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# The Use of Shape Memory Polymers as a Tool to Study Human Fibrosarcoma and Murine Mesenchymal Stem Cell Migration

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# The Use of Shape Memory Polymers as a Tool to Study Human Fibrosarcoma and Murine Mesenchymal Stem Cell Migration

A Capstone Project Submitted in Partial Fulfillment of the Requirements of the Renée Crown University Honors Program at Syracuse University

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## Abstract

Shape memory polymers (SMPs) are a class of “smart” materials that can transform between two distinct conformations through external stimuli, such as heat or electricity. Their usage in bioengineering has led to a promising field of research that lies at the interface of cell and mechanobiology, potentially providing insight into cancer therapies and tissue development—two processes that exist in dynamic environments in vivo. The present work involves creating new, shape changing, scaffolds for studies to analyze cell migration upon changes to the environmental topography. Specifically, this Capstone has been primarily focused on the development of a “half and half” fibrous scaffold, entailing 50% aligned and 50% random fiber alignments separated by a clear interface, to model and better understand how the migratory patterns of both human fibrosarcoma cells (cell line: HT-1080) and murine mesenchymal stem cells (cell line: C3H10T1/2) respond to this architectural change. For example, it is thought that upon metastasis, cancerous cells are able to reorganize the collagen fibers in the extracellular matrix, and use this reorganized architecture as a guide to invade other tissue areas.

Thermoplastic polyurethane (TPU) SMPs were prepared by electrospinning 700-900nm diameter fiber to serve as a cellular scaffold. Through the development of these scaffolds, we are interested in investigating two related, and simultaneously-tested, hypotheses comprised of static and dynamic polymers (each containing static unaligned, and aligned [control] scaffolds, in addition to a thermoplastic 50/50 unaligned-aligned [experimental]—“Half and Half”—scaffold). In the development of these experimental scaffolds, we have attempted to develop a scaffold that demonstrates the aforementioned properties. Through four primary methods to trigger the recovery in only half of the scaffold, we have made progress in minimizing imperfections that result from this process, some of which include: thermal buckling and incomplete recovery. Upon finalizing the protocol used to develop these experimental groups, we will analyze the cells’ migratory rate and how that rate is influenced by time. We anticipate that cells will preferentially migrate faster on scaffolds with aligned fibers than on scaffolds with unaligned fibers, due to the presence of a consistent track for the cells to migrate. Simultaneously, we also will examine whether cells seeded on randomly-oriented fibers will sense these fibers and in the direction of increased orientation.

## Executive Summary

The active properties of SMPs are predicated on their chemical (crosslinking) or physical (entanglement) connections between the chains, allowing for these materials to take on a permanent shape, heated to deform, fixed to a temporary shape, and then later recovered back to the permanent shape by an external stimulus, such as heat or electricity. This ability to change shape through a heat stimulus, for example, has held substantial promise in developing cytocompatible scaffolds to fill voids in tissue to stimulate growth *in vivo*, while their use *in vitro* has allowed for accurate study of human disease in a three-dimensional environment. For example, shape-changing scaffolds used to fill critical-sized bone defects in mouse models have been studied within the Henderson Lab and provided insight into how these materials can be used, osteoconductively. Furthermore, previous work in the Henderson Lab has shown that human adipose-derived stem cells initially seeded on SMPs with aligned fibers displayed cytoskeletal rearrangement tangential to the fiber alignment; however, upon shape memory actuation back to an unaligned fibrous state, the seeded cells no longer displayed this cytoskeletal organization – a testament to how cells' gene expression and behavior is highly influenced on environmental and mechanical cues[1].

The processes of metastasis are thought to be dependent on tumorigenic cells' diffusion along extracellular matrix (ECM) proteins, in particular collagen. In the context of oncological surgery, this aberrant division and migration is a critical point in the progression of the disease.

The spread of these mutated cells into remote organ systems hinders the complete removal of the tumor cells, as propagation at secondary sites away from the primary site can occur.

Cell migration is initiated by the reorganization of the cell membrane—via its actin cytoskeleton—in a highly integrated, multistep, process that is often regulated by the presence of external signals including mechanical and chemical signals[1]. Through G-protein coupled receptors or Receptor Tyrosine Kinases, which span the phospholipid bilayer via their hydrophobic domains, these stimuli react extracellularly and ultimately signal an alteration in gene expression to reorganize the actin cytoskeleton for movement. For example, in the context of metastasis and mechanical stimuli, Provenzano, et al., showed that, through primary tumor explants, the invasive properties of these cells are also highly dependent on their ability to radially reorient the collagen fibers in the ECM to migrate outward from the site of tumorigenesis and into surrounding tissue[2].

Through the use of electrospun SMPs and their ability to change shape via their nano-scale fibers, the reorganization of collagen seen by Provenzano, et al., can be intricately modeled and studied in detail through the fabrication of scaffolds with distinct fiber orientations. The process of electrospinning was used to prepare our thermoplastic polyurethane (TPU) scaffolds, as this process allows for recovery of the bulk shape, as well as internal fibrous architecture in response to the heat-

triggered recovery[1]. The goal of this study was to develop an experimental scaffold that demonstrates the aforementioned half-and-half properties. This mechanobiological investigation has proceeded under two related hypotheses—outlined below—that will be tested simultaneously on the same electrospun scaffold. Each test contained a static unaligned [control], a static aligned [control], and a 50% random and 50% aligned, "half-and-half" [experimental] electrospun scaffold. The goal in establishing a protocol to consistently develop these half-and-half scaffolds was to test the hypotheses that:

**A)** Cells will preferentially migrate at a faster rate onto scaffolds with aligned fibers than on scaffolds with unaligned fibers.

**B)** Cells seeded on random fiber topographies will sense the aligned fibers and migrate toward these aligned regions.

We believe that these two hypotheses will be verified due to the uniformity of the aligned fibers providing a consistent track for integrin attachment and cell movement, while the randomly-aligned fibers will not.

