

Melt Blown Poly(lactic acid) for Application as a Tissue Engineering Scaffold

A Thesis Presented for the
Master of Science
in
Polymer Engineering
The University of Tennessee, Knoxville

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December 2012

Acknowledgements

I would like to acknowledge all of the help I received throughout my work at UT from my lab mates, Ryan Hammonds, Kaan Serpersu, Pelagie Favi, and Russell Hallman, as well as the aid from Dr. Biggerstaff's group and their generous donation of A375 cells. I would also like to acknowledge the help from the members of the Textile and Non-Woven Development Center, specifically Josh Fogle. Finally, I would like to thank the UT maintenance staff for lending me tools and insight throughout my time at UT.

Dedication

I dedicate this thesis to Dr. Roberto Benson for providing me with an opportunity to succeed and the valuable tools necessary to complete the job properly.

Abstract

Poly(lactic acid) (PLA) was melt blown (MB) under varying processing conditions to create webs with micro and nano-architecture. Processing parameters varied were primary air flow rate and collector distance. In total, twenty-one webs were produced and the physical properties of the webs were investigated including, mean fiber diameter and fiber diameter distribution, mean pore diameter and pore size distribution, web thickness, degree of crystallinity, tensile modulus and degradation rate. Four webs, two with micro and two with nano-architecture, thought suitable for use as tissue engineering scaffolds were selected for seeding with A375 human malignant melanoma cells. Cell culture was conducted for 24hrs and the scaffolds were examined using scanning electron and confocal microscopy to investigate cellular attachment and migration on the surface and into the bulk of the material.

Results from the investigation show that it is possible to create a wide variety of architectures with both micro and nano-features through variation in primary air flow rate and collector distance. Physical properties of the webs can be tailored during processing and by post-processing treatment. Seeding select scaffolds with A375 cells demonstrated that both micro and nano-architectures were successful in facilitating cellular attachment, and migration on the surface and into the bulk of the scaffolds. Although the methods by which the cells attached and migrated on the scaffolds were different, bulk migration into the two different architectures was the same.

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1. Introduction

1.1 Tissue Engineering and Regenerative Medicine

The most common approach used today to repair damaged or failed organs or tissue are allografts, xenografts, autografts or implant a biomedical device to replace the biological damaged component. Although these methods have proved to be successful they have large limitations including, donor availability, infection, poor integration and even rejection by the host. In order to combat these concerns, the field of regenerative medicine (RM) was created with the goal being to treat tissue diseases by recreating and mimicking the host's natural tissues in order to restore full function. It is important to define the term tissue engineering (TE) and that it is a subfield of RM. Langer and Vacanti gave the first definition of TE as "an interdisciplinary field that applies the principles of engineering and life sciences toward the development of biological substitutes that restore, maintain, or improve tissue function or a whole organ"¹. The basic steps in tissue engineering are the creation of a porous scaffold, the culturing of cells in vitro, and then seeding the cells onto the scaffold for cell growth and proliferation. The scaffold can then be implanted into the host's damaged area and eventually the scaffold should be bioresorbed and replaced with the host's own living tissue.

1.1.2 Tissue Scaffolds

According to ASTM F2150-07 a scaffold is defined as "a support, delivery vehicle or matrix for facilitating the migration, binding or transport of cells or bioactive molecules used to replace, repair or regenerate tissues"². In order to be successful the scaffold should meet the following criteria:

- It must be biocompatible;
- It must have mechanical properties that are similar to the host tissue it will be replacing;
- It must have surface properties that facilitate cellular attachment, growth, proliferation and differentiation;

- It must facilitate extracellular matrix (ECM) production;
- It must have the proper pore size, porosity, pore interconnectivity, and pore size distribution in order to sufficiently deliver nutrients and remove waste as well as promoting cellular infiltration into the depths of the scaffold;
- It must be bioresorbable;
- It must degrade at a rate that matches the creation of new tissue.

In order to meet all of these goals several points must be taken into account prior to the scaffold fabrication. First off, certify the area of the body attempting to be mimicked by the scaffold. The physiological and mechanical properties of the implant site will vary greatly depending on the location in the host's body. Second, identify the time table for repair of the damaged tissue. Does the scaffold need to harbor the developing cells for several weeks or months? Finally, how will the degradation products produced by the scaffold interact with the surrounding host tissue? These three points will be the main drivers behind materials selection for the scaffold. Once the material has been selected, the fabrication process needed to create the proper scaffold architecture will have to be decided upon.

1.1.2.1 Scaffold Porosity

The structural design of the scaffold is critical to its success as a cell nurturing environment, because cell colonization depends both on surface chemistry and internal structure. The porosity of the scaffold is of the utmost importance because it directly affects the transport of nutrients to the cells and the removal of waste products. Also, uniform tissue growth on the surface and into the bulk of the scaffold is controlled by the pore size and connectivity between pores¹. As most human single cells are larger than 10 μ m in diameter it is important to select a fabrication technique and process parameters that will produce a final product with pores that are larger than the maximum cell size³. Conversely, if the pore size is too large, greater than 300 μ m in diameter, then the cells will not be able to colonize the entire scaffold due to difficulties in bridging large gaps⁴.

