Nuclear Rupture in Progeria Expressing Cells

Kranthidhar Bathula

Follow this and additional works at: https://scholarscompass.vcu.edu/etd

Part of the Molecular, Cellular, and Tissue Engineering Commons, and the Musculoskeletal Diseases Commons

© The Author

Downloaded from
https://scholarscompass.vcu.edu/etd/5479

This Thesis is brought to you for free and open access by the Graduate School at VCU Scholars Compass. It has been accepted for inclusion in Theses and Dissertations by an authorized administrator of VCU Scholars Compass. For more information, please contact libcompass@vcu.edu.
NUCLEAR RUPTURE IN PROGERIA EXPRESSING CELLS.

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at Virginia Commonwealth University.

by

Kranthidhar Bathula, B.S.

Virginia Commonwealth University, 2016

Director: Daniel Conway, Ph.D.,
Assistant Professor
Department of Biomedical Engineering

Virginia Commonwealth University
Richmond, Virginia

May, 2018
Acknowledgments

First and foremost, I would like to give my deepest regards and thanks to Dr. Conway for his support through the years. This journey started in undergrad and all along he encouraged my interest in the field and gave me the opportunities to be successful. I would also like to thank my committee members Dr. Lemmon and Dr. Guven for their feedback and advice. Also, Paul Arsenovic who mentored me early on and was there to help and give me guidance. Also, Lindsay LaFratta who aided with various projects that were key to this paper. Also, the rest of my lab mates who motivated me. Finally, I would like to thank my friends and family for their support in my graduate career.
Abstract

NUCLEAR RUPTURE IN PROGERIA EXPRESSING CELLS

Kranthidhar Bathula

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at Virginia Commonwealth University.

Virginia Commonwealth University, 2018.

Major Director: Daniel Conway, Ph.D., Assistant Professor, Department of Biomedical Engineering

Cells regularly take on various types of force in the body. They have structures that are able to mediate, transfer and respond to the forces. A mutation in force regulating proteins such as lamin in the nucleus or the KASH domain which connects the nucleus to the cytoskeleton of the cell can cause catastrophic events to occur. The aims of this study were to better understand the response of the nucleus when structural proteins are mutated or are not present while under force. Progeria, a rare disease where an additional farnesyl group is attached to lamin was used in this study. Different types of forces were used to represent similar conditions in the body. Confinement of endothelial cell width showed an increase of surface defects. When restricting proteins such as actin was removed the nucleus appeared to rupture. This was shown to occur at a higher rate in the progeria groups. Endothelial cells under shear force showed high amount of nuclear rupture in progeria expressing group. prolonged exposure showed more rupture which eventually cased cell death and cells to come off the surface. Progeria expressing smooth muscle cells under cyclic stretch also showed similar results as endothelial cells. The amount and rate of deformation of the nucleus when the cytoskeleton is connected and not was looked at. When the
connected the rate of deformation was higher. The high rate of nuclear defects and rupture while under force in progeria expressing cells shows that the nuclei have different structural properties and are weaker. It’s also been shown that the LINC complex contributes to nuclear deformation when stretching.
Table of Contents

Chapter 1: Introduction & Background ........................................................................................................... 8

Chapter 2: Force on the Nucleus in Progeria expressing cells ................................................................. Error! Bookmark not defined.
  2.1. Introduction .............................................................................................................................................. Error! Bookmark not defined.
  2.2. Methods ................................................................................................................................................ Error! Bookmark not defined.
  2.3. Results and Discussion ......................................................................................................................... Error! Bookmark not defined.
  2.4. Conclusions .......................................................................................................................................... Error! Bookmark not defined.

Chapter 3: Nuclear Rupture caused by External Forces (Shear & Cyclic Stretch) on Progeria expressing Cells ........................................................................................................................................ 28
  3.1 Introduction .............................................................................................................................................. Error! Bookmark not defined.
  3.2 Methods ................................................................................................................................................ Error! Bookmark not defined.
  3.3 Results and Discussion ......................................................................................................................... Error! Bookmark not defined.
  3.4 Conclusions .......................................................................................................................................... 28

Chapter 4: Cell vs. Nuclear Elongation in live stretched Control and DN KASH cells .................... 29
  4.1 Introduction .............................................................................................................................................. 29
  4.2 Methods ................................................................................................................................................ 30
  4.3 Results and Discussion ......................................................................................................................... 32
  4.4 Conclusions .......................................................................................................................................... 33

Chapter 5: Conclusions and Future Directions ......................................................................................... Error! Bookmark not defined.
  5.1 Conclusions .............................................................................................................................................. Error! Bookmark not defined.
  5.2 Future Directions ................................................................................................................................ Error! Bookmark not defined.

Bibliography ......................................................................................................................................................... Error! Bookmark not defined.

Figures ......................................................................................................................................................... 40-65
# Table of Figures

FIGURE 1: Depiction of the LINC Complex ................................................................. 40
FIGURE 2: Progeria Expressing Cell ........................................................................ 41
FIGURE 3: Nuclear Rupture .................................................................................... 42
FIGURE 4: Confocal Images of Progeria Cells on Lines ......................................... 43
FIGURE 5: Angle of Folds on Cells on Lines .......................................................... 44
FIGURE 6: Length and Angle of Folds .................................................................... 45
FIGURE 7: Variation of Length of Folds Based on Line Width ............................... 46
FIGURE 8: Nuclear Lamina Model of Progeria Cell ............................................... 47
FIGURE 9: Cell Analysis Counting Process ............................................................. 48
FIGURE 10: Number of Sheared Cells .................................................................. 49
FIGURE 11: Percentage Rupture for Sheared Cells .............................................. 50
FIGURE 12: Stained Images of Sheared Control Cells .......................................... 51
FIGURE 13: Stained Images of Sheared Overexpressed Lamin A Cells ............... 52
FIGURE 14: Stained Images of Sheared Progerin Cells ......................................... 53
FIGURE 15: Number of Stretched Cells ................................................................. 54
FIGURE 16: Percentage Rupture for Stretched Cells .......................................... 55
FIGURE 17: Stained Images of Stretched Control Cells ........................................ 56
FIGURE 18: Stained Images of Stretched Overexpressed Lamin A Cells ............ 57
FIGURE 19: Stained Images of Stretched Progerin Cells ....................................... 58
FIGURE 20: Lamin A/C Stain of Ruptured Endothelial and Smooth Muscle Cells .. 59
FIGURE 21: Cell Stretcher ...................................................................................... 60
FIGURE 22: Top View of Cell ................................................................................ 34
FIGURE 23: Stretched Control Cells .................................................................... 62
FIGURE 24: Stretched DN KASH Cells ................................................................. 63
FIGURE 25: Strain of Control Cells ....................................................................... 64
FIGURE 26: Strain of DN KASH Cells .................................................................. 65
Chapter 1 Introduction & Background