The process of development of this experimental half-and-half scaffold progressed through four primary methods, all centered around the implementation of heat energy into one half of the strained sample to restore its original fibrous architecture, while fixing the other half via cooling plates. The optimization and characterization of these four methodologies were the central focus of this Capstone. The different mechanisms through which heat was introduced were: an aluminum plate

“sandwich,” a heat gun, an $H_2O$ pipetting method, and an $H_2O$ dipping method. Throughout the course of the study, scaffolds with aligned fibers were uniaxially strained to 100% (twice the original size) in the direction perpendicular to the inherent alignment to generate a temporarily-fixed unaligned fiber orientation throughout the scaffold. Through the heating scaffold, the portion exposed to heat is recovered back to its original form and restored the original aligned fiber orientation. Through the aforementioned methods to trigger this specific spatial recovery, progress has been made in minimizing imperfections, such as thermal buckling and incomplete recovery, that result from the spatial recovery process and hinders the quality of data. Upon successful establishment of this scaffold, assays involving the cell migratory behaviors of human fibrosarcoma [cell line: HT-1080] and murine mesenchymal stem cells [cell line: C3H10T1/2] on fibrous scaffolds with different architectures will commence via a recently developed cell tracking algorithm[5].

Although a high variability in the quality of the scaffolds was seen amongst the replicates created through each methodology, we report that important steps were made in creating a wrinkle-free (or minimally-wrinkled) scaffold with a distinct ( < 100μm) interface between the aligned and unaligned fibers. This width, along with a smooth surface, is critical to establish prior to cell studies for the following reasons: when wrinkles appear on the surface of the scaffold during recovery (due to the exposure to heat), cells can presumably sense these inclines and depressions,

resulting in inconclusive cell motility events due to the presence of more than one variable: fiber alignment and surface wrinkles. Further, a distinct interface measuring less than 100µm is also needed when taking into account cells' rate of migration and duration of standard imagining assays. With standard live cell imaging assays lasting 24 hours, it has been recorded that glioblastoma cells (cancer of the nervous tissue) on aligned scaffolds migrated at an effective velocity of 4.2µm/hr (+/- 0.39 µm/h) compared to 0.8µm/hr (+/- 0.08µm/h) on random scaffolds[3]. Therefore, based on this study, within a 24h time frame, it can be assumed that the approximate maximum distance a cell on an aligned scaffold can travel is about 100.8µm. With an interface smaller than this maximal value, cells would be more likely to fully cross over the transition and begin to respond to the new fiber alignment. The presence of large- and small-scale wrinkles seen in all samples, in addition to an interface consistently measuring around 1mm—approximately 10x larger than the ideal model—has required further investigation and experimentation into methods tailored to address these current imperfections.

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## Acknowledgements

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## Introduction

Shape Memory Polymers (SMPs) have the ability to transform between two distinct conformations through an external stimulus, such as heat or electricity. Their usage toward applications in biomedical engineering has led to a promising area of research that lies at the interface of cell biology and mechanobiology, potentially providing insight into specific fields such as cancer and tissue engineering. Specifically, this thesis involves creating new tools that are anticipated to help increase the understanding of dynamic processes, such as cancer and tissue development. One of the chief goals behind the usage of SMPs is to culture cells in a changing environment, since the environment during tissue development and disease progression is dynamic. Thus, one of the many appealing features that highlight the usage of shape memory polymers is their dynamic functionality. When compared to the traditional static scaffolds, these polymers allow for accurate studies on how cells truly behave within the dynamic topographical environments of the human body, especially during aberrant cell growth, or metastasis.

With the number of breast cancer cases on the rise, the intricate nature of cellular processes has driven the progression of these “active” culture technologies, allowing for intricate investigation on the way fiber alignments and environmental topographies affect cell migration. These alterable scaffold conformations and materials have opened many avenues on the potential ways cell/SMP constructs can be utilized for precise modeling of the human body and its potential defects.

### *Study Goals*

This study has been primarily focused on the development of the cytocompatible scaffolds needed to explore the migratory patterns of both human fibrosarcoma (malignant fibroblasts) and murine mesenchymal stem cells. Previously, work has shown that changes in SMP topography preferentially drive cells' cytoskeleton to orient in tandem with the applied topographical change of the scaffold[1]. Here, 3D, cytocompatible, TPU SMPs have been prepared by electrospinning at a diameter of approximately 700-900nm to serve as a scaffold for the human fibrosarcoma (cell line: HT-1080) and murine mesenchymal stem cell (cell line: C3H10T1/2) lines of interest; this specific diameter range is critical in allowing for cell migration to be carried out in a fashion that facilities integrin adhesion and minimizes the overall environmental stress. Utilizing these polymers, the goal of the study is to investigate cell migration and behavior on fibrous scaffolds with different architectures. This mechanobiological investigation has been set under two related hypotheses that will be tested simultaneously on the same biological samples. Each test, with either static or dynamic polymers, contains unaligned [control], aligned [control], or 50% unaligned and 50% aligned [experimental] electrospun scaffolds.

The first hypothesis analyzes the overall speed of migration, in that cells will preferentially migrate at a faster rate onto scaffolds with aligned fibers than on scaffolds with unaligned fibers. We believe that this will be the case due to aligned fibers providing a consistent track for cell movement by way of cell migration, while the unaligned fibers will not. Furthermore, the second hypothesis states that cells seeded on random

fiber topographies will drive migration onto aligned topographies, as these allow for a more fluid migration. This fluidity is characterized by either movement in the direction of increasing fiber alignment, or movement without a change in direction once they attach to aligned fibers. In carrying forward both hypotheses, the reactions of cells to the topographical changes will be recorded and analyzed by way of live imaging (or time lapse) of fluorescently-labeled cells and a recently developed cell tracking algorithm[5].

## Materials and Methods

### Aligned Nanofiber 14hr Electrospin

*Preparation of Electrospin Solution*

A solution of 33% (weight per unit volume) B100HP3-060914 (name of TPU polymer) was prepared. With the feeding rate of the solution into the apparatus [seen below in **Figure 1**; adapted from JM Bourget, M. Guillemette, et al. (2013)][8] set at 0.2 mL/hr, and a spin time of 14hr, a theoretical volume of 2.8 mL was needed; however, 3.6 mL was prepared in order to account for loss/human error. In order to create this volume, 33% of the total volume (3.6 mL), calculated to be 1.188 g, was added to a glass vile. Further, the polymer crystals were diluted in a 1:2 mixture of DMF:Chloroform, respectively. Thus, 1.2 mL of DMF and 2.4 mL of Chloroform was also put into the same vile, along with a stir bar, and set to mix/dilute overnight.