1.1.2.2 Mechanical Properties

Cells migrate by exerting force on the substrate on which they are located in order to move forward. This movement is facilitated by the action of myosin II on the cytoskeleton through integrin-extracellular matrix linkages to create traction⁵. The tensile modulus of scaffolds has been shown to affect cellular activity at both the micro and nanoscale levels. Specifically, cells show reduced spreading in scaffolds made of weak hydrogels. This is most likely caused by the response to tractional forces between the cells and the scaffolds⁴. Ideally, the materials used to create the scaffold should be able to withstand the contractile forces imposed upon them by the cells, with a maximum force generated by the cell on the scaffold being 10-15% of the substrate's modulus⁶. Previous studies conducted have supported the theory that cell movement can be guided by substrate rigidity and that cells prefer areas of higher modulus to adjacent areas of lower modulus^{5, 7}. Evidence used to draw these conclusions was the recording of cell traction generated based on substrate modulus (kdyn/cm²), as well as cellular speed(μm/min) versus substrate modulus. This phenomenon of cell guided movement based on rigidity is referred to as durotaxis. The proposed mechanism of durotaxis is that as the cell receives feedback from its surroundings the input signal is detected and proceeds down the signal transduction pathway eventually resulting in an intracellular response. This mechanism is similar to the response produced by a foreign chemical. The intracellular response produced is either an increase or decrease in cellular traction forces. As the modulus of the substrate increases so does the tractional force produced by the cell which leads to a direct increase in spreading and bias of directional movement⁷.

1.1.2.3 Fiber Diameter

Extrinsic signals from the ECM are a major guiding force for cellular development. The cell membrane is littered with carbohydrate structures and more than half a dozen different types of receptor systems

that respond to interactions with adjacent cells, the ECM and signaling molecules⁸. Therefore, fiber diameter is a key feature that needs to be tailored as the attachment of integrins and the creation of focal adhesions is strongly affected by the scale of the surface's topographical features. Studies have been carried out on porous scaffolds with both micro and nanoscale features, and it has been concluded that cells respond more favorably to nanotopography by increasing the number of filopodia and microspikes on the cell membrane surface⁹. Incorporating technical nanoscale features into the scaffold such as pillars, ridges or steps has proven to be effective, but a simple decrease in fiber diameter down to the nanoscale has also been found to increase cellular attachment and proliferation. Cells that bind to microfiber scaffolds tend to flatten and spread as if being cultured on a 2-dimensional surface, while cells that bind to nanofiber networks tend to spread over multiple fibers and migrate into the bulk of the scaffold⁹. The mechanism behind this phenomenon is that nanofiber networks have more surface area that better facilitate protein absorption and create more binding sites that are easily accessible. Figure 1 illustrates the difference between attachment of a single cell to a micron sized fiber and nano-sized fibers.

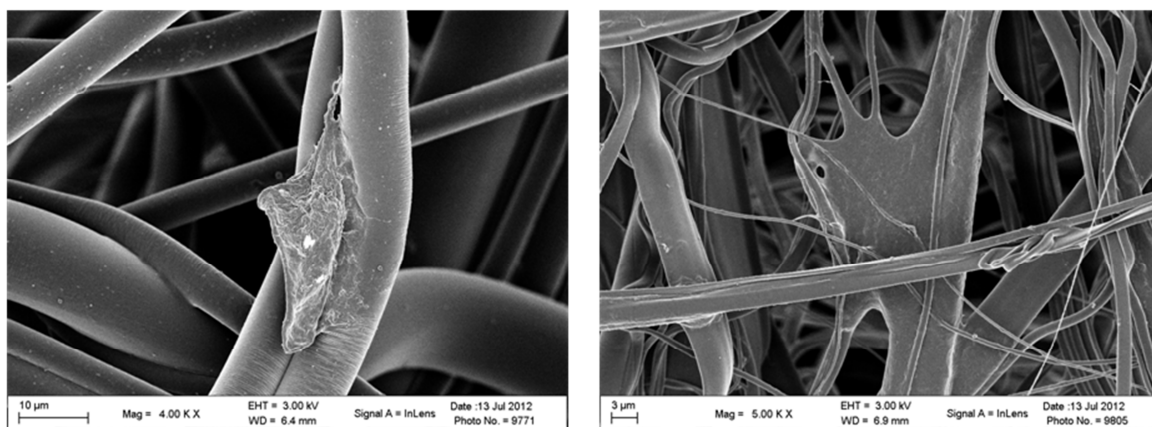


Figure 1. (Left) Single cell attached to a micron sized fiber. (Right) Single cell spread out over multiple nano-sized fibers

1.1.2.4 Biocompatibility

When choosing a fabrication process that will create a scaffold with desirable properties it is important to consider its effect on the biocompatibility of the final product. Several scaffold fabrication techniques require the use of toxic solvents that can be released after implantation and cause harmful effects. The fabrication technique used can also alter the rate of degradation of the scaffold material. Degradation rate depends primarily on the polymer properties such as, molecular weight, chemical composition, poly dispersity index, and microstructure, but processing conditions can dramatically affect the rate of decomposition by inducing premature degradation during processing. Also, the scaffold architecture, i.e., micro or nano-structure can change the rate of degradation of a material.

