterning of colloids with a scanning laser," *Electrophoresis*, Vol.33, pg1931-1937, (2012).
4. **V Velasco** and SJ Williams. "Electrokinetic concentration, patterning, and sorting of colloids with thin film heaters," *Journal of Colloid and Interface Science*, Vol.394, pg 598-603, (2013).

5. W Asghar, **V Velasco** et al., "Selection of Functional Human Sperm with Higher DNA Integrity and Fewer Reactive Oxygen Species." *Advanced Healthcare Materials*, Vol.00, pg 1-8, (2014).
6. **V Velasco**, MJ Gruenthal, E Zusstone, SJ Williams, JM Thomas, E Berson, R Keynton, "An Orbital Shear Platform for Real-Time, *In Vitro* Endothelium Characterization," *Biotechnol. Bioeng.*, Vol.113, pg 1336-1344, (2016).

Journal Publications in Preparation:

1. **V Velasco**, R Keynton, SJ Williams, "Microfluidic Platform for the Impedance Characterization of Endothelial Cells under Fluid Shear Stress."
2. W Asghar, H Shafiee, **V Velasco**, et al., "Toxicology Study of Single-walled Carbon Nanotubes and Reduced Graphene Oxide in Human Sperm."

Abstracts and Invited Presentations:

1. S Day, **V Velasco**, SJ Williams. "Development of an Opto-electrowetting Platform," *IMECE*, (2011). Poster Presentation
2. **V Velasco**, K King, SJ Williams, and R Keynton, "Development of an Automated Microfluidic Platform for Impedance Analysis of the Endothelium under Flow-Induced Shear Stress," *BMES*, (2011). Poster Presentation
3. **V Velasco**, AH Work, Jr., and SJ Williams, "Rapid Electrokinetic Self-Assembly and Patterning of Colloids," *KY Nanotechnology Symposium*, (2012). Oral Presentation
4. AH Work, Jr., **V Velasco**, and SJ Williams, "Electrokinetic Manipulation of Colloids with a Scanning Laser," *IMECE*, (2012). Poster Presentation
5. **V Velasco**, AH Work, Jr., and SJ Williams, "Electrokinetic Concentration and Patterning of Colloids with Thin Film Resistors," *IMECE*, (2012). Poster Presentation
6. AH Work, Jr., **V Velasco**, and SJ Williams, "Rapid Electrokinetic Patterning (REP) of Hydrosol Colloids at a Planar Electrode Surface," *AICHE*, (2012). Poster Presentation
7. M Gruenthal, **V Velasco**, RE Berson, R Keynton and SJ Williams "Impedance Analysis of Endothelial Cells in Development of an Orbital Shear Platform," *AICHE*, (2012). Poster Presentation
8. **V Velasco**, M Gruenthal, and SJ Williams, "A Microfluidic Platform for Impedance Analysis and Characterization of Human Umbilical Vein Endothelial Cells," *AICHE*, (2012). Oral Presentation
9. AH Work, Jr., **V Velasco**, and SJ Williams, "Patterning Simple Geometries with Colloids Using a Scanning Laser," *AICHE*, (2012). Oral Presentation
10. MJ Gruenthal, **V Velasco**, RE Berson, RS Keynton, and SJ Williams, "Impedance analysis of endothelial cells cultured on an orbital platform," *ASME/IMECE*, (2013). Poster Presentation
11. SJ Williams, **V Velasco**, A Mishra, J-S Kwon, and ST Wereley, "Rapid electrokinetic patterning (REP): manipulating colloids from nanoparticles to bacteria," *2013 Kentucky Nano Symposium*, (2013). Oral Presentation
12. W Asghar, VR Sah, H Shafiee, **V Velasco** et al., "Toxicology of Carbon Based Nano-materials on Human Sperm: A Concentration and Time

Dependent Study,” *Graphene and Related Technologies: from laboratory to Industry*, (2014). Oral Presentation

13. **V Velasco**, MJ Gruenthal, SJ Williams, JM Thomas, E Berson, R Keynton, “An Orbital Shear Platform for in-Vitro Real-Time Endothelium Characterization” *AIChE*, (2014). Oral Presentation
14. V Velasco, SJ Williams, “Microfluidic Platform for Impedance Characterization of Endothelial Cells Under Fluid Shear Stress”, *AIChE*, (2014). Poster Presentation
15. V Velasco, R Keynton , SJ Williams, “Microfluidic Chip for Impedance Analysis and Characterization of Human Umbilical Vein Endothelial Cells Under Fluid Shear Stress”, *Physics & Chemistry of Microfluidics GRC*, (2015). Poster Presentation

Professional Affiliations:

- Society of Hispanic Professional Engineers (SHPE)- *Member*
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- American Society of Mechanical Engineers (ASME)- *Member*
- American Electrophoresis Society (AES) – *Member*
- American Institute of Chemical Engineers (AIChE) – *Member*

Awards and Scholarships:

- Awarded Carl Storm Underrepresented Minority (CSURM) Fellowship, \$600, Spring 2015.
- Awarded University Graduate Student Research Fund, \$300, Fall 2014.
- Aided in the writing of an awarded University internal grant (IRIG), "The Project Completion Grant", \$4,000, Summer 2014
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- Southern Regional Education Board State Doctoral Fellowship, Fall 2010.
- Hispanic Scholarship Fund, Fall 2003.