*Electrospinning*

Upon setting up the apparatus with a 25G needle, a feeding rate of 0.2 mL/hr, center-hole translation, a 4K drum rotation, and a voltage source of -

500~18kV (drum~needle, respectively), no fiber projections were seen. With this, the feeding rate was increased to 0.3 mL/hr and allowed to run for 14hr, generating an aligned scaffold by way of the high RPM of the rotating mandrel, as shown to the right of **Figure 1**[6]. At the conclusion of the 14hr electrospin, the mat, collected on aluminum foil, was gently peeled off of the mandrel, put in the vacuum oven overnight, and then stored in the vacuum desiccator at 25˚C.

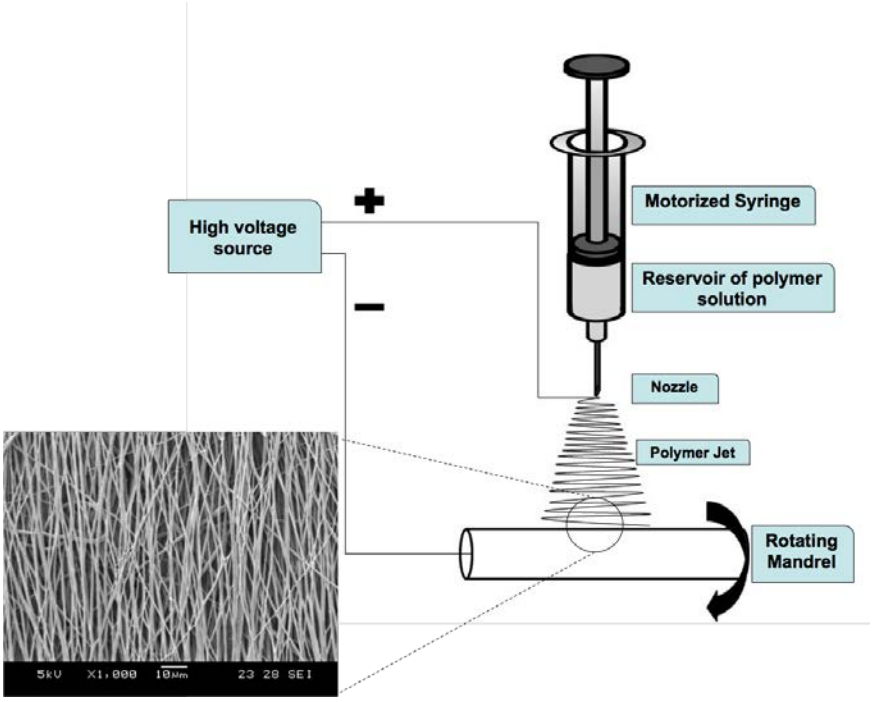


**Figure 1**: Schematic representation of the electrospinning process used to generate nanofiber SMPs. Scaffolds of aligned fibers were prepared through the procedure illustrated above. To produce this aligned scaffold, the rotational speed of the collecting drum was set to 4000 rpm. This high rate of rotation preferentially orients the fibers along the circumferential direction of the mandrel. Furthermore, the rotating drum was charged to -0.5 kV, while the nozzle was charged to +16 kV, allowing for the projection of fibers within the diameter range of interest—between 700-900nm.

## SMP Thermal Treatment and Programming

### *SMP Thermal Treatment*

The process of obtaining polymeric scaffolds through electrospinning has been well-documented to stretch amorphous polymer chains, such as the TPU

polymeric scaffolds used in this study. Upon exposing a newly-spun scaffold to heats near/above its polymer glass transition temperature, the polymeric TPU chains release their molecular-level strain, resulting in dimensional changes to/shrinking of the scaffold[1]. In order to prevent this phenomenon—which complicates material programming (discussed below) and the polymer's general shape-changing properties—a method previously developed by the Henderson Lab was used[1]. Via this method of thermal treatment, the aligned fibrous scaffold was wrapped around a conical tube evenly covered in a 65 wt.% Pluronic F127 hydrogel for adhesion. Upon liquifying a separate volume of the hydrogel at 4˚C, this solution was poured into another conical tube of a larger diameter allowing for the scaffold (wrapped around a smaller-diameter conical tube) to be completely immersed in between two hydrogel layers upon cooling. This assembly was then held isothermally at 70˚C for 3h. Upon completion, the mat was submerged in deionized water (changing the water approximately every 30min) for 12hr and kept at 4°C to remove the hydrogel.

*SMP Programming*

To implement the shape-memory actuation into the thermally-treated mat, the aligned scaffold was stretched to 100% strain (twice its original length) at 60˚C via a Dynamic Mechanical Analyzer (DMA; TA Instruments Q800). Through a systematic study of parameters of the stretching procedure were established. The rate of stretching used was 0.004N/min until a total force of 0.6N was applied on the sample, while an abort option was programmed to abort once a strain greater than 95% was recorded. The scaffold programming was achieved

through uniaxial stretching perpendicular to the alignment of the fibers in order to temporarily establish a random fiber orientation upon fixing at 0˚C.

**SMP Recovery**

The original protocol used in the preparation of the experimental, half and half scaffolds utilized a 65%/35% pluronic hydrogel bilayer followed by isothermal heating to induce recovery in one half of the scaffold. However, this method did not consistently yield a distinct interface between the random and aligned portions of the scaffold and showed difficult reproducibility. The following methods were employed in an attempt to establish structural integrity to the half-and-half scaffolds through a distinct interface under 1μm. The extent of recovery was precisely measured through the use of a digital caliper.

*Aluminum Plate Method*

Through this method in triggering SMP recovery, a heated aluminum plate "sandwich," was used as a means to restore the aligned fiber architecture in one half of the scaffold. Trials were held at 75˚C and 85˚C and kept below 100˚C, as temperatures above 100˚C melted the polymeric material and/or fusion of the polymeric fibers. Conversely, the fixation of the other half of the scaffold through a frozen aluminum plate "sandwich" was done to prevent conduction of heat and maintain the random architecture in the other half of the scaffold. These plates were kept frozen overnight prior to use and remained on an ice block throughout the recovery process to maintain the temperature of the sample below its $T_g$ at 0˚C.

