Mesenchymal Stem Cell Response to Static Stretch on Electrospun Nanofibrous Scaffold

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***Abstract*— Mesenchymal stem cells (MSCs) are multipotent stem cells that have been considered for an increasing list of therapeutic practices, not simply because of their inherent ability to differentiate into connective tissues including bone, fat and cartilage, but furthermore due to their trophic and anti-inflammatory effects which contribute to healing and tissue regeneration. MSCs are often affected by the growth factors they encounter as well as the physical cues from their cellular microenvironment. These microenvironmental cues are important for tissue engineering, where stem cells must be able to differentiate down specific lineages and organize into tissue-like structures. One way of investigating the effects of MSCs' microenvironment is through seeding of polymer nanofibrous scaffolds. These scaffolds can be generated with a highly aligned nanofibrous structure that mimics the native microenvironment of tendons, ligaments, and fibrocartilages. Further, these scaffolds can be engineered to have a crimped or wavy structure, which is a property of native tissue microarchitecture that is known to be important for the bulk tissue mechanical response and is likely a regulator of cellular mechanotransduction. In this study, we examine the effects of static stretch on MSCs using aligned or crimped electrospun polymer scaffolds. We also examine the role inhibitors or activators of cellular contractility play in regulating nuclear deformation on MSC seeded scaffolds. Here, we report that the Poly-L-Lactide (PLLA) crimped scaffold is capable of reducing nuclear deformation under static stretching. We also report that increasing contractility, with Lysophosphatidic acid (LPA), or inhibiting the contractility and the actin cytoskeleton, with Y27632 or Cytochalasin D (CytoD), further prevents nuclear deformation. Due to the low yield of PLLA scaffold, dynamic loading of MSCs has not yet been explored. Future studies must be conducted in order to evaluate MSC response at higher static strain as well as MSC response to dynamic loading on PLLA scaffolds..**

***Index Terms*— Mesenchymal stem cells, Scaffold, PCL, PLLA, Tissue Engineering**

I. INTRODUCTION

Cells have the innate ability to sense and respond to mechanical forces in ways that can be both beneficial and harmful to tissue function as well as its structure [1]. Many factors play into how cells will respond to these forces, including the type of and magnitude of the particular

mechanical cues and mechanotransduction mechanisms that occur naturally [2]. Mechanical forces play an important role in the maintenance and degradation of orthopedic tissues, especially where changes with development and degeneration can result in a different cellular interpretation of mechanical forces. These mechanisms are vital in various native tissues that are prone to degeneration, often requiring surgical interference.

One orthopedic tissue prone to damage and degeneration is ligament. There are four major ligaments in the knee. The ligaments in the knee connect the femur (thighbone) to the tibia (shin bone), and include the following: Anterior cruciate ligament (ACL). The ligament located in the center of the knee, controls rotation and forward movement of the tibia (shin bone). Posterior cruciate ligament (PCL). The ligament located in the center of the knee controls backward movement of the tibia (shin bone). Medial collateral ligament (MCL) gives stability to the inner knee. Lateral collateral ligament (LCL). The ligament that gives stability to the outer knee [3].These ligaments play a significant role in musculoskeletal biomechanics. They represent an important area of orthopedic treatment for which many challenges for repair remain [4]. Ligaments can adapt to changes in the physical microenvironment due to injuries, diseases, or exercise [4]. Many of the challenges we face have to do with restoring the normal mechanical function of these tissues. Natural healing typically leads to the formation of scar-like tissue retaining inferior mechanical properties. Many ligament injuries are repaired through surgery, however as with any surgical procedure, complications can occur. Some possible complications may include, but are not limited to bleeding, infection, and blood clots in the legs or lungs. Some individuals experience pain, limited range of motion in the knee joint, and occasional swelling in the knee after surgical ligament repair. Other attempts at ligament repair have been made to restore these mechanical functions including using tissue engineered constructs for tissue replacement. However, progress has been slowed due to the complexities of tissue structure and their mechanical properties[5]. This study aims to further understand the way cells respond to mechanotransduction using electrospun nanofibrous scaffolds. Moreover, this study aims to study cell mechanotransduction and the importance of contractility in this process.

II. BACKGROUND

2.1 Mesenchymal Stem Cells:

Mesenchymal stem cells (MSCs) are adult stem cells

traditionally found in the bone marrow. Mesenchymal stem cells have long thin cell bodies with a large nucleus. As with other stem cell types, MSCs have a high capacity for self-renewal while maintaining multipotency. Thus, mesenchymal stem cells have enormous therapeutic potential for tissue repair. MSCs have been shown to be capable of differentiating into multiple cell types including adipocytes, chondrocytes, osteocytes, and cardiomyocytes. [6] MSCs can be expanded *in vitro* and, under appropriate conditions have remarkable immunoregulatory properties; because of this, their potential use as therapeutic agents *in vivo* is being extensively studied [7].