The nucleus is one of the most important organelles in the cell; it connects to the cytoskeleton of the cell and is able to take on physical forces. When these forces are mis-regulated due to mutated structural proteins catastrophic events can occur. Recent work has been done to better understand the nucleus’ response under high force in these situations. By testing an altered form of the Lamin A protein, known as progerin, its role and importance can be understood. The aims of this study were to put cells in high force states through: constriction, shear, and stretch to observe structural proteins’ response from a control vs altered state. Constricting cells in lines places high force on the nucleus, this showed a higher amount of wrinkles on the surface of progeria cells. Variation in wrinkle length and direction was also observed. External protein actin which restrains the nucleus was removed and the time for loss of wrinkles was observed. Progeria showed slower and less loss. It was also shown to produce high amounts of nuclear rupture in progeria cells. To further look at nuclear rupture various types of external forces were applied such as shear and stretch to a monolayer of cells. Various groups and time points showed high amount of nuclear rupture and cell death occurring at longer time points in progeria cells; this was observed in endothelial and smooth muscle cells. To understand the link between the nucleus and the cytoskeleton the LINC complex was studied. Individual cells were stretched where the LINC complex was connected and disconnected. The nucleus was shown to stretch more when the LINC complex is disconnected. Overall, this research gave insight into the role of Lamin A when placed under external force; the connection between the nucleus and cytoskeleton is also better understood.
LINC Complex

Cells are able to take on force and transfer them to the nucleus, this is done via chromatin and LINC complex interactions. Early studies have shown tugging on integrin receptors in the cell membrane causes the nucleus to move\(^1\), proving that there is a physical link between the two. The linker of nucleoskeleton to cytoskeleton (LINC) complex is vital to performing this action as it links the nucleus to the cellular cytoskeleton\(^2,3\) as shown in Figure 1. It is a conserved system in all eukaryotic cells\(^4\), which emphasizes its importance to cell function. The LINC complex anchors the cytoskeleton to the nuclear envelope through SUN and KASH domain proteins\(^3\). The SUN domain consists of proteins SUN1-4, though SUN1 & 2 are the most common in most tissue\(^1\). The SUN proteins go through the inner nuclear membrane into the perinuclear space\(^3\). Within the perinuclear space the SUN proteins connect to the KASH domain proteins, which are Nesprin 1-4, LRMP, and KASH5\(^1\). They travel through the outer nuclear membrane and eventually connect to actin, microtubules, and intermediate filaments\(^3\). As shown in Figure 1 the components of the LINC complex can be seen traveling through the nuclear membrane and eventually connecting to the cytoskeleton. Nesprin 1-2 are able to bind to actin, dynein and kinesin\(^1\). Nesprin 3 binds to plectin which connects to intermediate filaments\(^1\). Nesprin 4 can bind to dynein which binds to microtubules though mostly expressed in epithelial cells\(^1\). LRMP does not connect to the cytoskeleton, KASH5 is only found in meiotic cells and binds to dynein\(^1\).

The LINC complex has many roles including maintaining nuclear structure, position, and allows for migration\(^1\). It has been shown that knocking down Nesprin 1 causes the nucleus to increase in height\(^1\), showing that the nucleus is under tension. It has also been shown that disturbing nesprin-2, SUN2 or the connecting actin prevent the nucleus from being moved and
the centrosome from being correctly oriented\(^5\). Recently, studies have shown that there is an increase in nuclear force by confinement of the cell, the more confined the cell the more force was placed on the nucleus, this was measured through a nesprin-2 FRET sensor\(^2\). It has also been shown that physical forces across the LINC complex can regulate certain gene expression\(^6\). TAN (Transmembrane Actin-associated Nuclear) lines are produced from nesprin 2 and SUN2 proteins that connect to actin bundles that bind across the top of nuclei\(^1\). This actin going across the top of the nucleus is considered the actin cap and keep the nucleus under tension. Experiments where cells were elongated on rectangular micro-patterns produced elongated nuclei and showed nuclear lamina deformations\(^7\). ‘Indentation sites’ were observed deep in the nucleus where force was placed when actin and chromatin co-localized\(^7\). It was shown that apical actin put a compressive force on the nucleus from the top\(^7\). The density of LINC complex proteins was higher at indentation sites, the indents are deep and the nuclei unable to escape the cage created\(^7\). The indents here could be produced by the actin cap that keeps the nucleus under tension.

**Lamins**

The nuclear lamina is a 40-60 nm thick layer of proteins, mostly lamins that reside between the inner nuclear membrane and chromatin\(^8\). Lamins are intermediate filaments which are 10-12 nm thick depending on the type; they are coiled-coils, meaning a single coil of monomers is made and again coiled over itself to produce a filament\(^9\). Lamins reside in the nuclear envelope and produce the nuclear lamina which has structural properties which are key to nuclear function\(^9\). The nuclear lamina also interacts with chromatin in the nucleus which may allow it to alter genetic expression\(^9\). There are two type of lamins: A&B-type lamins\(^9\). A-type
lamins include Lamin A/C which are coded for in the *LMNA* gene, B-type lamins include Lamin B1/B2 which are coded for in *LMNB1 & LMNB2*. A-type and B-type have as similar protein Head and α-Helical rod Domain, the tail is where they vary. Lamin B1 and B2 are similar in structure though B2 is longer, Lamin A and C are the same where A is longer. Some organisms produce a single type Lamin but mammals produce both types; B-type lamins are in all somatic cells but A-type lamins are expressed mostly in differentiating tissue.

Lamins provide structural support to the nucleus, studies have previously shown that when lamin is depleted the nucleus is smaller and fragile. This is also supported by experiments done with mutated *LMNA* where the nucleus has been shown to have varying properties. Cells that lack *LMNA* are easier to deform and deform to greater extents. These nuclei also deform in an isotropic manner compared to the anisotropic deformation of normal nuclei, meaning nuclei deform the same way when perturbed compared to responding differently depending on the location of perturbation. Oddly loss of just Lamin B1 shows blebbing but not doesn’t affect the mechanical properties of the nuclei, this could mean that Lamin A/C take on the majority of the load and give the nuclei its structural properties. Lamins being a part of the LINC complex interact with SUN1&SUN2 which travel through the inner nuclear membrane. Thus it is possible that forces can be transmitted bi-directionally through the cell, reaching from the cell membrane to inside the nucleus. On the other side of the lamina, lamin interacts with heterochromatin. Lamin has binding sites on its rod domain and tail region and is able to effect chromatin organization. Cells with lamin mutations have shown heterochromatin to be disengaged or lost.

Laminopathies are diseases involving *LMNA* mutations, there are over 400 disease causing mutations that have been observed, some of which are inheritable. Some mutations
affect single types of tissue while others can target multiple; they are mostly found in mesenchymal tissue and bone\textsuperscript{9}. There are only 2 diseases related to Type-B lamin mutations\textsuperscript{9}.