*Heat Gun Method*

In utilizing a heat gun, SMP recovery was triggered through the standard principles of convection. One half of the samples were fixed below $T_g$ at 0˚C through the use of frozen aluminum plates, while the other half of the sample remained exposed to the heat. Samples were recovered through both the “hi” and “low” settings on the instrument at different heights to control the amount of exposure to heat through dissipation. Trials included: hi and low settings at distances ranging from ~35mm, ~50mm, ~85mm, ~155mm and > 155mm, as measured by a digital caliper.

*$H_2O$ Pipetting Method*

Analogous to the Aluminum Plate Method, the conduction of heat was carried out and exposed onto the sample through the stable pipetting of heated deionized water, while the other half was fixed under 0˚C aluminum plates stationed on an incline to prevent water from entering the other half via capillary action. Replicates were recovered at 79.4˚C and 100˚C, and a temperature increase to such an extent was done to account for the cooling (low volumes of water) between uptake, transfer, and pipetting. The deionized water was then pipetted as evenly as possible onto the exposed half of the scaffold, where recovery estimated by the use of the caliper.

*$H_2O$ Dipping Method*

To provide more uniformity, samples were fixed between two aluminum plates at 0ºC and dipped into water as a means to establish a more even recovery along the scaffold. The deionized water used to recover the samples

was heated to 88.5˚C. Samples were then dipped evenly into the water, where recovery was estimated by eye.

**Scaffold Analysis**

Fiber quality and architecture were primarily analyzed by a scanning electron microscope (SEM), upon sputter coating with gold. Images were generated with a 5kV accelerating voltage and a spot size of 24. The fiber alignment on select samples were further characterized by way of a 2-D Fast Fourier Transform (FFT) image analysis on the Java-based image processing program, ImageJ.

## Results

*Aluminum Plate Method*

The overall schematic of the hot plate method is shown below (**Figure 2**). Through the Aluminum Plate Method shown on the following page, the experimental setup revealed variable results primarily characterized by incomplete recovery.

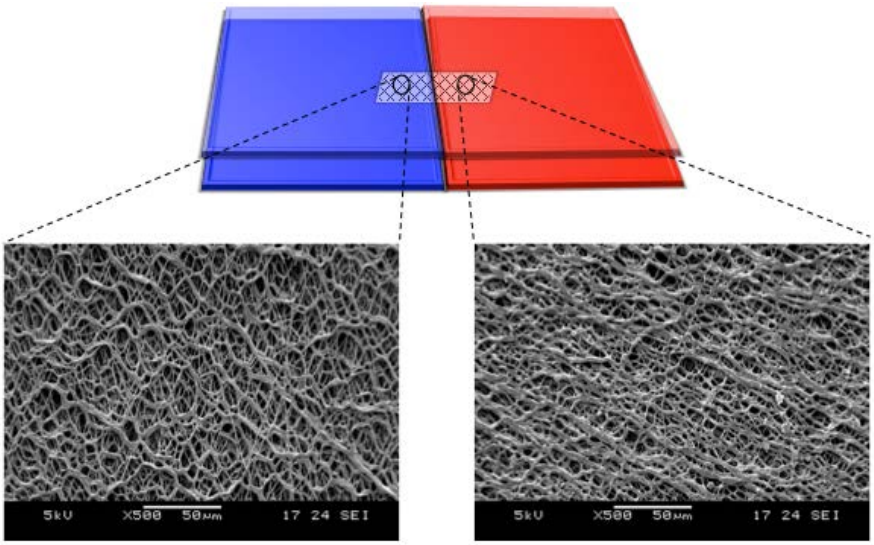


**Figure 2**: The Aluminum Plate Method

Despite not exhibiting thermal buckling, this incomplete recovery resulted in the retainment of the random fibers in the region of expected alignment and made the samples prepared under this method unideal due the likelihood of recovering once in the hydrated state and in cell culture. As shown in **Figure 3**, the 2D-FFT of the sample above quantitatively analyzes the fiber alignment in both regions of the sample. As shown below via the pixel intensity along either 90˚ or 270˚, the recovered region, initially expected to return to back to the aligned fiber orientation, failed to exhibit this fibrous architecture as compared to the aligned control. This resulted in a scaffold without two distinct fiber alignments, hindering the ability to analyze how cells are affected by a distinct change in fiber architecture.