2.2 Electrospun Scaffolds:

Electrospun scaffolds provide a three-dimensional nanofibrous environment that allows for structural modulation of MSC differentiation while maintaining MSC's multipotent capacity. [8] These scaffolds can be generated using biocompatible polymers such as poly (epsilon-caprolactone)(PCL), with a highly aligned structure that directs cell alignment and induces an elongated cell morphology [8]. On these aligned scaffolds, directed matrix deposition occurs with extended time in culture and results in anisotropic mechanical properties in engineered constructs similar to those of native tendons, ligaments and fibrocartilages [8]. Altering the biocompatible polymers result in various scaffolds to better resemble their native characteristics. These scaffolds provide an ideal method to study differentiation of MSCs and the influence of cellular alterations under mechanotransduction.

2.3 Cytoskeleton:

The cytoskeleton is a series of intercellular proteins that provide a cell with shape, support, and movement. There are three main types of cytoskeletal polymer: actin filaments, microtubules and intermediate filaments. Together, these polymers control the shape and mechanics of eukaryotic cells. All three are organized into networks that resist deformation but can reorganize in response to externally applied forces, and they have important roles in arranging and maintaining the integrity of intracellular compartments [9]. The cytoskeleton is connected to the nuclear interior by LINC (linker of nucleoskeleton and cytoskeleton) complexes located in the nuclear envelope. The LINC-complex is a protein complex associated with both inner and outer membranes of the nucleus and actin microfilaments of the cytoskeleton. The LINC complex is involved in the anchoring of both nuclear membranes and nuclear lamina to the actin cytoskeleton via transmembrane proteins. These complexes consist of SUN proteins and Nesprins present in the inner and outer nuclear membrane respectively. Whereas SUN proteins can bind the nuclear lamina, members of the nesprin protein family connect the nucleus to different components of the cytoskeleton.[10] Nesprin-1 and -2 are located on the outer nuclear membrane bind to actin filaments, a protein essential for cellular contraction.

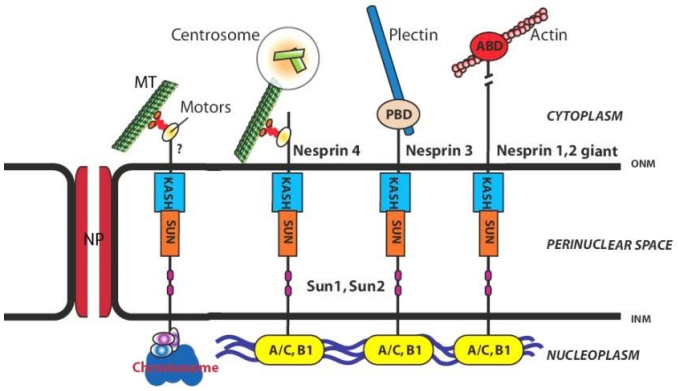

Figure 1-Illustration of the cytoskeleton and the LINC Complex.

2.4 Contractility:

Cellular contractility represents the fundamental ability of cells to shrink. The changes in a cells' ability to produce force during contraction result from incremental degrees of binding between myosin (thick) and actin (thin) filaments [11]. Cellular contractility is essential for many biological phenomena such as cytokinesis, movement, differentiation and substrate adherence [12]. These changes in cell shape are achieved by deviations of the cytoskeleton, most importantly the actin cytoskeleton via the actomyosin network. This network is regulated mainly through the activity of proteins from the Rho-GTPase family that regulate both actin nucleation and myosin activity through downstream effectors such as mDia and Rho kinase (ROCK) [12]. Manipulation of the actomyosin network allows for manipulation of contractility. This manipulation will allow for the examination of the relationship between inhibitors and contractility as well as the relationship between contractility and nuclear deformation.

2.5 Regulation of gene expression:

The function of a cell is regulated by gene expression and mechanical forces can lead to both activation and inhibition of gene expression. Gene activation enhances the interaction between RNA polymerase and a particular promoter, encouraging the expression of the gene. Activators do this by increasing the attraction of RNA polymerase for the promoter, through interactions with subunits of the RNA polymerase or indirectly by changing the structure of the DNA [13]. During gene inhibition repressors bind to the Operator, coding sequences on the DNA strand that are close to or overlapping the promoter region, impeding RNA polymerase's progress along the strand, thus impeding the expression of the gene [13]. Prior to activation or inhibition of genes mechanical signals can be relayed through the cell by phosphorylation of proteins. One such protein that is phosphorylated in response to loading is the MAP kinase ERK 1/2. These phosphorylation events lead to downstream activation or inhibition of gene expression.