**Progeria**

Hutchinson-Gilford Progeria Syndrome (HGPS) is a rare laminopathy affecting 1 in 8 million births in the world\textsuperscript{11}. Affected individuals appear to age at a drastic rate though are unable to grow to full physical stature; they experience balding, wrinkling of skin, and bone lesions among other physical triatis\textsuperscript{11,12}. Most, typically die in their teenage years due to arthrosclerosis\textsuperscript{11}.

Mutations in the LMNA gene which encodes for Lamin A and C are responsible for the effects of the disease\textsuperscript{12}. For most cases nucleotide position 1824 in LMNA is altered (Cytosine substituted to Thymine); this produces a truncated prelamin A which is 50 amino acids short\textsuperscript{12,13}. Progeria expressing prelamin lack the cleavage site required to remove an attached farnesyl group, thus the whole body including the uncleaved portion misalign along the wall of the nuclear envelope which causes the blebbing phenotype of progerin nuclei\textsuperscript{12,14}.

The physical properties of progeria expressing nuclei are different compared to normal cells. The differences in the lamins of progeria and normal cells are shown in Figure 2. Expression of progeria in isolated xenopus oocytes nuclei tested through atomic force microscopy (AFM) shows that they have a higher elastic modulus than wild type nuclei\textsuperscript{15}. This shows that progeria nuclei are stiffer compared to wild type, and is further supported by AFM tests on isolated nuclei from dermal fibroblasts which showed similar results\textsuperscript{16,17}. The increased stiffness may be related to the blebbing of the progeria nuclei.
Nuclear Rupture

The nuclear envelope (NE) is a membrane that encapsulates the nucleus; it has the job of protecting and regulating nuclear function\(^\text{18}\). It is made of two lipid bilayers, known as the inner and the outer nuclear membrane (INM & ONM). The space between the membranes is the perinuclear space\(^\text{18}\). The NE contains various proteins with varied functions. Nuclear pore complexes are channels that run through the NE where bidirectional transport occurs, RNA and ribonucleoproteins smaller than 40nm exchange between the nucleoplasm and cytoplasm\(^\text{18}\). Inner nuclear membrane proteins interact with lamins and chromatin; they play a role in chromatin organization, gene expression, and DNA metabolism\(^\text{18}\). Outer nuclear proteins all have a KASH domain which interacts with SUN domain proteins of the inner nuclear membrane protruding to the perinuclear space\(^\text{18}\). The INM and ONM proteins form connections that travel through the perinuclear space; these proteins could allow for physical connections between the cytoskeleton of the cell to the chromatin of the nucleus\(^\text{18}\). The nuclear lamina consists of lamins that reside on the inside of the INM, they have been shown to play major roles in the structural integrity of the nucleus when exposed to mechanical force, it is also said to play roles in chromatin function and gene expression\(^\text{18}\).

Nuclear integrity is compromised when the NE breaks. This happens where the NE is weakened, such as areas with protrusions or lacking in lamins where breakage can easily occur\(^\text{19}\). Though ruptures occur the nuclei can still repair itself and remain viable even after multiple ruptures\(^\text{20}\). Cells expressing laminopathies have been shown to be prone to nuclear rupture\(^\text{19}\), likely because they are unable to regulate force properly which results in mechanical failure. Intra-nuclear pressure has been shown to be the cause of rupture rather than the envelope being ripped apart; KASH and SUN domains, proteins that anchor the nucleus to the cell were altered
and knocked down, ruptures were still shown to be present, through a smaller amount of ruptures were observed this is more likely due to not binding to actin rather than the nucleus being pulled apart. To further prove this LINC complex proteins which are attachment points on the nucleus were looked for in areas of nuclear blebs and were not found to localize there. Lesions in the lamin network cause chromatin herniation which leads to NE ruptures, which clearly exhibits the importance of an intact lamina for NE stability. Figure 3 shows the different stages before nuclear rupture occurs as well as the different observed causes of nuclear rupture.

Studies were performed where lamin type A proteins were knocked out and observed, the nuclei of these cells were shown to deform easier, this shows that lamins play a role in nuclear rigidity. If nuclei are able to deform easier then they can cause areas of higher stress and are more prone to rupture that way. It was shown that fibroblasts expressing HGPS had less ruptures than lamin A/C deficient cells, nuclei with a mutated lamin are able to regulate forces than having a lack of lamins. A correlation for rupture size and time of repair were looked at, it can’t definitively be said that there is a direct correlation as even small ruptures took a long period of time to repair but a positive correlation was shown. It was also shown that nuclei that ruptured repeatedly were able to repair themselves faster after multiple ruptures. This supports the idea of force strengthening, where a cell is ready to perform a function that has previously occurred.

The amount of nuclear rupture occurring in cells increases when the cells were confined. A study looked at cells that had reduced Lamin B1 levels that were prone to rupture; it was shown that disruption of the actin cytoskeleton reduced rupture. Here the nucleus is less confined by actin filaments in the cell further supports the idea that confining the nucleus is the
reason for NE failure to occur. Cells with mutated type A lamin have been grown on soft
substrates and rupture less than on regular hard surfaces which produce misshaped nuclei\textsuperscript{22}.
Chapter 2: Force on the Nucleus in Progeria expressing cells

2.1. Introduction

HGPS is associated with an aberrant lamina network structure and abnormal nuclear morphology, redistribution of the heterochromatin, alterations in gene expression and nuclear structural instability\(^{12,17,23-25}\). The HGPS mutation or the exogenous expression of progerin leads to an accumulation of progerin in the lamina at the INM. Progerin expressing cells have altered nuclear morphologies that have been described as blebs, wrinkles or folds\(^{12,25-27}\). In addition to altered nuclear shape, HGPS is associated with increased lamina stiffness\(^{12,17,25}\). It is unclear how an increase in a structural protein and a stiffening of the lamina could lead to the blebbed nuclear lamina, which is seemingly related to lamina fragility.

In this study, we investigate the mechanical dysfunction of the nuclear lamina under strains imposed by endogenous cellular forces, by forces in cells under confinement. In considering our data and models correlating the thickening of the lamina with the change in the two dimensional bending modulus of the lamina network, we suggest that the reason for the formation and propagation of wrinkles in progerin expressing nuclei is from the formation of microaggregates of progerin. In addition to suggesting the mechanism of lamina wrinkles associated with HGPS, we also comment on how this altered lamina nano- and micro- structure may impact cytoskeletal force transmission through the cell. These combined structural effects may have important functional consequences in the disease and highlight the benefit of applying physical models to study biological systems to determine aspects of disease states.
*It has been shown that nuclear ruptures decrease when there is a loss of actin filaments, contractile actin filaments or LINC complex, but if the nucleus height is controlled for then nuclear rupture returns, suggesting that confinement is a cause of nuclear rupture \(^{21}\).