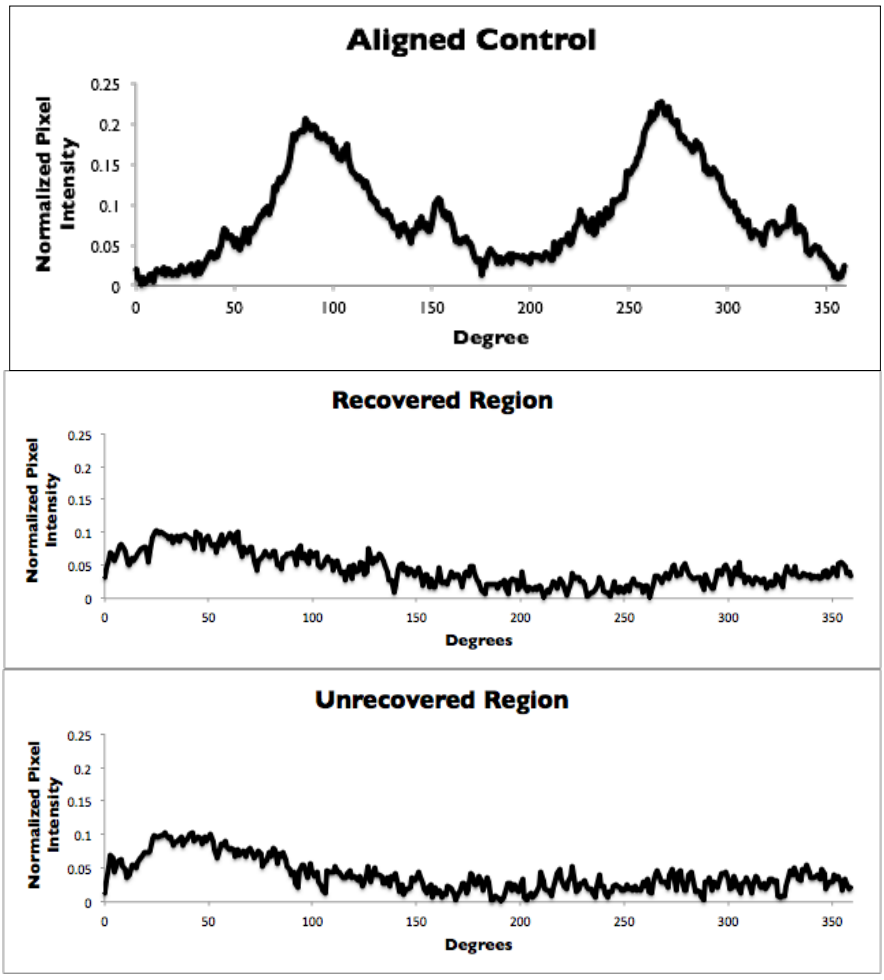


**Figure 3**: 2D-FFT of sample yielded from the Aluminum Plate Method.

*Heat Gun Method*

In an attempt to quell the issue of incomplete recovery, the use of a direct and stable heat source onto the scaffold was implemented via a heat gun in an analogous fashion of the Aluminum Plate Method. As shown below in **Figure 4**, this methodology was used as a means to trigger recovery of the scaffold in a quick and steady manner through the exposure of heat.

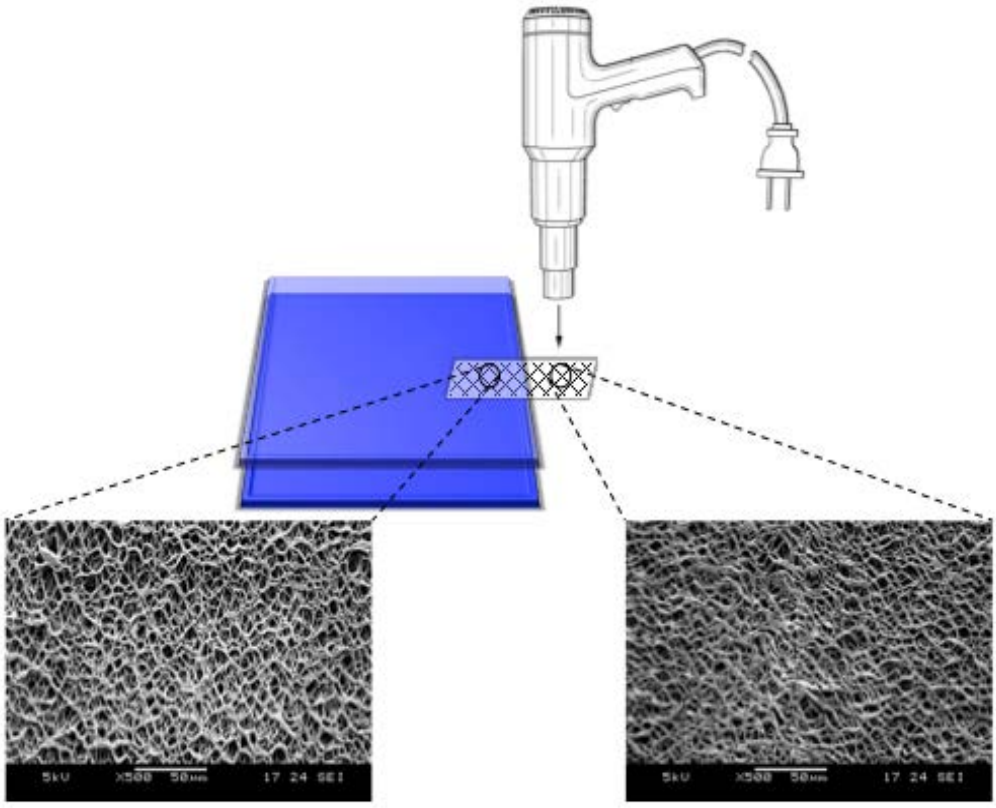

**Figure 4**: The Heat Gun Method

The samples generated through this method consistently resulted in recoveries > 95%, but exhibited poor macro-scale quality primarily due to thermal buckling of the scaffold during recovery and lack of overall control during recovery. Furthermore, as shown by both regions displayed above, these samples failed to exhibit the half-and-half fibrous architecture, as nanoscale analysis of the fibers

failed to recover back to their original aligned morphology. Another replicate is shown below to further illustrate the lack of district fiber architectures (**Figure 5**). Similarly, this resulted in a scaffold without two distinct fiber alignments, hindering the ability to analyze how cells are affected by distinct fiber architectures.