2.6 Aggrecan

Aggrecan is a major proteoglycan found in the extracellular matrix (ECM) of cartilage. This molecule is important in the proper functioning of cartilage because it provides a hydrated gel structure that endows the cartilage with load-bearing properties[14]. Aggrecan, combined with collagen, create a stiff network within the ECM providing resistance to deformation.

2.7 Scleraxis

Scleraxis is a basic helix-loop-helix (bHLH) transcription factor and a highly specific marker for all the connective tissues that mediate attachment of muscle to bone. It is important for development and maintenance of tendon and its expression is known to be regulated by mechanical forces.

III. MATERIALS AND METHODS

3.1 Nanofibrous scaffold fabrication

3.1.1 Poly-L-Lactide

The preparation of Poly-L-Lactide (PLLA) nanofibers by electrospinning was performed under sterile conditions. A 14.3% w/v solution of PLLA was prepared in a 1:1 mixture of chloroform and N, N-. This solution was ejected via syringe pump at 2.5 mL/h through a spinneret charged to +16 kV. Fibers were collected for 3 hours on a grounded rotating mandrel with a surface velocity of 10 m/s (schematic shown in Figure 1). This resulted in a scaffold sheet of ~600 μm thickness from which rectangular samples (5 × 60 mm) were cut with respect to the prevailing fiber direction. Samples were stored in a desiccator until mechanical testing or cell seeding.

3.1.2. Dual: Poly-L-Lacitde / Poly(Ethylene-Oxide)

Dual electrospun scaffolds were also created by co-electrospinning PLLA and PEO at the same time and collecting both polymers on a single spinning mandrel. The PLLA solution used was the same as above and the PEO solution was 10% w/v PEO in 90% ethanol. Polymers were electrospun as above.

3.1.3 Poly(epsilon-caprolactone)

Aligned nanofibrous poly(ε-caprolactone) (PCL) scaffolds were fabricated by electrospinning as described previously [15]. Briefly, a 14.3% w/v solution of PCL (BrightChina, Hong Kong, China) was prepared in a 1:1 mixture of tetrahydrofuran and *N*,*N* dimethylformamide (Fisher Chemical, Fairlawn, NJ). This solution was ejected via a syringe pump at 2.5 mL/h through a spinneret charged to +13 kV. Fibers were collected for 10 h on a grounded rotating mandrel with a surface velocity of 10 m/s. This resulted in a scaffold sheet of ~600 μm thickness from which rectangular samples (5 × 65 mm) were cut. Samples were stored in a desiccator until mechanical testing or cell seeding.

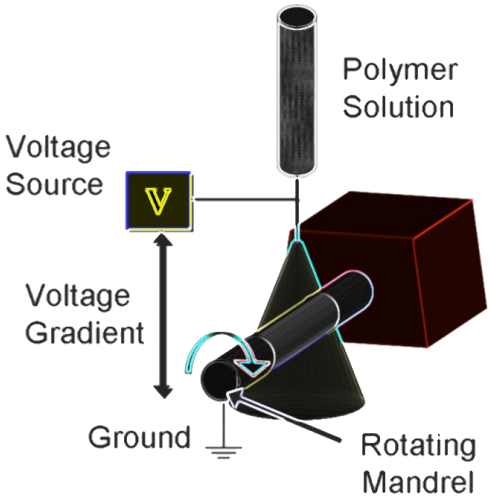

Figure 2- Schematic of electrospinning setup

3.2 Scaffold Treatment

PLLA scaffold mats were treated two different ways: 1) scaffold was positioned between two glass plates and heated to 65°C using a hot plate for 15 minutes or 2) no treatment. Dual scaffold mats were treated three different ways:1) scaffold was positioned between two glass plated and heated to 65°C using a hot plate for 15 minutes prior washing out PEO with water (HW). 2) PEO was washed off prior to being heated between two glass plates at 65°C using a hot plate for 15 minutes (WH). Or 3) PEO was washed off (W). Heating Dual scaffolds mats was found to increase crystallinity from 30% to 52%.

3.3 MSC Harvest

Mesenchymal stem cells were harvested from juvenile bovine tibias and femurs. Legs were soaked in soapy water until harvesting and then rinsed with water. Legs were then soaked in 70% ethanol (EtOH) for approximately 10 minutes. Excess tissue from the joint including muscles, tendons, and ligaments that connect the femur to the tibia was removed. The joint capsule was then opened and the menisci were dissected from the surface of the tibia. Each leg was then sprayed with 70% EtOH before harvest. Legs were cut using an electric handheld saw (Black and Decker RS500K, New Britain, CT) in separate patterns to isolate porous bloody trabecular bone. The exposed trabecular bone was then cut into cubes and placed into 50ml cylindrical tubes. 15ml of dissection medium(1g of heparin in 500mL of Basal Medium[100 mL FBS and 10mL of PSF in 1L of DMEM] at 2% PSF). Tubes were shaken and the fused liquid was poured into new 50 mL tubes. Another 15ml of dissection medium was added to each tube and centrifuged at 300 rcf for 5min. to pellet cells. The supernatant was aspirated and cells were suspended in 25mL of basal medium. Cells were incubated on treated 15 cm plates at 37°C. Media was changed every three days and MSCs were grown up to passage 2 before seeding.