1.1.3 Scaffold Fabrication

There are many available techniques for the fabrication of TE scaffolds, but only those most prevalently used will be discussed here including, solvent casting and porogen leaching, thermally induced phase separation, solid free form fabrication, gas foaming, and electrospinning.

1.1.3.1 Solvent Casting and Porogen Leaching

Solvent Casting and Porogen Leaching is used to create porous scaffolds by dispersing particles of predetermined size into a polymer solution. The particles dispersed can make up a wide range of materials including, mineral salts, organic particles like sugar, paraffin spheres, or ice particles. The solution is then poured into a mould and cast. The solvent used to create the polymer solution is removed by air or vacuum drying. Finally, the added particles are leached out by selective dissolution leaving a porous material. This is a valuable technique because the pore size may be changed with ease, it is relatively simple, and allows for a maximal obtainable pore size of 500µm and 90% porosity. The disadvantages of this technique are that there can be lingering particles in the final scaffold that were

not removed by leaching, the chance of having residual solvent, and poorly connected and irregularly shaped pores. Also, due to gravity the pore distribution throughout the whole scaffold is only uniform in very thin cast scaffolds less than a few millimeters thick^{1, 10}.

1.1.3.2 Thermally Induced Phase Separation (TIPS)

As the name implies, TIPS uses thermal energy as the driving force to induce a phase separation in a polymer solution or a polymer solution with bioactive ceramic particles. This method is commonly used with biodegradable polymers such as, PLA or PLGA. The phase separation induced can be either solid-liquid or a liquid-liquid and is achieved by lowering the temperature¹⁰. The solvent found in the solvent rich phase is then removed by sublimation and a highly porous structure is formed. This process is advantageous in that it can create highly porous structures in a short amount of time that display good interconnectivity and can be tailored relatively easily. Changing the processing parameters only slightly such as, polymer concentration, solvent/nonsolvent ratio or the thermal quenching method will significantly alter the morphology of the final structure¹¹. As with the solvent casting technique, TIPS uses solvents that may be toxic to biological tissue and cannot always be fully removed from the finished scaffold.

1.1.3.3 Solid Free Form Fabrication

Solid Free Form Fabrication is a relatively new technique that uses a 3-dimensional printer to layout software designed porous structures layer by layer to its designers specifications. Conventional CAD software, such as SolidWorks, can be used to design the scaffold¹². The scaffold is assembled from the bottom up and can take on very complex architecture. Unfortunately at this time the technique is not capable of producing nano-sized features and is relatively expensive.

1.1.3.4 Gas Foaming

Gas foaming is a technique that utilizes the properties of super critical CO₂ in order to produce microporous scaffolds from biodegradable polymers such as PLA, PGA and PCL. Super critical CO₂ has a density similar to that of liquid CO₂, but still maintains a gas like velocity. This allows it to be a powerful solvent with a high rate of diffusion. The technique is employed by dissolving super critical CO₂ into a powder comprised of solid ground polymer at high pressure. The subsequent mixture is a low viscosity liquid. When the pressure in the chamber is released two events occur. First, the solubility of the CO₂ in the polymer decreases substantially, and second the super critical CO₂ undergoes a phase transition from liquid back to gas. This causes the development and growth of CO₂ bubbles that generate pores within the solidifying polymer. The gas is evacuated from the polymer melt as its viscosity increases with decreasing content of CO₂¹. The size and abundance of pores can be controlled by altering the amount of super critical CO₂ and by regulating its rate of escape from the solidifying mixture. This method works very well for creating porous scaffolds and its effectiveness is highlighted by the fact that it does not use any solvents that may be toxic to mammalian cells. Still, the method does have its draw backs in that pore interconnectivity is often subpar due to the rapid escape of CO₂ from the mixture and phase separation techniques generally create a non-uniform pore size distribution⁴.

1.1.3.5 Electrospaying and Electrospinning

Countless articles and reviews have been published citing the effectiveness of electrospinning in the fabrication of porous scaffolds for tissue engineering. Still, the fundamentals of electrospinning will be reviewed here in order to highlight its similarities and contrast its differences to meltblowing non-woven tissue scaffolds. A typical electrospray setup is comprised of a syringe pump containing a dilute polymer solution, a positively charged syringe needle tip, high voltage power supply, ground, and negatively charged collector plate. Figure 2 contains a schematic of the typical setup. When the collector is rotating

the process is termed electrospinning rather than electrospraying. High voltage is applied to the system and when the cohesive force between the polymer solution and the needle tip is overcome by the electrostatic force between the needle tip and the collector plate, a jet of polymer solution moves towards the collector plate. On its way to the collector plate the fluid jet is accelerated and stretched and simultaneously the solvent evaporates from the system⁴. More than 200 different types of synthetic and natural polymers have been used to successfully create fibers ranging in diameter from a few nanometers to several microns¹. The fiber properties can be controlled by both the processing parameters and the solution parameters. Processing parameters include applied voltage, polymer solution flow rate, and capillary collector distance. Solution parameters include solvent vapor pressure, molecular weight of the polymer, polymer concentration, solution viscosity, and solution conductivity.