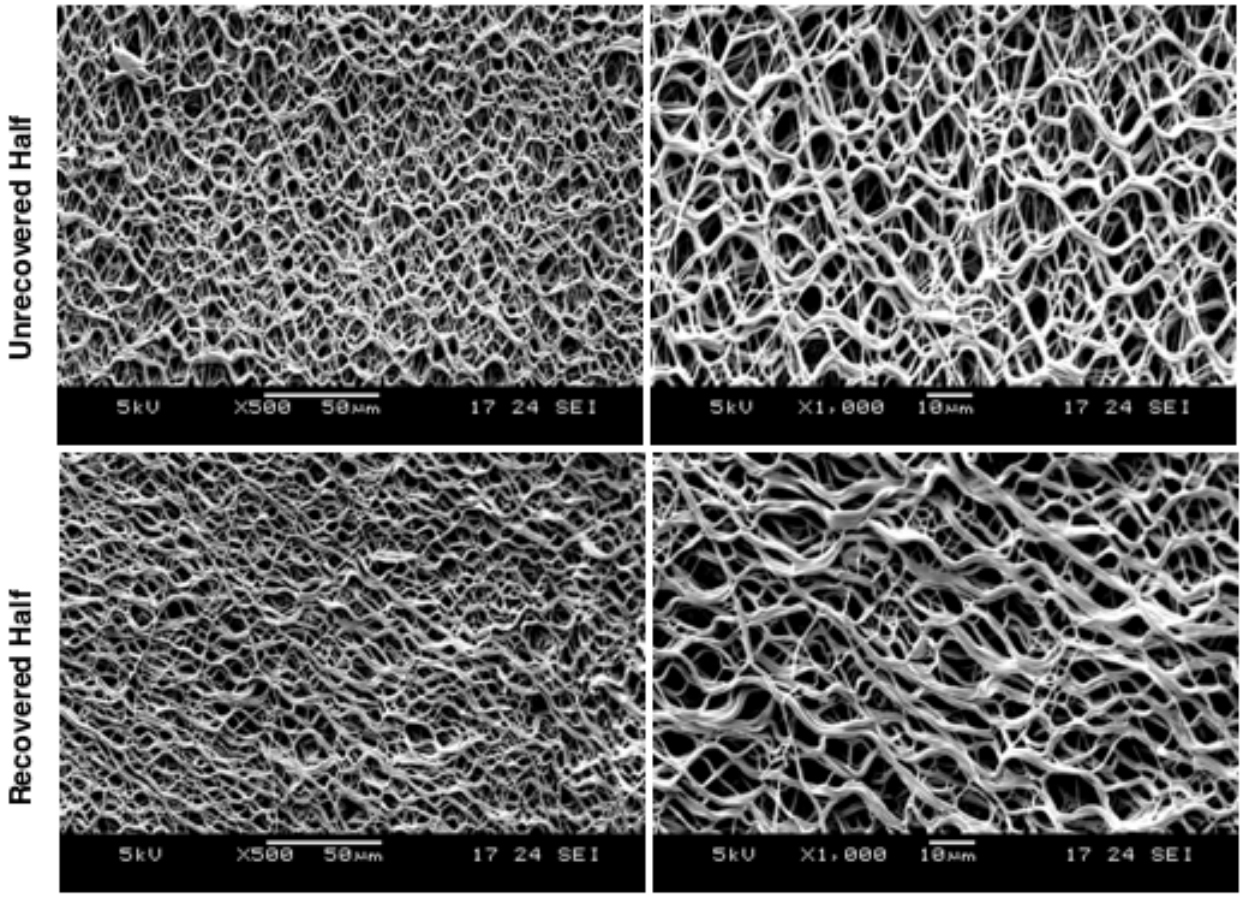


**Figure 5**: Technical replicate from the Heat Gun Method illustrating lack of distinct fiber alignments

*$H_2O$ Pipetting Method*

With the poor control exhibited through the use of the heat gun, the design in **Figure 6** below was established as a means to control where scaffold recovery would take place, while maintaining an interface through cold Aluminum plate

fixation. An incline was implemented as a means to inhibit water from entering the fixed part of the scaffold in between the cold plates via capillary action.

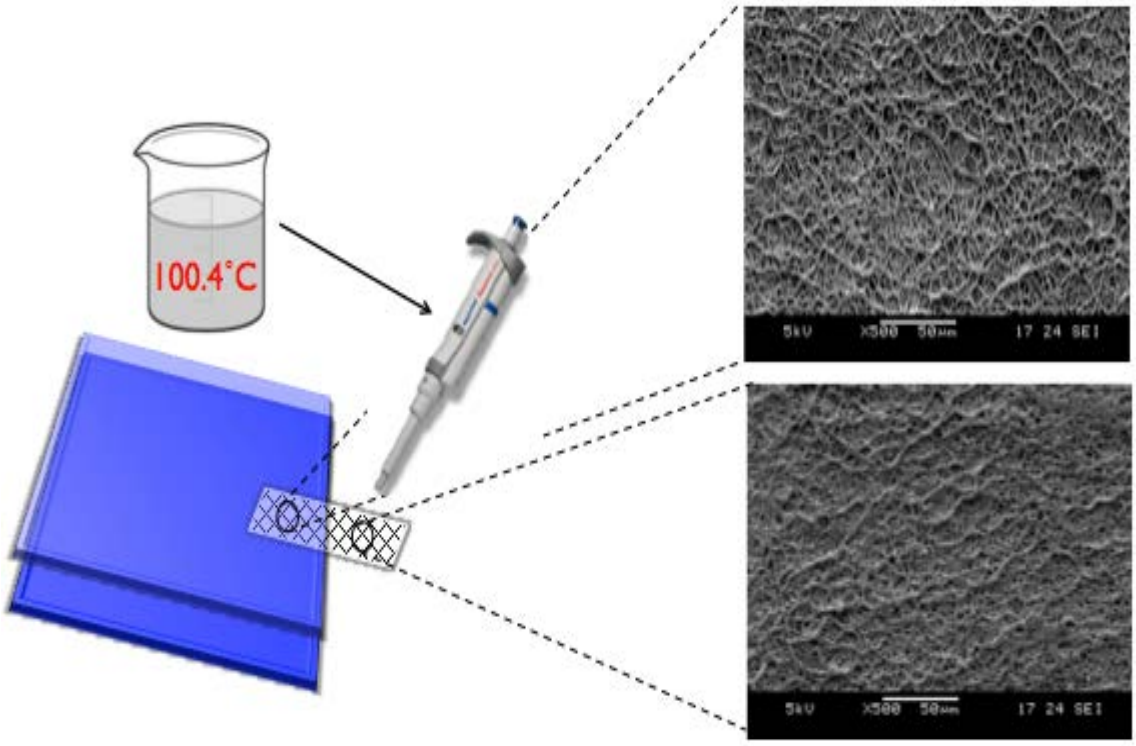


**Figure 6**: Schematic of the $H_2O$ Pipetting Method

While the control of recovery was able to be maintained through the volume of water dripped onto the scaffold, thermal buckling was seen to develop, increasing with each drop pipetted onto the sample. Additionally, as shown in the schematic above, and in **Figure 7** below, the scaffold replicates also failed to show distinct fiber alignments, and thus, this method determined to be ineffective in yielding the architecture of interest.