3.4 Cell Seeding

Electrospun scaffolds were hydrated through decreasing increments of ethanol (100%, 70%, 50%, 30%, H_20). Scaffolds were then soaked overnight in fibronectin solution (20 ug/ml) . Upon aspirating the fibronectin, scaffolds were washed in Phosphate Buffer Saline (PBS) 2x before cell seeding. Plated MSCs were trypsinized and suspended in DMEM Basal medium. Cells were then placed into three 50ml conical tubes and spun down at 300rfc for 5 minutes. Media/Trypsin was aspirated and cells were combined into one tube via resuspending in Basal medium and pipette transfer. Cells were then counted using a hemocytometer and diluted to a concentration of 1,000,000 cells/ml. Each scaffold was seeded with approximately 100,000 MSCs and stored in an incubator at 37°C for an hour. Once removed from the incubator scaffolds were suspended in Chemically Defined Medium (CM-) overnight.

3.5 *Activators/inhibitors*

3.5.1 Y-27632:

Y-27632[(+)-(R)-trans-4-(1-aminoethyl)-N-(4-pyridyl) cyclohexanecarboxamide dihydrochloride] is widely used as a specific inhibitor of the Rho-associated coiled-coil forming protein serine/threonine kinase (ROCK) family of protein kinases [16]ROCK Inhibitor (Y-27632) is a selective, ATP-competitive inhibitor of Rho-associated protein kinase (ROCK) including p160Rock (Ki=140nM) and Rock-II. Also acts as a potent inhibitor of agonist-induced Ca2+ sensitization of myosin phosphorylation and smooth muscle contraction. [17]

3.5.2 Lysophosphatidic acid (LPA):

Lysophosphatidic acid (LPA) is an endogenous glycerophospholipid signaling molecule and ligand activator. Signaling associated with LPA has been reported to stimulate growth of fibroblasts, vascular smooth muscle cells, endothelial cells, and keratinocytes. LPA has been indicated as both a proliferative and anti-apoptotic factor, signaling for Pl3K-mediated regulation of cell activity. LPA has also been described to potentiate acetylchloine receptor currents by G-protein-mediated activation of Ca^{2+}-dependent/independent protein kinase C [18].

3.5.3 CytoD:

Cytochalasin D (CytoD), a widely used inhibitor of actin dynamics. It binds to barbed end of actin filaments, inhibiting the association and dissociation of subunits. It also causes the inhibition of actin polymerization [19].

3.5.4 ML7:

ML-7 has been shown to inhibit Ca2+ dependent and independent smooth muscle MYLK (myosin light chain kinases) via competitive inhibition of ATP and also inhibits PKC and PKA. This compound also inhibits cell transporters activated by shrinkage and affects the osmotic volume regulation of cells. Mechanistic studies suggest that this is done via stimulation of K-CL transport, a cell volume regulating agent. ML-7 has also been reported to affect the superoxide O2--producing system of human neutrophils in a myosin light chain kinase independent manner [20].

3.6 Knockdown with lentivirus

RNA interference of Nesprin 1 giant (directed at the N-terminal calponin homology domain) was performed using the BLOCK-iT Lentiviral Pol II miR RNAi Lentiviral expression system. MSCs were infected at an MOI of ~20 and cultured for 96 hours prior to seeding. Cells were seeded on PCL scaffolds (200k/side) for 2 days and then statically or dynamically stretched.

3.7 Western Blot

Following dynamic stretch, total protein was isolated in RIPA buffer and quantified using the Lowry assay. Samples were run on 4-15% gradient poly-acrylamide gels and protein was transferred to a nitrocellulose membrane. Membranes were blocked with 5% w/v BSA in PBS-tween (0.1% w/v) and western blots were performed for ERK 1/2 (cell signaling 1:1000) and phospho-ERK 1/2 (cell signaling 1:1000).

3.8 Mechanical testing

Unseeded scaffolds were loaded onto an Instron tensile machine (Model 5848; Instron, Norwood, Massachusetts) at a gauge length of 20 mm with a 10N load cell. Samples were stretched to 30% strain at a rate of .02 mm/second (.1%/s). Stress-Strain curves were created using Wavemaker (Instron) and analyzed in MATLAB (Fig.8, 9).