### 2.2. Methods

#### Cell Culture and Transfection

For HUVEC studies, Primary HUVEC (Lonza, passages 3-6) were grown in EGM-2 medium (Lonza). To express progerin in HUVEC an adenovirus was developed to express HA-tagged progerin (HA-progerin was a gift of Bryce Paschal\(^{28}\), adenovirus was prepared by Vector Biolabs, Malvern PA). The lowest level of adenovirus that infected nearly 100% of cells was used. To overexpress wild-type lamin A in HUVEC, lamin A adenovirus (based on RefSeq BC014507) was purchased from Vector Biolabs and used at an identical titer level as progerin. For actin depolymerisation studies, latrunculin A (Tocris) was added at 10 µM for reported times before cell fixation and labelling.

#### Micropatterning

HUVEC were seeded on micropatterned lines of width 20 or 40 µm, as previously described\(^2\). Briefly, the stamps used to micropattern fibronectin lines of 20 or 40 µm were made with polydimethylsiloxane (PDMS). Stamps were coated with fibronectin and were pressed onto a prepared coverslip. Once stamped, the coverslips were washed and treated with Pluronic F-127 to limit cell adhesion to only the fibronectin lines. Cells were then seeded onto the coverslip.
**Cell Fixation and Labelling**

Cells were fixed using 4% formaldehyde in phosphate buffered saline (PBS) and permeabilized using 0.2% Triton X-100 in PBS. For fluorescence microscopy experiments, cells were stained with 0.1 µg/mL Hoechst 33342 (ThermoFisher) for DNA staining. HUVEC were stained with anti-lamin A antibody (cat # sc-7292, Santa Cruz) for control cells or anti-HA antibody (cat # 901501, Biolegend) for progerin-expressing cells with an Alexa Fluor 488 fluorescent secondary (cat # A-21202, Thermo Fisher). HUVEC were also stained with rhodamine phalloidin (cat # PHDR1, Cytoskeleton).

**Imaging and Analysis**

HUVEC cells were imaged using a Zeiss 710 LSM confocal at 63x and 1.4NA. Images were processed using ImageJ.

**2.3. Results and Discussion**

To examine the role of extracellular perturbation on nuclear lamina reorganization, we considered how cells respond to growth on patterns. Endothelial cells were grown on patterned lines of 20 µm or 40 µm in order to ascertain the extent of deformation of the lamina network under cell confinement. A sample size of 12-15 cells were used for each group. Previously, patterning on lines of this thickness has been shown to exert forces on the nucleus from the cytoskeleton\textsuperscript{2,29,30}. On 20 µm lines nuclei are oblate and orient in the direction of the actin filaments. This orientation has been shown to be a direct function of the cellular confinement to patterning\textsuperscript{29}. There are some folds in control lamina, but these coincide with actin filament
structures (Figure 4A). Progerin expressing cells show numerous folds and wrinkles in the nuclear lamina, but these dysmorphic structures do not correlate with actin filament structures (Figure 4E).

We quantified the dysmorphic structures, or wrinkles observed in the lamina, visualized in Figure 4 along the length of the nucleus and compared them to the orientation of the nucleus. (Figure 5) Earlier studies have suggested that cells under extreme loading conditions or, in this case confinement, may propagate wrinkle or fissure formations. Lamin networks that are healthy have been found to deform uniformly under similar conditions. For cells patterned on 20 µm stripes, wrinkles observed in the lamina (seen in Figure 4) were not statistically different for control and progerin-expressing endothelial cells (Figure 6A). As an additional control, we also overexpressed wild-type lamin A in cells to ensure that the results were from progerin expression and not from either increased lamin A or from viral treatment. Levels of exogenous lamin A, measured from confocal immunocytochemistry, were 204 +/- 43% higher compared to wildtype cells. Endothelial cells grown on wider, 40 µm stripes without progerin did not show any wrinkles whereas progerin expressing cells had wrinkles statistically similar to cells without progerin grown on 20 µm stripes (Figure 7A).

In cells on 20 µm stripes, we also considered the orientation of the wrinkles. Our data indicates that the most deformations in control nuclear lamina structures lie in the direction of the primary orientation of the cells with more than half at 0-20° (Figure 6B). This is in agreement with the organized actin cytoskeleton visible along the length of the stripes visible in the overlays (Figure 4). Conversely, progerin expressing cells displayed angles ranging from 40-90° for many of these folds. For progerin expressing cells on 40 µm stripes, there is an increased
number of wrinkle formations in the range of 80-90° which is nearly normal to the applied force from the actin cytoskeleton (Figure 7B).

To compare control versus progerin expressing cells, we considered cells on 20 µm stripes and quantified the wrinkles in the nuclei. In cells confined on the stripes, we depolymerized actin using latrunculin A, fixed cells at increasing time, and imaged the nuclei lamina in control and HA-progerin expressing cells. The actin depolymerized within a minute as expected but the wrinkles in nuclei took some time to be removed, likely based on the stiff mechanics of the nucleus. We plotted the length of wrinkles versus time after actin depolymerization treatment to determine if there was a difference in the loss of wrinkles. From the plot (Figure 6C), the wrinkle loss from both cases can be modelled as an exponential decay. After 1 hour of latrunculin A treatment, there is nuclear rupture in a small number of nuclei of nuclei (7% of control nuclei, 0% of lamin A expressing nuclei) as visualized by DNA present outside the nucleus. Interestingly, more of the progerin expressing nuclei (90%) rupture. Fits of exponential decay of control, exogenous-lamin A and HA-progerin are shown in Figure 6D. Progerin expressing cells show a slower loss of wrinkles on a timescale of 111 min versus statistically similar scales of 45 min and 55 min for control and exogenous-lamin A, respectively. Thus, despite the fact that wrinkles are maintained longer, progerin expressing cells appear to be more susceptible to rupture under these conditions.

Nuclei in cells from patients with HGPS can exhibit protrusion of the nucleus towards the cytoplasm\textsuperscript{23} as well as many other gross nuclear morphological changes\textsuperscript{12,26}. There are many structural changes associated with HGPS including reduced lamin B1 levels\textsuperscript{31}, loss of heterochromatin\textsuperscript{24}, changes in chromatin-lamin binding\textsuperscript{32}, altered lamin-nuclear envelope association\textsuperscript{33}, altered nuclear pore complex\textsuperscript{34} and changes in how the nucleus binds to the
cytoskeleton\textsuperscript{30}. Here, we have tried to examine lamina-specific defects through cellular manipulation of cells exogenously expressing progerin. Of note by our group and others is that the exogenous expression of progerin, by plasmid such as DsRed-progerin or virus such as HA-progerin, is not the same as HGPS. Defects that result from exogenous expression appear to be more severe from the higher expression levels. However, the physical models appear consistent since similar force-induced wrinkling behaviour is observed in nuclei from patients with HGPS\textsuperscript{12}.