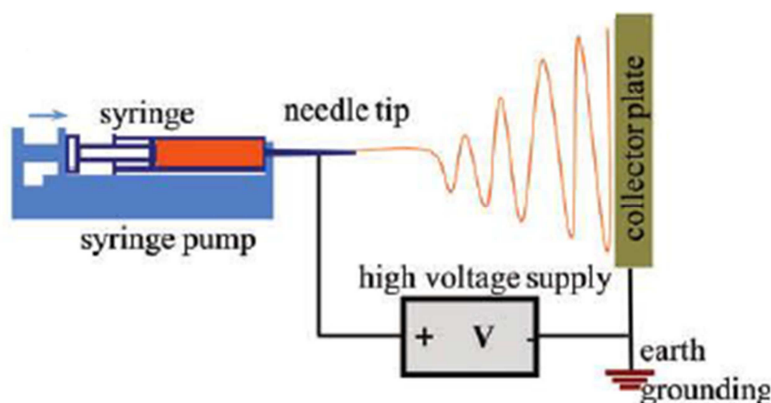


Figure 2. Schematic illustration of a standard electrospinning setup (adapted from Hong et al. 2011)

An initial increase in the applied voltage will cause a decrease in the fiber diameter, but continued increase will result in an increase in the fiber diameter. An increase in the flow rate and/or concentration of the polymer in solution will cause an increase in the fiber diameter. Moving the collector plate further away from the needle tip will result in a decrease in fiber diameter, as well as increasing the solution conductivity. These guidelines need to be taken as blanket statements because each polymer solvent system will respond differently⁴. The largest problem associated with

electrospinning is controlling both the porosity of the non-woven mat as a whole, as well as the porosity and morphology of the fibers themselves. The solvent evaporation rate will affect the pores located in the fibers, and even small changes in things such as ambient temperature and humidity can have a large effect of the morphology and porosity of the individual fibers. Despite these downfalls, many novel solutions have been thought up to tailor the properties of electrospun scaffolds including alteration of the nozzle configuration, using two nozzles simultaneously, and collector plate changes in size and shape and speed of take up.

1.2 Meltblowing Non-Woven Porous Scaffolds

1.2.1 Meltblowing Basics

Meltblowing is a one step process that converts solid polymer material directly into fibers. The process is completed by loading polymer pellets into a hopper that are run through an extruder, which ultimately forces the polymer melt through a die. At the die exit the molten polymer is taken up by forced hot air and deposited onto a collector at high speeds. During this stage the polymer streams are rapidly attenuated by the drawing force created by the high velocity air¹³. The typical setup for this process can vary in size from a bench top model to industrial production standards which can produce webs at speeds up to 30,000m/min. A typical melt blowing setup at The University of Tennessee is presented in figure 3, while figure 4 contains an image of a MB die as wells a schematic of the inner die features. Dies are typically created with a series of micro or nano-sized holes numbering from tens of holes to half a thousand across the die width.

In meltblowing the fibers have motion in 3-space, meaning that not only are the fibers moving towards the collector in the z-direction, but they also possess vibrational motion in the x and y-directions. These motions cause a web structure to be formed due to the fibers entangling within 1cm of the die exit¹⁴. Close to the die exit the fibers are placed under an extreme amount of force as they are

accelerated from a standstill, to the velocity of the forced air in only a few centimeters. When the fibers reach their maximum velocity, which is consequently equal to the air velocity at that time, a zone known as the stopping point is reached. The stopping point is generally 8-10cm away from the die. At the stop point the stress generated in the fibers by their rapid acceleration goes to zero, with the maximum stress occurring in the fibers at a distance of about 2cm from the die¹³.

Shielding Gas Hopper Extruder Metering Pump Die Collector



Figure 3. Photo capturing a typical meltblowing setup at The University of Tennessee, Knoxville