3.9 Actin/Dapi Staining

Seeded scaffold was washed in PBS and fixed overnight in 4% Paraformaldehyde (PFA) at 4°C. Scaffolds were then washed in PBS 3x before being incubated for 5min at 4°C in Permeabilizing solution (PS) [26ml PBS, 130µl Triton X-100, 2.8g Sucrose, 78ul of 200 mM $MgCl_2$). After aspiring the PS, scaffold was then washed in PBS 3x before adding Labeling Solution (LS) [26ml PBS, 260 mg BSA, 26ul ALF488-Phalloidin] and incubating for an hour at 37°C. LS was aspirated and scaffold was washed in PBS 3x then mounted on a slide using a DAPI mounting agent. Stained scaffold were imaged on a fluorescent microscope. Nuclei were imaged on the Dapi channel, Actin on the GFP channel.

3.10 Static Stretch

Seeded aligned Electrospun PCL scaffold were stretched using a custom tensile stretch machine (figure 3) under a fluorescent microscope (Invitrogen). Scaffolds were stretched in increasing increments (3%, 6%, 9%, 12%, 15% strain).

Pictures were captured at every strain point using a fluorescent microscope on the DAPI channel and analyzed (Fig. 4).

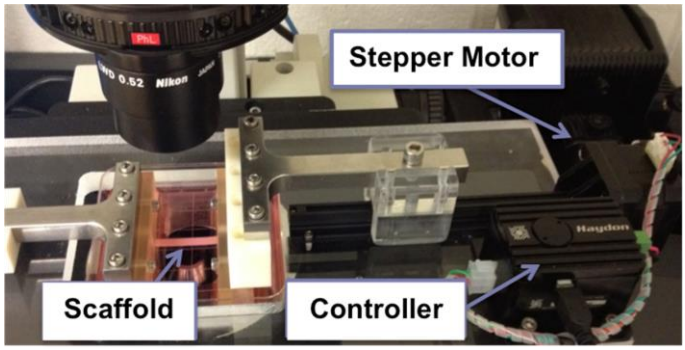

Figure 3- Image of micro-tensile device used for stretching MSC seeded, Hoechst stained scaffolds and quantifying nuclear deformation

3.11 Dynamic Stretch

After two days of preculture MSC seeded PCL scaffolds were loaded into a custom tensile bioreactor and stretched to 3% strain at 1Hz for 15min (protein analysis) or 6 hours (mRNA analysis).

3.12 Analysis

Experimental analysis was conducted using custom MATLAB (Mathworks) programs to determine modulus, strains, cross sectional Area, and nuclear deformation (Fig.4(A,B)), GraphPad Prism(GraphPad, San Diego, CA) for statistical analysis (Figs. 4-9) and Image J for photographic files.

IV. Experimental Results

4.1 Nuclear Deformation under Static Stretch

Images of PCL scaffold under static stretch were analyzed. Nuclear aspect ratios were calculated and normalized to unstrained control for nuclear deformation analysis. Results indicate that MSCs, when treated with CytoD, Y27, or LPA show reduced nuclear deformation when compared to a control; Whereas MSCs treated with ML7 show increased nuclear deformation when compared to a control (*Fig. 4*).

4.2 Nesprin 1 Giant Knockdown

Normalized nuclear aspect ratios taken from analyzed images of PCL scaffold under static stretch show that knocking down Nesprin 1 Giant (N1G) reduces nuclear deformation under applied strain(Fig.5A). Analysis of dynamic loading of PCL scaffold and Aggrecan expression shows that MiRneg virus (viral control) as well as the control (non viral treated control) significantly increases over time whereas knocking down Nesprin 1 Giant results in a much smaller increase. However, Scleraix expression increased significantly with N1G knockdown virus, miRneg virus as well as control.

4.3 Mechanical testing of PLLA scaffold[T1]

Results of mechanical testing of PLLA scaffold using an instron show that PLLA Normal contains the largest linear modulus(*Fig. 9B*) however, Dual Wash Heat contains the largest transition strain (*Fig. 9C*).