One particularly important implication for the progerin-expressing nucleus would be structural integration of the cytoskeleton with the nucleoskeleton called the LINC (linker of nucleus to cytoskeleton) complex. The LINC complex is important in balancing forces throughout the cell and transmitting forces across the cell (Figure 8C)\textsuperscript{35,36}. Severing the LINC complex prevents forces from being transmitted to the inside of the nucleus\textsuperscript{17} and forces from being transmitted from one side of the cell to the other\textsuperscript{4}. We suggest that improper distribution of forces across the nuclear lamina from the non-isotropic distribution of lamins associated with progerin expression could modify propagation of force throughout the cell (Figure 8D). This may be in-part why the cracks form away from the direction of the actin filaments in progerin expressing cells (Figure 4, 6). Thus, in HGPS premature aging, and in aspects of normal cellular aging\textsuperscript{27}, accumulated nuclear lamina defects may prevent proper force transmission through cells.
2.4. Conclusions

Our findings reveal that the abnormal nuclear morphology observed in HGPS and progerin expression is a consequence of both structure and mechanics. Excessive accumulation of progerin at the nuclear lamina causes wrinkles and invaginations observed in numerous cellular conditions. We suggest that these altered shapes are a result of microaggregates rather than just a uniform stiffening of the lamina.
Chapter 3: Nuclear Rupture caused by External Forces (Shear & Cyclic Stretch) on Progeria expressing Cells

3.1. Introduction

Cells in the body are exposed to various types of forces that test their mechanical stability and endurance. Laminopathies where lamin proteins have mutated such as in Hutchinson–Gilford progeria syndrome (HGPS) cause mis-regulation of forces in the nucleus which can lead to catastrophic failure of the nuclear envelope (NE). Nuclear rupture occurs naturally during interphase and in cancer migration studies has shown to repair itself\(^{37}\); though in high stress environments the nucleus can rupture and cause cell death. Laminopathies make the nucleus more prone to ruptures\(^{21}\). Recent work has been done to understand causes of nuclear rupture. Recent experiments where 3T3 fibroblasts were loaded with a lamin A/C knockdown have shown a rupture rate near 50% compared to 0% in wild type\(^{38}\). Similarly, cells taken directly from progeria patients have shown 13.6% rupture rate\(^{38}\). There is obviously a relation between altered lamin and nuclear rupture.

In this study we engage cells through external forces (Shear Stress and Cyclic Stretch) by simulating conditions in the body to look at their effect on the nucleus of progeria expressing cells. Shear stress is put on vascular endothelial cells which line the inner walls of arteries to simulate blood flow. Cyclic stretch is put on smooth muscle cells, the layer below endothelial cells to simulate their contraction and dilation. Looking at the nuclei at various time points of being engaged we see that there is an increase in nuclear ruptures and cell death that occurs the
longer cells exposed to the external forces. Since this was observed in both cell types it further proves that progeria compromises that structural integrity of the nucleus.

### 3.2. Methods

**Cell Culture and Transfection**

For HUVEC studies, Primary HUVEC (Lonza, passages 3-6) were grown in EGM-2 medium (Lonza). To express progerin in HUVEC an adenovirus was developed to express HA-tagged progerin (HA-progerin was a gift of Bryce Paschal; adenovirus was prepared by Vector Biolabs, Malvern PA). The lowest level of adenovirus that infected nearly 100% of cells was used. To overexpress wild-type lamin A in HUVEC, lamin A adenovirus (based on RefSeq BC014507) was purchased from Vector Biolabs and used at an identical titer level as progerin. Cells were seeded onto glass for shear experiments or PDMS plates (FlexCell) for stretch experiments.

**Cell under Shear Force**

Shear stress experiments used a parallel plate flow chamber. Cells were seeded on slides coated with 10µg/mL bovine fibronectin. Cells were exposed to approximately 15dynes/cm² or 1.5 Pa laminar shear force in complete media for varying times of 0hr (Static), 2hr, 6hr, or 24hr.

**Cell under Cyclic Force**

Cyclic stretch was applied to a monolayer of cells by seeding cells onto a UniFlex culture 6-well plate with a PDMS bottom coated with 10ug/ml bovine fibronectin. A Flexcell FX-5000
tension system was used to regulate the pneumatic system and apply 5% strain to the cell monolayer.

**Cell Fixation and Labelling**

Cells were fixed using 4% formaldehyde in phosphate buffered saline (PBS) and permeabilized using 0.2% Triton X-100 in PBS. For fluorescence microscopy experiments, cells were stained with 0.1 µg/mL Hoechst 33342 (ThermoFisher) for DNA staining. HUVEC were stained with anti-lamin A antibody (cat # sc-7292, Santa Cruz) for control cells or anti-HA antibody (cat # 901501, Biolegend) for progerin-expressing cells with an Alexa Fluor 488 fluorescent secondary (cat # A-21202, Thermo Fisher). HUVEC were also stained with rhodamine phalloidin (cat # PHDR1, Cytoskeleton).

**Imaging and Analysis**

HUVEC cells were imaged using a Zeiss 710 LSM confocal at 20x and 1NA. Images were processed using ImageJ. A median filter was placed on the original DAPI channel image, then a threshold was taken to separate the background from the cells, the image was then converted to binary, the watershed tool was used to separate any overlapping cells, and then an ellipse fit was used to fit the nuclei. Image processing steps shown in Figure 9. We were able to calculate the number of ellipses’ in a total image, the area, perimeter, and angle. The number of rupture events that occurred were counted manually using the nuclear GFP and the Lamin A/C stained channel.
3.3. Results and Discussion