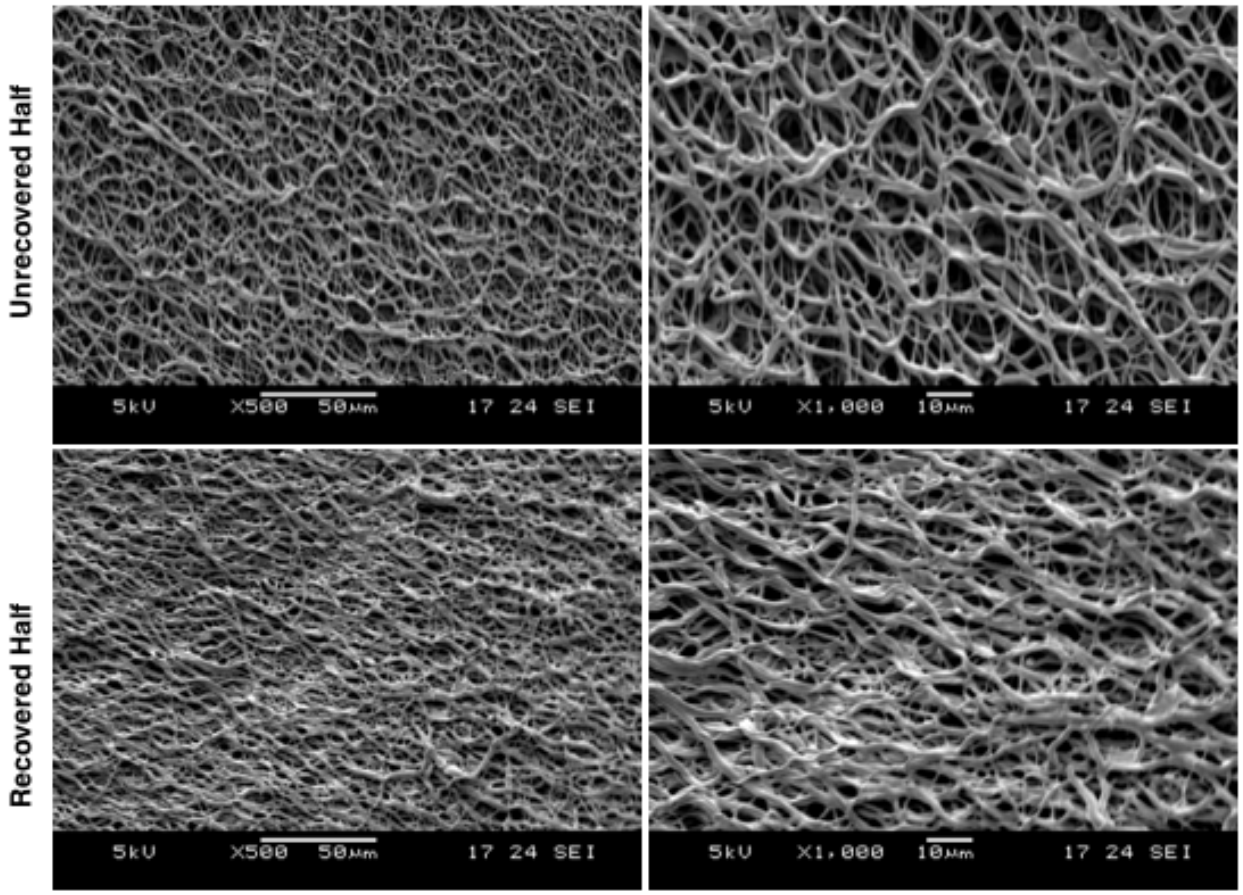


**Figure 7:** Technical replicate from the $H_2O$ Dipping

*$H_2O$ Dipping Method*

Based upon the lessons learned during testing of the prior approaches, the $H_2O$ Dipping method was carried out (shown below in **Figure 8**) in an attempt to avoid the previously seen imperfections, such as thermal buckling, proper fiber alignment, and low % recovery. The sample processed through this method showed to better the quality of the scaffold in

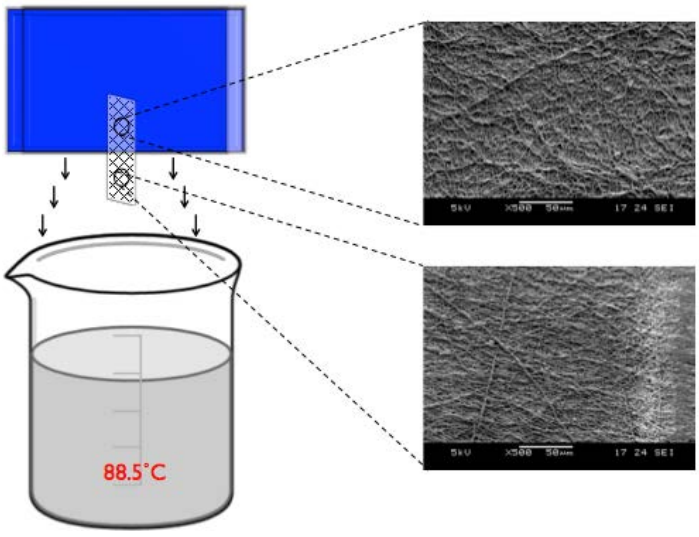


**Figure 8:** Technical replicate from the $H_2O$ Dipping Method

these areas and of the four total methods, exhibited the most promising results based on the distinct fiber alignments (**Figure 9**). However, the scaffold still displayed wrinkling, curling (presumably due to one half being suspended freely in the water during the active recovery process) and an interface on the order of approximately a millimeter, as opposed to the ideal width of 100μm or less — all properties that may hinder cell studies.