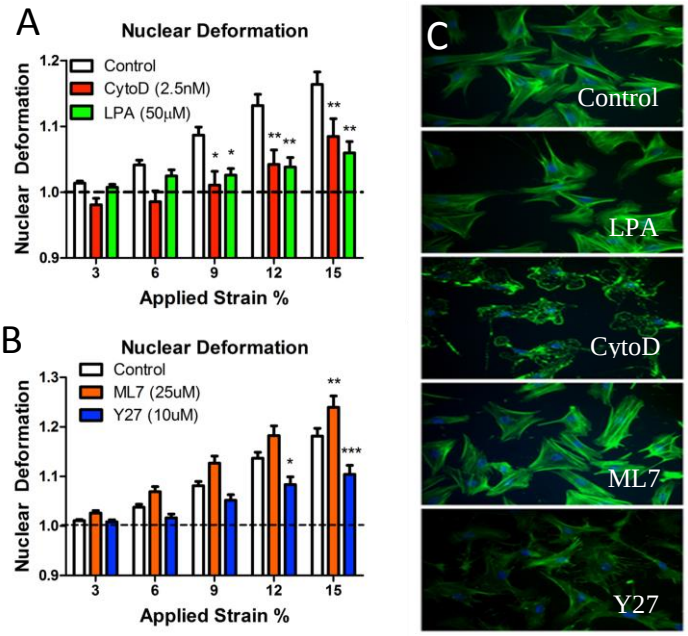

Figure 4- Quantification of nuclear deformation, defined as the ratio of the nuclear principal axis (long/short) normalized to the unstrained state. Cells were treated with the contractility activator Lysophosphatidic Acid (LPA) or the F-actin inhibitor Cytochalasin D (CytoD) (A). Additionally, cells were treated with contractility inhibitors ML7 and Y27632 (B). Other cells were seeded on glass slides, treated with phamacologic agents and stained with phalloidin (green) and Dapi (blue) to visualize F-Actin and nuclei, respectively(C). Error bars indicate mean +/- SEM (n=45-60 nuclei). *=p<0.05, **=p<0.01, ***=p<0.001, compared to control at the same strain, Two-way ANOVA with Bonferroni post hoc.

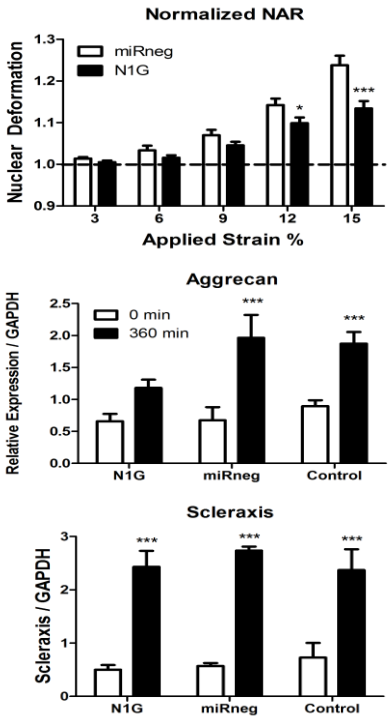

Figure 5- Quantification of nuclear deformation, defined as the ratio of the nuclear principal axis normalized to the unstrained state. Cells were treated with Nesprin 1 Giant miRNA lentivirus and miRneg virus (A). In addition, Aggrecan expression under dynamic loading with knockdown virus was quantified, defined by the relative expression divided by GAPDH (B). A similar comparison is quantified for Scleraxis (C). Error bars indicate mean +/- SEM (n=45-60 nuclei). *=p<0.05, **=p<0.01, ***=p<0.001, compared to control under the same conditions, Two-way ANOVA with Bonferroni post hoc.

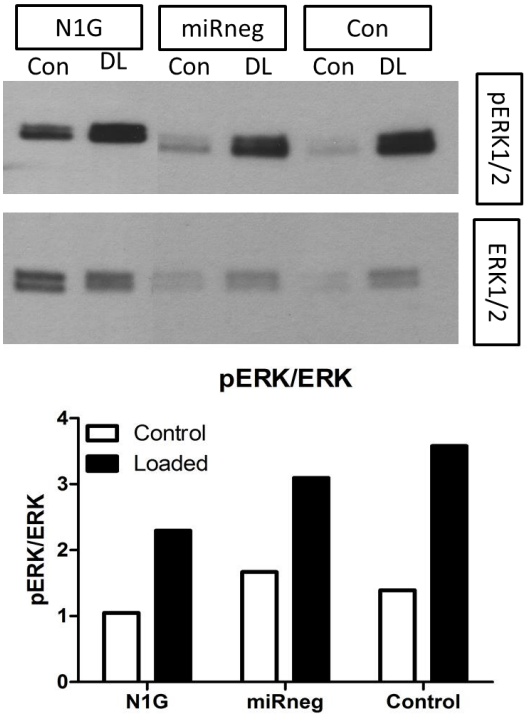

Figure 6- Results from western blot comparing ERK 1/2 to its phosphoralted state with or without loading (A) when Nesprin 1 Giant is knocked down (N1G) and a quantification of the blot (B).

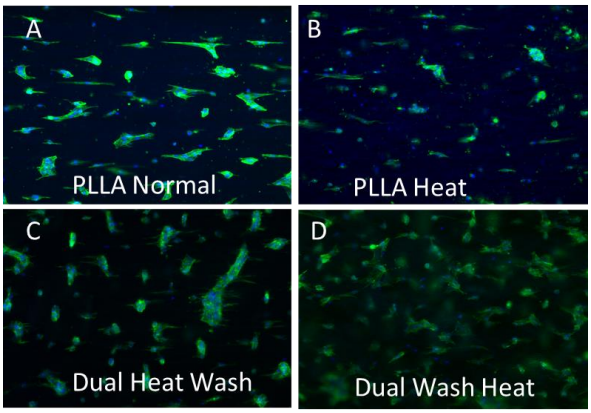

Figure 7-Seeded PLLA scaffolds images under the fluorescent microscope. Scaffolds were stained with phalloidin (green) and Dapi (blue) to visualize F-Actin and nuclei, respectively. A) PLLA Normal B) PLLA Heated C) Dual Heat Wash D) Dual Wash Heat