Endothelial cells line the inner walls of blood vessels and are constantly exposed to shear forces from blood flow; to simulate this environment human vascular endothelial cells were exposed to 15 dynes/cm² or 1.5 Pa of force via media flow. We looked at three groups: Control, Progeria, and Overexpressed Lamin A at 0hr (Static no shear), 2hr, 6hr, and 24hr time points. The control group represents wild type cells that have not been altered. Progeria cells have a modified lamin with an attached farnesyl group to simulate cells from a progeria patient. Overexpressed lamin allows us to compare an abundance of lamin in the nucleus against the other groups and see how that affects the results obtained. Figures 10&11 represents the number of cells and percent of ruptures for endothelial cells in each group at each time point. The control and overexpressed lamin groups showed a fairly consistent number of cells from 0hr to 2hr; from 0hr to 24hr to there was a 7.73% difference for control and a 22.46% difference for overexpressed lamin cells. This may be due to prolonged exposure to shear forces where cells may have died; the higher difference in overexpressed lamin may be due to an abundance of the structural protein lamin where the cell is unable to regulate forces properly causing cell death. Progerin expressing cells showed a high percent difference from the same time points at 64.09% which was significantly higher than any other group. This is most likely due to progeria cells being sheared off the surface because they were unable to regulate force properly and nuclear rupture occurred and caused the cells to die. This is supported by the number of nuclear ruptures observed. We calculated percent ruptures based on the number of nuclei counted versus the number of ruptures observed. Both the control and overexpressed lamin showed little to no rupturing occurring within at any time point, any ruptures that did occur in the groups were
considered insignificant when compared to the progeria group. The progeria groups showed high percentage rupture at all time points including static, this shows that even when growing in a monolayer where no outside forces are present the nuclei are delicate enough to fracture and leak. There was no difference between the percent of ruptures from 0hr to 2hr showing a percent difference of only .30%, 0hr to 6hr showed a percent difference of 56.41% meaning a huge increase in the percentage of ruptures, and 0hr to 24hr showed a 50.45% difference. We believe the reduction from 6hr to 24hr is due to the number of cells as there is significant difference between the two time points; the cells that had ruptures had sheared off, leaving a majority of live resilient cells at 24hr, this would explain the reduction in number of cells and the reduction in percent of ruptures. Examples of stained images from which the values were obtained are shown in Figures 12, 13 and 14; each represents a different cell group.

After looking at the endothelial cell layer we decided to look at a layer below at smooth muscle cells. Smooth muscle cells contract and dilate blood vessels; to simulate this environment smooth muscle cells were placed on an elastic dish where they were elongated and contracted at 5% strain at 1Hz. This was again done with the same groups as the previous experiment: Control, Progeria, Overexpressed Lamin A at time intervals: 2hr, 24hr and 24hr Static (no strain applied). Figures 15&16 represents the number of cells and percent of ruptures for smooth muscle cells in each group at each time point. We showed similar results from this experiment as the shear experiment in that there was increase in nuclear rupture the longer the cells were exposed to outside force. Both the 2hr and static progeria group showed similar amounts of percentage rupture where 24hr showed the most. There was a percent difference of 91.96% between static and 24hr; this can be seen in Figure 15. The number of cells remains roughly the same for all groups except for the 24hr progeria group though it has a high amount of error. The number of
cells may have remained the same, smooth muscle cells tend to be larger and have more focal adhesions that bind to the surface. Also, the shear system setup allows for cells to be taken away from the plate they are adhering to, the strain system can cause detachment but the cell would still be in the area so it is possible that it can reattach. Examples of stained images from which the values were obtained are shown in Figures 17, 18 and 19; each represents a different cell group.

Quantitatively the endothelial cell ruptures are less catastrophic compared to the smooth muscle cell rupture. Endothelial cells rupture at certain points where the nucleoplasm leaks out. The rupture events for smooth muscle cells are extremely violent in that the nucleoplasm fills the entire cell giving an outline of the cell body. It is possible that the nucleus for smooth muscle cells have a rupture at a point but the repeated cyclic motion causes the rupture to increase in size which leads to releasing and spreading of the contents throughout the cell. This can be seen in Figure 20.

3.4. Conclusions

Our findings show that mechanical stimulation of progeria expressing cells cause nuclear rupture in multiple cell types. HGPS weakens the nucleus and can cause eventually cell death in occur with repeated stimulation. Endothelial nuclear rupture is different than smooth muscle cell rupture based on qualitative observation.
Chapter 4: Cell vs. Nuclear Elongation in live stretched Control and DN KASH cells

4.1. Introduction

Cells regularly take on force in the body and can be misshapen by it. One of the connections that is crucial for transferring force and allowing the cytoskeleton and the nucleus to connect is the linker of nucleoskeleton to cytoskeleton (LINC) complex. It is a combination of various proteins that anchor in the nuclear envelope and connect to the cytoskeleton.

Previous experiments where the LINC complex was decoupled and the cell was manipulated showed the nuclei moved less compared to when the LINC complex was intact\textsuperscript{39}. Another experiment similar to ours where cells were stretched on a membrane showed qualitative showing that the nuclei did not stretch as much at low strains compared to control cells\textsuperscript{39}. The differences between this study is that we are looking at single cells rather than a monolayer where extracellular forces can contribute to the deformation of the cell. By looking at individual cells the contribution of the LINC complex to the cells shape and deformation can be understood.

Here we stretched NIH 3T3 fibroblasts on micro-patterned rectangles (30x100µm) at regular intervals at high strains to understand the deformation of the nucleus compared to the cell. We compared control cells with cells where the LINC complex was no longer connected using DN KASH. The limited rectangular area allowed us induced constriction of the cell as it elongated.
4.2. Methods

Uniaxial Stretch Device Setup and Micropatterning

PDMS membrane (Class VI Silicone, Gloss, 12"x12", 0.005" thickness, 40 durometer) was cut 5cm x 40cm. The cut membrane was wrapped around the bottom of the tumblers and locked into place via zip-ties. When the knob is turned the gear system is engaged and causes the tumbler to turn which stretches the membrane. Once membrane is fairly taught it is sterilized with 70% ethanol. A PDMS chamber roughly 2cm x 2cm x 1cm with a 1.5cm diameter bored hole is attached to the center of the membrane with a thin layer of Vaseline.

The center of the membrane, in the hole of the chamber was stamped with fibronectin rectangles 30x100µm. The stamps used to stamp fibronectin rectangles were made with polydimethylsiloxane (PDMS). Stamps were coated with fibronectin and were pressed onto the prepared membrane. Once stamped, the area was coated with 0.2% BSA-647 (Molecular Probes, Eugene, OR) to visualize the surrounding area of the rectangles. The stamped area was washed and treated with Pluronic F-127 to limit cell adhesion to only the fibronectin rectangles.

Cell Culture and Transfection

NIH 3T3 mouse fibroblast cells were grown in Dulbecco's Modified Eagle Medium (DMEM) with 10% fetal calf serum and 500µg/mL of Penicillin-Streptomycin antibiotics. Dominant negative KASH expressing NIH 3T3s were developed to use for the DN KASH group. Cells were maintained in an atmospherically controlled incubator at 37º C and 5% CO₂ atmosphere. Media were changed regularly. The cells were seeded directly onto the center of the chamber and allowed to attach overnight. The cell body was labeled with CellTracker Green.
CMFDA (Invitrogen) which was diluted to a final working concentration of 20µM in DMSO then media, the solution was placed on the cells for 15 minutes at 37º. The nucleus was labeled with Hoescht which at a final working concentration of 1.62µM in media and was placed on cell for 5 minutes. The growth media was changed to live cell imaging media with 10% fetal bovine serum prior to imaging.

**Stretching and Imaging**

The uniaxial live cell stretcher device is placed directly into the stage of the 710 Zeiss Confocal Microscope. The knobs (Figure 21) on the stretcher can be turned to stretch the membrane and elongation of a cell can be visualized in real time. The cells were stretched for a brief strain, a z-stack in all channels was taken, this process was repeated at regular intervals till desired strain was reached.