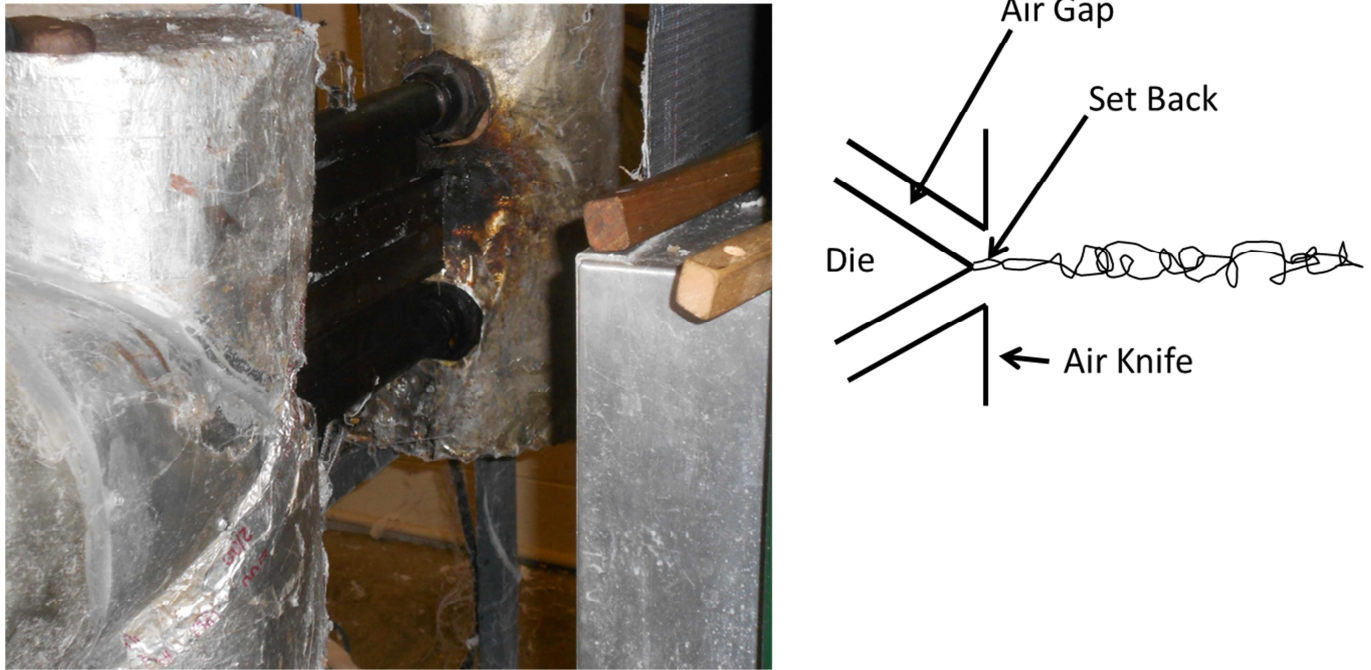


Figure 4. (Left) Photo capturing the MB die. (Right) Schematic representation of the inner workings of the MB die.

It is important to note that over this range of distance the air velocity decreases by several orders of magnitude, while the fiber velocity only increases to a few m/s. Figure 5 shows how the fiber velocity and air velocity are inversely proportional to one another until the stopping point is reached.

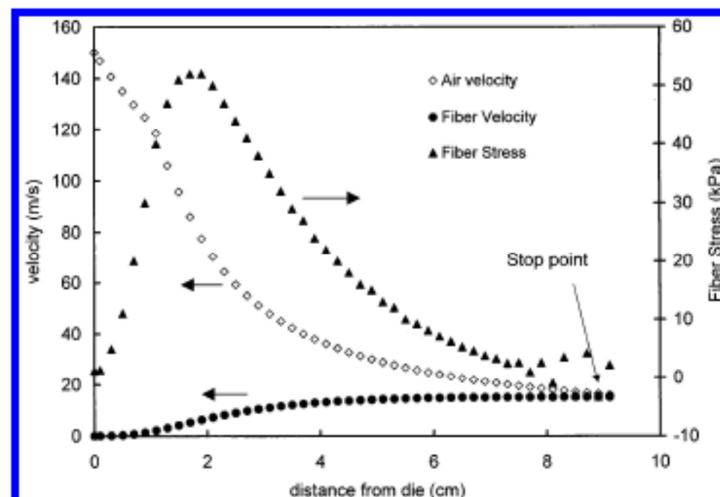


Figure 5. Computational model of the stopping point in melt blown polypropylene (Marla et al 2003)

The fibers remain in the molten state until they contact the collector, therefore processing conditions such as throughput, air flow rate, die to collector distance (DCD), and die hole diameter all have a large effect on the final web structure produced. The two processing variables that will be most vigorously discussed are air flow rate and DCD, and their effect on pore size, fiber diameter, fiber entanglement and fiber orientation. It is important to note that although each parameter will be discussed in its own section, the properties of the final web are ultimately linked to all of these parameters, and reaching the target goal for one property will alter the values of the others. For example, it is difficult to discuss the changes in pore size without mentioning its affect on fiber diameter and fiber entanglement. Initially, it is important to describe the concept of basis weight (BW) as the measure of the amount (g) of material being laid down per unit area. The larger the BW, the more material is present per square meter.

1.2.2 Fiber Diameter

Researchers have shown that in general the fibers produced via MB do not solidify until they are laid down on the collector. Techniques employed to investigate this occurrence include the use of online infrared cameras, fiber shrinkage monitoring, and crystallinity studies using online X-ray characterization¹⁵. Therefore, it is possible for the diameter of the fibers to change throughout their entire journey from die to collector. As the majority of the stress imposed on the fibers occurs 2-4cm from the die it is obvious that the majority of fiber attenuation occurs here. However, it has been observed that fiber diameter continues to change, albeit minimally, at large distances away from the die¹³. From this it is suggested that there is another mechanism, besides aerodynamic drag, that is at work in fiber attenuation. The current consensus is that fiber attenuation occurs due to both aerodynamic drag and fiber-fiber collisions during the MB process¹⁶. On the contrary to a decrease in fiber diameter at large distances, fiber diameter may increase during MB by fiber-fiber fusion.