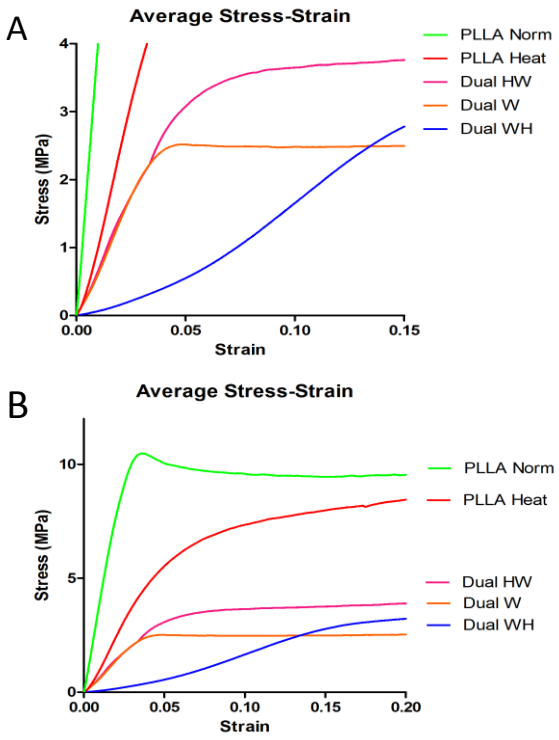

Figure 8- Stress strain curves of PLLA electrospun scaffolds with or without a PEO component (to increase porosity when washed (W) out and with or without a heating (H) cycle (to increase crimp by increasing crystallinity). Average stress-strain curves for n=3 samples/ group. (A) Strain up to 15% (B) Strain up to 20%

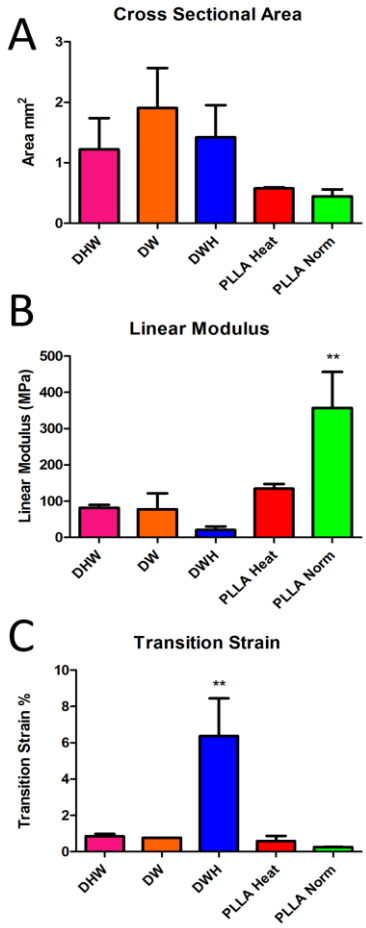

Figure 9-Quantified mechanical properties of PLLA scaffold with or without PEO component and with or without heating cycle. One-way ANOVA with Tukey's post hoc, n=3/group.(A) Average cross sectional areas measured using a custom laser device and analyzed in MATLAB.(B) Linear Modulus calculated as the best fit line to the stress-strain curve and (C) transition strain calculated using bilinear fits of the curves. ** = $p<0.05$ vs. all other groups (B)

Discussion and Conclusion

By seeding aligned Electrospun nanofibrous PCL scaffold and treating them with inhibitors or activators, we can conclude that increasing contractility with LPA prevents nuclear deformation. This may be because the tensed actin network is pre-stressed enough to shield the nucleus from strain transmission. We can also conclude that by inhibiting contractility and the actin cytoskeleton with Y27632 or CytoD we can further prevent nuclear deformation, indicating that the structure of actin is important for transfer of strain to the nucleus. Furthermore, decreasing contractility while maintaining the structure of actin with ML7 can result in an increase in nuclear deformation. This is likely because the base line stress acting on the nucleus is decreased in this case but the actin necessary to transmit strain is still present. We can also conclude that through static stretching of PCL scaffold nuclear deformation is reduced with knock down of

N1G, likely because these Nesprin connections to the nucleus are important for transmission of strain. Through dynamic loading of PCL scaffold we can conclude that knocking down N1G will reduce the induction of Aggrecan expression but not of Scleraxis. Additionally it seems that ERK 1/2 activation is relatively normal with knockdown of N1G. This suggest that N1G has very little effect on ERK 1/2. By mechanical testing PLLA scaffold, we can conclude that Dual Wash scaffold had the highest transition strain, which would imply that it contained a larger amount of fiber crimp. Also, we can conclude that increasing porosity through washing PEO reducing the linearity of stress strain curves as well as reduce the modulus of the scaffold. By stretching Electrospun nanofibrous scaffolds we can determine which treatments create stronger material for future in vivo implementation. We can also determine which cellular components are important for nuclear deformation under strains, and weather these components regulate how cells respond to dynamic mechanical forces. Future directions include dynamic loading of PCL scaffold with inhibitors (ML7, Blebistatin) and activators (LPA) as well as static and dynamic stretch of PLLA crimped scaffolds.