**Analysis**

ImageJ FIJI was used for image analysis. A z-stack was converted to a z-projection image where all the slices were summed, this produced an image with maximum boundaries. The X and Y dimensions of each channel were calculated along the direction of elongation. Figure 22 gives an example of how the dimensions for the nuclei were measured. This produced the length and width parameter of the nucleus and cell body. From this information the strain was calculated. The reported values were recorded and plotted in MatLab.
4.3. Results and Discussion

We wanted to understand the LINC complex’s contribution and connection to the deformation between the cell membrane and the nucleus under stretch. To do this we stretched fibroblasts to very high strains and measured its effect on the cell. Figures 23 & 24 show the cells and nuclei of both control and DN KASH cells at 0 and 238623 Pa stress. The deformation of all the cell and nucleus can be seen here.

Figure 25 & 26 represent the fit graphs’ strain on the cell when the LINC complex is present or detached. The cell membrane X axis slope is .016 for control and .016 for DN KASH. The nucleus X axis slope is .011 for control and .008 for DN KASH. The cell membrane Y axis slope is -.005 for control and -.004 for DN KASH. The nucleus Y axis slope is -.004 for control and -.004 for DN KASH. All the slopes are similar except for the X slope of the nucleus where control cells are higher meaning that they are elongating more per increment of stretch. This is supported by the fact that the control cell nuclei are connected to the cell membrane via the LINC complex, as the cell elongates so does the nucleus due to this connection. Since the DN KASH nuclei are not connected their shape change is mediated by other methods. We hypothesize that the DN KASH nucleus’ elongation is mediated by the cell membrane flattening when stretching. It is also possible that at high strains the control and DN KASH cells behave the same as the LINC complex can break at higher strains leaving both groups with a disconnected LINC complex.

The Y axis slope of the cell and nuclei for both groups have the same slope and are very close to each other. When the nucleus is stretched in the X axis the Y axis comes inward showing that Y axis of the nucleus is controlled by the cell membrane.
4.4. Conclusions

The LINC complex plays a role in the elongation of the nucleus when the cell is stretched. It connects to the cell membrane and the nucleus and strains the nucleus more when stress is applied. The Y axis slope of the nucleus is led by the cell membrane.
Chapter 5: Conclusion & Future Directions

5.1. Conclusions

Mechanical forces are common in the body. Cells that make up the blood vessels regularly experience shear and stretching forces. These forces the pass through cells to internal structures such as the nucleus. This study tries to better understand the structural properties of the nucleus by altering specific proteins that either contribute to nuclear integrity or its connection to the rest of the cell through loading them in different ways. Previous studies looking at laminopathies where Lamin A is mutated have shown that the nucleus behaves differently compared to when normal lamin is present. By looking at Progeria where Lamin A is mutated the importance and properties of the protein can be better understood.

High force on the nucleus has shown to cause folds in the nucleus, even when the constricting protein actin is removed the progerin expressing nuclei remain deformed to an extent. The relaxation of the nucleus is slower as well where the folds don’t completely disappear, showing that these folds may be permanent. The nuclei of progeria cells has been said to be stiffer, this data supports that claim. The structural mechanics of mutated lamin causes the nuclei to have different properties. The change in stiffness here also affects the elastic nature of the nuclei. The areas where folds occur may be due to a buildup of progerin lamin; some areas have a higher buildup of progerin than others, it is not uniformly distributed. The relaxation of the nucleus causes high amount of rupture to occur in progeria cells, this could be due to the quick changes in forces causing a catastrophic event to occur where the nucleus bursts open. This is supported by the tensegrity model of the cell.
To look further into nuclear rupture cells were placed under shear and stretch forces to simulate bodily conditions. High amounts of rupture was observed in the progeria group. It was also shown that prolonged exposure causes more rupture to occur; the 24hr time point for shear showed a low cell count suggesting that when the nucleus ruptured the cells died and came off the surface. Normal amounts of force on these cells cased nuclear rupture, again showing that progeria nuclei are fragile.

By stretching the cell and looking at the deformation in the cell membrane and nuclear membrane the link between the two can be understood. The rate of deformation for the X axis of the cell and nucleus are different which could mean the cytoskeleton that connects the two could have elastic properties. The Y axis of the cell and nucleus are about the same which shows that the deformation of the nucleus in the Y axis is driven by the cell; this is further supported by imaging where at higher strains the Y axis of the nucleus and cell are touching. When comparing control to DN KASH the only difference was in the X axis of the nucleus. The LINC complex does not contribute to the deformation of the cell, the outside forces pulling on the cell only contribute to that. The nuclei when connected and disconnected have different properties, when disconnected the rate of stretch is slower.
5.2. Future Directions

In the future we plan to take the knowledge gained by the studies done and build on them to better understand the structural properties of the nucleus in various states. It is possible to test nuclear rupture in progeria cells while the nucleus and cytoskeleton are disconnected. This can be done through DN KASH. We would be able to see whether the intermediate proteins that transfer force from the outside of the cell to the nucleus causes less rupture. The results would be measured through the counting of surviving vs ruptured cells, similar to the experiments done previously.

Drugs that have previously been used to aid in progeria patients can be tested on a cellular level to see if they are effective in eliminating the cellular defects such as the fold in lines. This can also be used in the shear and stretch systems by looking at the amount of rupture. The data gained from this experiment will determine how effectively a specific drug is to treating the root cause of progeria.

It would be interesting to look at the individual contributions of structural proteins rather than to whole cytoskeleton to nuclear deformation under stretch. By disrupting actin, microtubules, and intermediate filaments and stretching the contribution of each set of proteins can be determined. From this data in conjunction with known knowledge of the size and shape of these proteins a model can be built to replicate the structural properties of the cell.
Bibliography


Figure 1: A depiction of the Linker of Nucleoskeleton and Cytoskeleton complex. It shows the various SUN and KASH domain proteins that are between the inner and the outer nuclear membrane. It also shows how the nuclear lamina and the cytoskeleton are connected.

Figure 2: A and B represent progeria nuclei, C and D represent control or normal nuclei. Panel A shows that the defect in the LMNA gene that prevents a farnesyl group to detach from prelamin. This produces a blebbled nuclear lamina which leads to an oddly shaped nucleus as shown in a live cell in panel B. Panel C shows how a normal lamina without the extra farnesyl group aligns to the nucleus. This produces a normal nuclear lamin which leads to a circular nucleus as shown in a live cell in panel D.

progeria family circle: cause. Available at:

Figure 3: Panel A shows the different stages of nuclear rupture and how it occurs. Where there is an initial force which causes a membrane bleb, a chromatin herniation, and eventually leads to a nuclear rupture. Panel B shows previously tested causes for nuclear rupture where a cell sitting on a flat, rigid substrate with an intact actin network and LINC complex causes nuclear rupture but with a disturbed actin or LINC complex does not. This same experiment where the cell is confined shows that whether the actin network and LINC complex are disrupted or not causes rupture.