1.2.2.1 Aerodynamic Drag

At the die exit the fibers experience large aerodynamic drag forces in the direction of the collector and the transverse direction. Coinciding with this, the fibers are also at their maximum temperature as they leave the die face. This makes the distance of the first few centimeters the prime area for fiber attenuation, and both online measurements and computational simulations have shown this to be the case^{13, 16}. The amount of parallel force (force in the machine direction) exerted on the fibers by the air velocity was modeled by Marla et al as¹³;

$$F_{PAR} = C_f \rho_a v_{a,eff,PAR}^2 \pi d_f L_f \quad (1)$$

where;

- L_f is the length of the fiber unit;
- C_f is the drag coefficient ($C_f = \beta Re_{DN}^{-n}$);
 - β is the Matsui Coefficient which is the relationship between the drag coefficient and the Reynold's number determined empirically from collected data¹⁷
 - Re_{DN} is the Reynold's number based on fiber diameter and the component of the air velocity perpendicular to the fiber axis
- ρ_a is the density of the air (kg/m^3);
- $V_{a,eff,PAR}$ is the component of the effective air velocity parallel to the fiber axis (m/s)
- d_f is the diameter of the fiber (μm).

Similarly, the amount of force exerted onto the fibers in the perpendicular direction is;

$$F_N = C_N \rho_a v_{a,eff,N}^2 \pi d_f L_f \quad (2)$$

As can be seen from both equations, the largest contributor to force acting on the fibers in either direction is the squared velocity component. Experimental data has verified that the velocity component is the largest contributor to fiber diameter reduction. An increase in air velocity of 100cfm has shown to decrease mean fiber diameters by $1\mu m$ ¹⁶. This trend makes sense as increasing the air velocity increases the amount of stress experienced by the fibers near the die exit. Figure 6 shows that doubling the air velocity can cause a 4 fold increase in the stress experienced by the fibers.

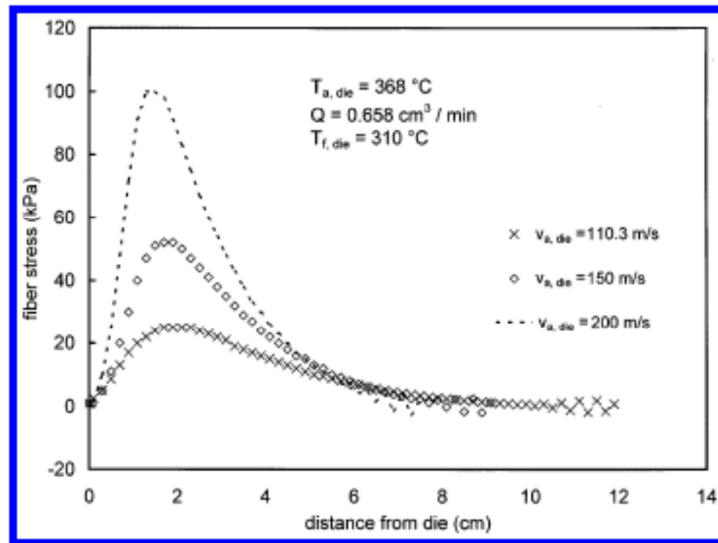


Figure 6. Computational modeling of the effect of air velocity on the stress generated in MB fibers near the die exit (Adapted from Marla et al. 2003)

However, it should be noted that this effect levels off when air flow rates are increased above 500cfm, and in some cases are reversed when the DCD is close. The most likely explanation for such an observance is that the fibers are reaching the collector before they have a chance to fully reduce in diameter. Although most fiber attenuation occurs within a few centimeters of the die, the fibers are still molten as far as 80cm away from the die, which allows for changes in diameter to still take place¹⁶. Increased air flow rate at shorter DCDs produces more turbulence in the air flow near the collector which in turn increases fiber divergence near the collector. This phenomenon can be visually observed when melt blowing at close DCDs by the increase in the spread of loose fibers at the edges of the collected webs. Increased divergence reduces the effects of fiber reduction by an occurrence deemed fiber contact.

1.2.2.2 Die-Collector-Distance

Researchers have shown that increasing the DCD results in a reduction in fiber diameter when operated under the same air flow and throughput rate¹⁶. As webs are frequently collected beyond the stopping point, it is safe to say that the increased reduction in fiber diameter seen as DCD increases is due to

fiber-fiber interactions. The actual decrease in fiber diameter with an increase in DCD is small, on the order of a few tenths of a micron for every 10cm the collector is moved away from the die. However, changing the DCD can affect the fiber size diameter distribution by increasing the number of fine fibers to coarse fibers. Also, increasing the DCD will increase the size of the largest fiber diameter present. Essentially, as the DCD increases there is more time available for fiber-fiber interactions which can lead to fiber acceleration or fiber fusion.