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REFERENCES

[1] Guilak, F., Sah, R.L., and Setton, L.A. Physical regulation of cartilage metabolism. In *Basic Orthopaedic Biomechanics*. Philadelphia, Pennsylvania, USA. V.C. Mow and W.C. Hayes, editors. Lippincott-Raven. 1997. pp.197–207

[2] Orr, A. W., Helmke, B. P., Blackman, B. R., & Schwartz, M. A.. Mechanisms of Mechanotransduction. *Developmental cell, 10*(1), 11-20.March 2006

[3] *Knee Ligament Repair.* Johns Hopkins Medicine. N.p., n.d. [Online] available: http://www.hopkinsmedicine.org/healthlibrary/test_procedures/orthopaedic/knee_ligament_repair_92,P07675/

[4] *Ligament and Tendon Structure and Function*." N.p., n.d. [Online] available: http://www.engin.umich.edu/class/bme456/ligten/ligten.htm

[5] Ge, Z., Yang, F., Goh, J. C., Ramakrishna, S., & Lee, E. H. Biomaterials and scaffolds for ligament tissue engineering. Journal of Biomedical Materials Research Part A, 77(3),pp. 639-652.March 2006

[6] M. F. Pittenger et al, Multilineage potential of adult human mesenchymal stem cells, *Science*, pp. 143-147, April 1999.

[7] César Nombela-Arrieta and Leslie E. Silberstein. *The identity and properties of mesenchymal stem cells*. Available: http://www.nature.com/nrm/posters/mscs/index.html.

[8] T.P.Driscoll, et al. "Fiber angle and aspect ratio influence the shear mechanics of oriented electrospun nanofibrous scaffolds." *Journal of the mechanical behavior of biomedical materials* 4.8: pp. 27-1636. November 2011

[9] T.Sumez, et al. "Molecular architecture of the Spire–actin nucleus and its implication for actin filament assembly." *Proceedings of the National Academy of Sciences*. September 2011

[10] M.Katima; A. Sonnenberg. "Nesprin-3: a versatile connector between the nucleus and the cytoskeleton." *Biochemical Society Transactions*. July 2011

[11] GM. Cooper "Actin, Myosin, and Cell Movement" in *The Cell: A Molecular Approach. 2nd edition*. Sunderland (MA): Sinauer Associates; 2000.

[12] Y. Cinnamon, et.al "Cellular contractility requires ubiquitin mediated proteolysis." *PloS one*. October 2009

[13] S. Austin, R. Dixon . "The prokaryotic enhancer binding protein NTRC has an ATPase activity which is phosphorylation and DNA dependent". *EMBO J.* **11** (6): 2219–28. PMC 556689.PMID 1534752 June 1992

[14] C.Kiani, et.al "Structure and function of aggrecan." *Cell research*, *12*(1),pp. 19-32. November 2002.

[15] B.M.Baker, et al. "The potential to improve cell infiltration in composite fiber-aligned electrospun scaffolds by the selective removal of sacrificial fibers."*Biomaterials* 29.15 May 2008

[16] T. Ishizaki, et al. "Pharmacological properties of Y-27632, a specific inhibitor of rho-associated kinases." *Molecular pharmacology* 57.5 May 2000

[17] "ROCK Inhibitor (Y-27632)." - *Millipore*. N.p., n.d. [Online]available:. http://www.millipore.com/catalogue/item/scm075

[18] "Lysophosphatidic Acid (CAS 22556-62-3)." *Lysophosphatidic Acid*. N.p., n.d.[Online] available: http://www.scbt.com/datasheet-201053-lysophosphatidic-acid.html.

[19] J.A.Cooper. "Effects of cytochalasin and phalloidin on actin." *The Journal of Cell Biology* 105.4 pp. 1473-1478. October 1987

[20] "ML-7 (CAS 110448-33-4)." *ML-7*. N.p., n.d. [Online] available :http://www.scbt.com/datasheet-200557-ml-7.html

Images

[F1] "Hodzic Lab- Projects." *Hodzic Lab- Projects*. N.p., n.d. [Online] available:http://vr-core.wustl.edu/Pages/Hodzic-Lab-Projects.aspx.