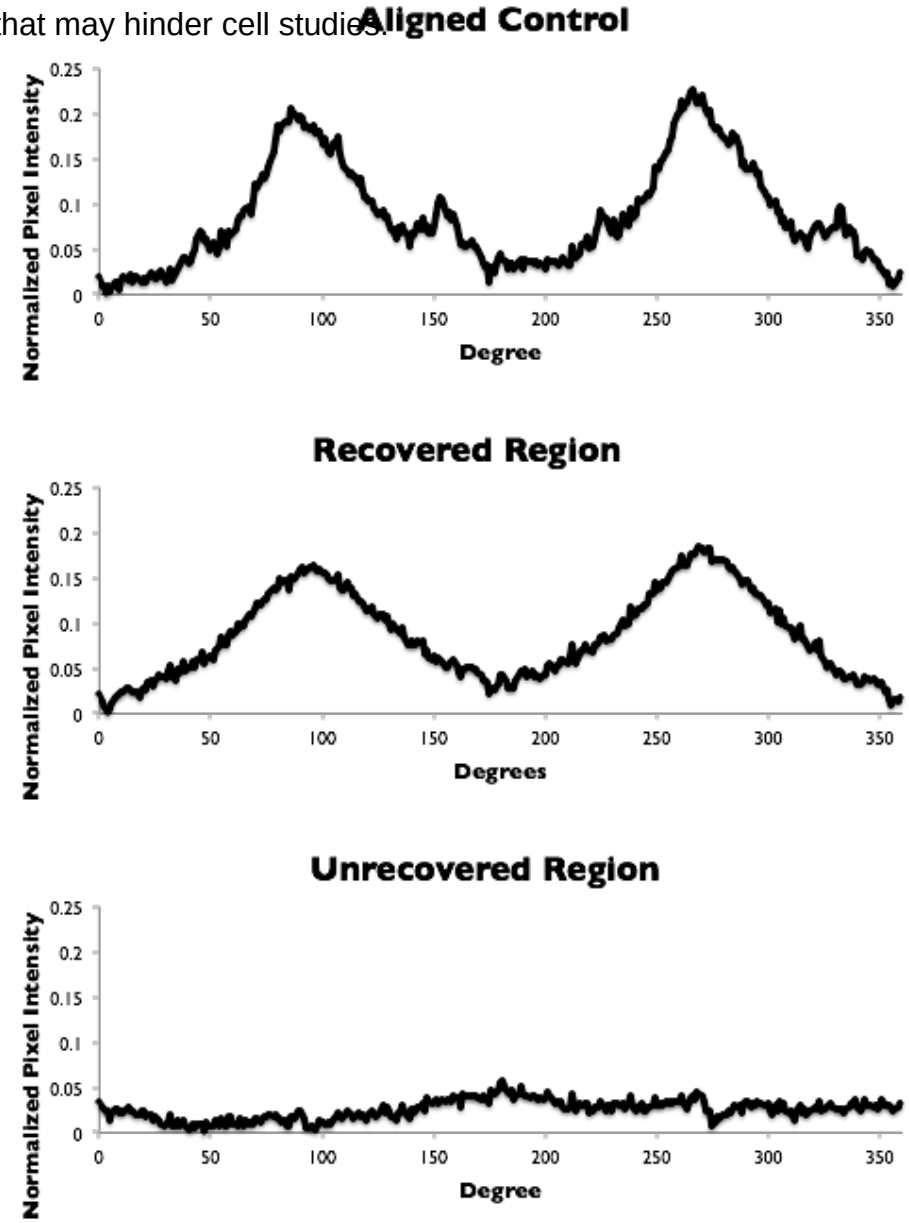


**Figure 9**: 2D-FFT of sample yielded from the $H_2O$ Dipping Method.

## Discussion

The application of heat energy into one half of the strained scaffold proved to be a promising method in the development of the half-and-half scaffolds. However, as mentioned above, two major imperfections were present amongst nearly all samples: thermal buckling of the scaffold in addition to a large interface.

The use of thermal buckling in thermoplastic scaffolds is actively used within the material sciences, where permanent wrinkles develop upon recovery due to a strain mismatch between two surfaces[4]. Further, our method in utilizing aluminum plates may have also played a role in further influencing wrinkle development, as the unrecovered half was constrained at the interface. The drive to eliminate these wrinkles is predicated on their presence hindering results acquired from cell studies. For example, when analyzing cell migratory data, a particular pattern in migration cannot be said to be definitively due to the alignment of the fibers, as more than one variable are present within the cell's environment: scaffold fiber alignment and scaffold inclines/depressions.

Furthermore, the presence of a large interface exceeding 100 μm between the two fiber alignments upon recovery complicates cell culture experiments as cells will not be able to cross over and interact with both topographies during a standard 24h time-lapse assay. As glioma cells on aligned scaffolds migrated at a velocity of 4.2μm/hr (+/- 0.39 μm/h) compared to 0.8μm/hr (+/- 0.08μm/h) on random scaffolds[3], theses rates of migration play large role in their ability to migrate across a transition region of a specific distance.

Understanding the mechanisms of metastasis within the human body lies at the peak of interests across a multitude of scientific disciplines. Outside the realm of cell signaling, the mechanical properties of tissues play significant roles in migratory patterns. In utilizing SMPs, the underlying mechanism of metastasis can be explored and hopefully down regulated through precisely mimicking of the collagen fiber alignment in the extracellular matrix. On the other hand, stem cell delivery, proliferation, and longevity equally depend on bodily topographies and ECM alignments. Thus, understanding and investigating how fiber alignment can stimulate their rate of migration for integration into tissues is just as significant for the future therapeutic modalities involved in tissue regeneration. Through coupling the biochemistry behind the two model systems of human fibrosarcoma cells and mouse fibroblasts with the bioengineering of SMPs, the human condition can be dramatically improved in a patient-specific, cytocompatible fashion.

## Conclusion and Summary of Future Work

Throughout the course of this Capstone, progress has been made on the development of the half-and-half scaffolds. However, thermal buckling and a large transition are two major imperfections that must be addressed through future experiments prior to the utilization of these scaffolds in vitro. One way in which these two phenomena can be addressed is potentially though immersing the scaffold in two Pluronic hydrogel layers of 35 wt.% and 65 wt.% followed by an isothermal heating at 70˚C for 3h. Similar to the SMP thermal treatment explained above, we hope to take advantage of the higher concentration

hydrogel layer as a means to fix one half of the sample, while allowing for the half of lower concentration to recover upon exposure to heat. Through a sharp hydrogel boundary, without the application of aluminum plates, this isothermal method will allow for a steady recovery (due to the presence of the hydrogel) in the half of lower concentration, while allowing for complete stabilization in the region of higher concentration.

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*Image Reference*

[6] Electrospinning Device Schematic; **Figure 1**:
Jean-Michel Bourget, Maxime Guillemette, et al. (2013). Alignment of Cells and Extracellular Matrix Within Tissue- Engineered Substitutes, Advances in Biomaterials Science and Biomedical Applications, Prof. Rosario Pignatello (Ed.), ISBN: 978-953-51-1051-4, InTech, DOI: 10.5772/54142. Available from: http://www.intechopen.com/books/advances-in-biomaterials-science-and-biomedical-applications/alignment-of-cells-and-extracellular-matrix-within-tissue-engineered-substitutes