1.2.2.2.1 Fiber Diameter Reduction due to Fiber-Fiber Interactions

As stated in the above section, the fibers reach the stopping point only a few centimeters from the die, but diameter attenuation continues at distances out to more than 80cm away from the die. Obviously there must be another factor that affects fiber diameter other than primary air flow rate. One plausible explanation is that fiber-fiber contact can cause acceleration of one fiber and lead to further elongation of that particular section of the fiber. Fiber-fiber contact is almost inevitable in the MB process as the fibers are moving in 3-dimensions and webs begin to form very close to the die exit. The web structures produce constrained sections of fiber and visual observations show obvious changes in fiber diameter on the same fiber between points of contact with constraining fibers. Figure 7 contains examples of such occurrences.

1.2.2.2.2 Fiber Diameter Size Increase by Fiber-Fiber Contact

Fiber-fiber interactions may also have the effect of increasing fiber size diameter due to fusion between the two molten streams of polymer. It is interesting to note that finer fibers have less of a chance to coalesce than coarse fibers at the same distance away from the die exit. The reasoning here is that fine fibers have less thermal inertia and cool faster than coarse fibers even though they also have less

surface area than the coarse fibers. Figure 8 shows nano-fibers wrapping around micro fibers and two micron-sized fibers fusing together.

1.2.2.3 Mass Throughput

Fiber diameter is affected by mass throughput. At any distance from the die where fiber attenuation occurs the diameter of the fiber will be larger for larger mass throughput compared to small mass throughput at the same location¹⁵. Although the increase in fiber diameter is not large, in fact the most substantial change is a shift to larger fiber size distribution rather than actual diameter increase (reduction in the number of fine fibers), the mechanism behind this is not fully understood. The data available shows that an increase in throughput rate keeps the fiber temperature hotter over a longer distance away from the die. Taking into account Newton's second law of motion reveals that the more massive fibers undergo less acceleration than smaller fibers do, when exposed to the same air velocity. Coupling these two ideas together creates the perfect storm to increase fiber diameter, as there is more mass force to be accelerated, and there is more heat over a longer distance to allow them to coalesce together.

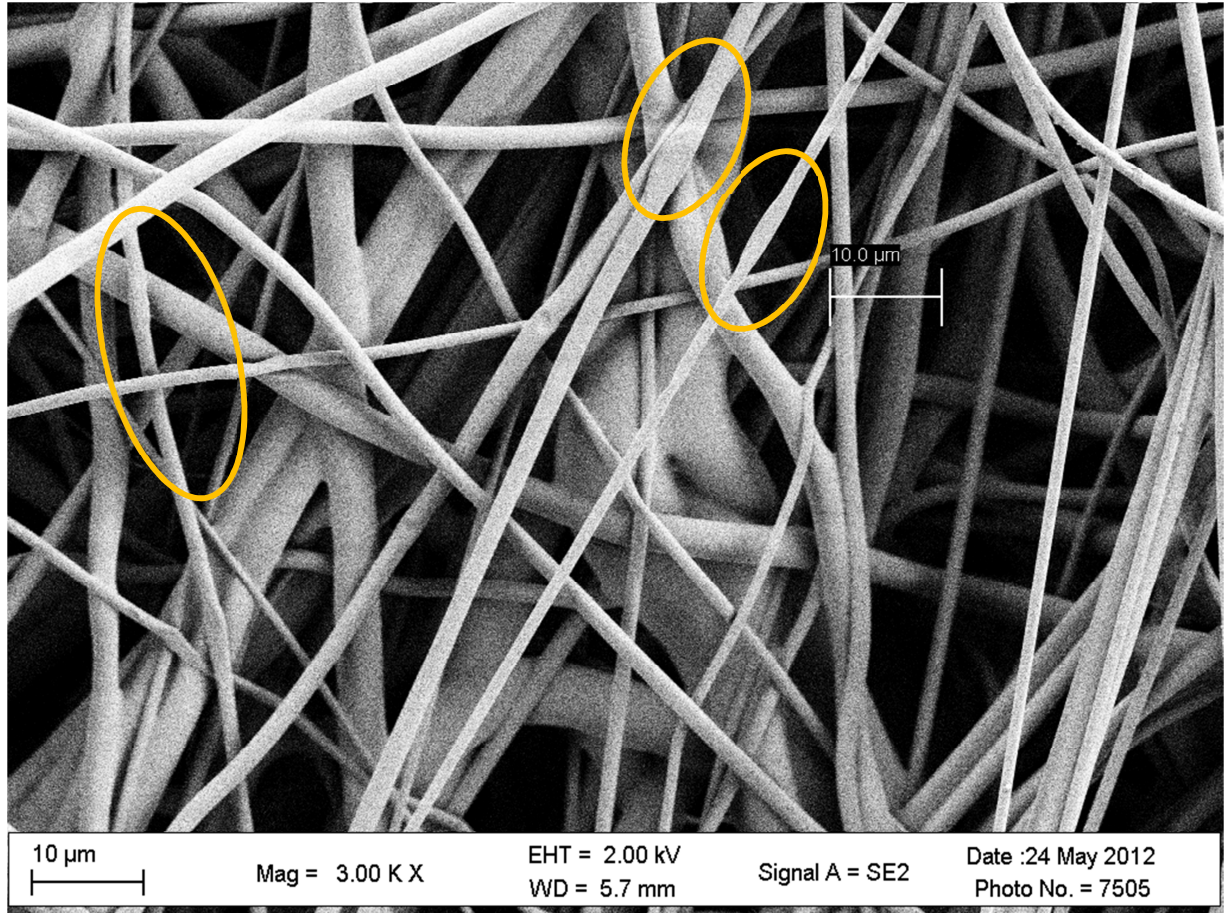


Figure 7. Electron Micrograph of an entangled MB web with highlighted areas of fiber-fiber contact points and changes in fiber diameter