**Figure 4:** Confocal fluorescence microscopy sections for cells patterned on lines. Fixed HUEVC were stained for Lamin (control) or HA (Progeria), and all cells were also stained for actin (phalloidin) and DNA (Hoechst 33342). A) Lamin A/C (control) stained with a lamin A/C antibody. B) Control cell stained for actin to check the orientation of folds against the filament structures. C) Lamin A control cells with Hoescht staining for DNA. D) Merge of the lamin and actin channels shows nuclear alignment with the stripes and lamin folds coincident with the actin filaments. E) Progerin cells stained with anti-HA to label HA-progerin express more wrinkles. F) Progerin cells stained for actin to show the orientation of folds against the filament structures. G) Progerin cells with Hoescht staining for DNA. H) Merge of the lamin and actin shows lamin folds distinct from actin filaments. For both conditions the z-resolution for the lamin channel (488nm) was chosen at 3.5 µm, actin channel (561 nm) 1.9 µm and DNA channel (405 nm) 1.3 µm.
Figure 5: Methodology of measuring the angle and length of cracks. A) We measure the length of the crack and the angle of the crack with respect to the stripe. B) Comparison of crack length versus crack angle shows no particular correlation.
Figure 6: Formation of wrinkles for cells under one-dimensional confinement. A) Length of deformations or wrinkles for control, exogenous lamin A or HA-progerin expressing endothelial cells cultured on 20 µm diameter stripes. B) On 20 µm width stripes, wrinkles in control cells and exogenous lamin A expressing cells (+ lamin A) primarily align with the stripe axis whereas HA-progerin-expressing cells do not show preferred orientation. C) On 20 µm stripes, treatment with latrunculin A and fixation at different time points shows an exponential decay. D) Fits of exponential decay shows the differential decay constants for control and exogenous lamin A versus HA-progerin cells. Fits same for 4 points as 2 points. 30-50 cells per condition considered. * indicates statistically different $p < 0.001$; ** indicates $0.001 < p < 0.05$; C and D no * indicates statistically different with $p > 0.05$ +LA is not included in panel C as initial experiments did not involve the group; it was added later on and included in panel D.
Figure 7: Wrinkle comparison on 40 µm stripes versus 20 µm stripes. A) Length of deformations or wrinkles for control or HA-progerin expressing endothelial cells on 20 µm or 40 µm diameter stripes. On 40 µm stripes, control cells show no wrinkles whereas cells expressing progerin do. B) For progerin-expressing cells, orientation preference of the wrinkles is further lost as the stripe diameter widens. 30-50 cells per condition considered.
Figure 8: Model of nuclear lamina under force. A) The nuclear lamina for control cells experiences a thinning of membrane and dilation of lamin A network. B) The nuclear lamina for progerin expressing cells experience high stress and buckle at the aggregates irrespective of force application. Cracks then emanate from the aggregate space. C) In control cells cytoskeletal forces are balanced through the nuclear lamina and are propagated from one side of the nucleus to the other. D) With defects and cracks in progerin-expressing cells, forces may be unbalanced and dissipated.
Figure 9: Represents the step by step analysis process done in FIJI ImageJ. The original DAPI channel is taken, a median filter is applied so the threshold cannot distinguish individual parts of the nucleus, a threshold of the image is taken to separate the nucleus from the background, the threshold is made into a binary image, the binary image has watershed applied which separates two nuclei which may have on top of each other, and then the features in the image are fit to an ellipse from which various data was obtained. The process was automated through a macro function in ImageJ which runs the steps. We were able to obtain number of cell count by running images through this process.
**Figure 10:** The number of cells for the groups Control, Progerin, and Overexpressed Lamin A for time points 0Hr, 2Hr, 6Hr, and 24Hr. There is a drastic decrease in the progerin group from 0 and 2 hr to 6 and 24 hr.
**Figure 11:** The percentage of ruptures given by comparing the number of ruptures and the number of cells. The progerin group shows the most percentage ruptures of any time point.
Figure 12: Stained endothelial cells from the control group at 0, 2, 6, and 24Hr time points. Row A represents nuclear GFP, B represents DAPI (nucleus), C represents Actin, and D represents Lamin A/C.
Figure 13: Stained endothelial cells from the overexpressed Lamin A group at 0, 2, 6, and 24Hr time points. Row A represents, nuclear GFP, B represents DAPI (nucleus), C represents Actin, and D represents Lamin A/C.
Figure 14: Stained endothelial cells from the Progerin group at 0, 2, 6, and 24Hr time points.
Row A represents, nuclear GFP, B represents DAPI (nucleus), C represents Actin, and D represents Lamin A/C.
**Figure 15:** The number of cells for the groups Control, Progerin, and Overexpressed Lamin A for time points 2Hr, 24Hr, and 24Hr Static. The number of cells tend to stay roughly the same for all groups except progerin 24 Hr static though it shows a high error.
Figure 16: The percentage of ruptures given by comparing the number of ruptures and the number of cells. The progerin group shows the most percentage ruptures of any time point.
Figure 17: Stained smooth muscle cells from the control group at 2, 24, and 24Hr static time points. Row A represents nuclear GFP, B represents DAPI (nucleus), C represents Actin, and D represents Lamin A/C.
Figure 18: Stained smooth muscle cells from the overexpressed lamin A group at 2, 24, and 24Hr static time points. Row A represents nuclear GFP, B represents DAPI (nucleus), C represents Actin, and D represents Lamin A/C.
Figure 19: Stained smooth muscle cells from the progerin group at 2, 24, and 24Hr static time points. Row A represents, nuclear GFP, B represents DAPI (nucleus), C represents Actin, and D represents Lamin A/C.
Figure 20: Zoomed images of progerin group lamina A/C stained channels. A represents endothelial cells, B represents smooth muscle cells.
**Figure 21:** 3-D rendering of the stretch device used to strain the cells. Fig. A represents a fully set up labeled device before being placed into the microscope. Fig. B is an exploded view of the main components.
Figure 22: A representation of the nuclei and how X and Y axis values were measured.
Control:

**Figure 23**: 3 Control cells before and after elongation. Column A is the light channel, B is DAPI (Nucleus), C is Cell Body, and D is Membrane.
DN KASH:

0 Stress

238623 Pa Stress

**Figure 24:** DN KASH cells before and after elongation. Column A is the light channel, B is DAPI (Nucleus), C is Cell Body, and D is Membrane
**Figure 25:** Plot of the fit lines of strain for the X and Y axis of the cell and nucleus for Control Cells
Figure 26: Plot of the fit lines of strain for the X and Y axis of the cell and nucleus for DN KASH Cells