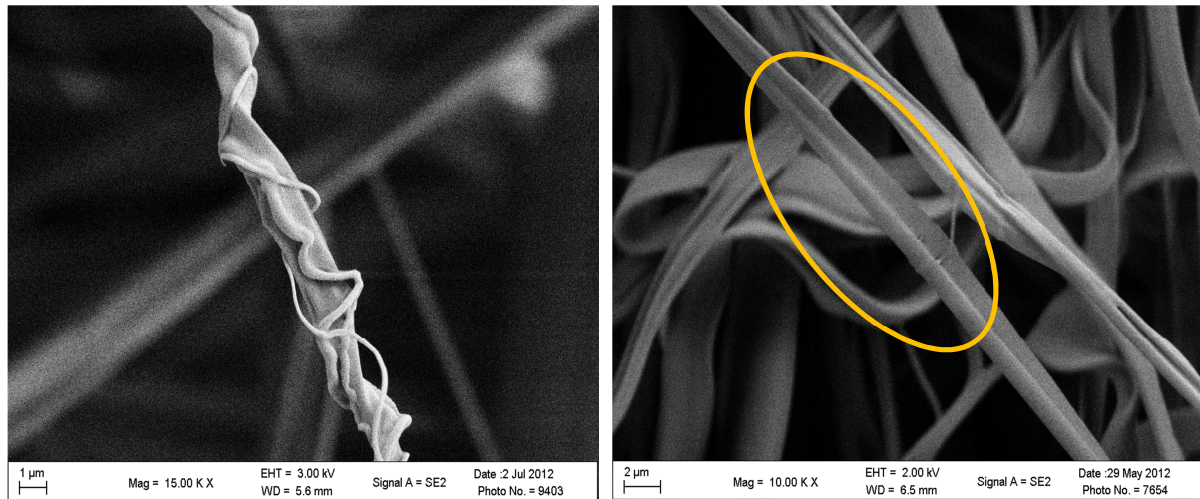


Figure 8. (Left) Fine fibers wrapping around larger diameter fibers. (Right) Fusion of two larger diameter fibers

1.2.3 Fiber Entanglement

Fiber entanglement occurs in the MB process due to drag forces imposed upon the fibers as they travel from the die to the collector. As mentioned above, the aerodynamic drag force is high at the die and at the collector. The result of fiber entanglement is an increase in the size and number of fiber bundles. Fiber entanglement is heavily dependent on the DCD. At all DCDs the percentage of fine fiber bundles out of the total population is always substantially higher than the percentage of coarse fiber bundles (larger diameter). As the DCD is increased, the percentage of fine fiber bundles decreases significantly, while the percentage of coarse fiber bundles increases slightly. A plausible explanation for such a trend is that close to the die exit the fibers are spaced relatively close to one another, the variance in spacing is dependent on the die architecture. The high density of fibers allows for easy entanglement to occur close to the die. As entanglement occurs and the fibers move away from the die, the probability of encountering an adjacent fiber decreases as the fibers and fiber bundles are now spaced further apart from one another. As DCD increases there is more distance allotted for possible fiber bundle-fiber bundle interaction, and when two or more bundles come together to form a coarse bundle the percentage of coarse bundles increases slightly, largely at expense of fine bundles. The increase in DCD also allows more time before collection for the entanglement of single fibers. Studies have shown that the mean fiber bundle size increases with DCD according to a second order polynomial that levels off as DCD becomes >40cm. Similarly, the maximum bundle diameter increases with DCD and levels off quickly as DCD increases¹⁴.

1.2.4 Fiber Orientation

Studies have shown that fiber orientation in MB webs prefers the machine direction (MD) compared to the transverse direction (TD)¹⁴. A logical explanation for this preferred orientation is that the direction of

air flow at the collector is in the MD. Fiber orientation in MB webs is measured based on the amount of fiber cross sectional area aligned in each direction, rather than on the alignment of individual fibers. This method of practice has been employed due to the large variations in fiber diameters. The ratio used to determine fiber orientation is the amount of fiber area aligned in the MD compared to the amount that is oriented towards the TD. A ratio of 1 represents isotropic fiber orientation, while a ratio greater than 1 shows preferred alignment in the MD.

Fiber orientation varies with DCD and air speed. As DCD increases fiber orientation decreases linearly to a ratio >1 . The mechanism behind this change in orientation is based on the drag forces imposed upon the fibers during the MB process. The air speed in the MB process is high near the die, while the fiber speed is relatively low causing an increase in aerodynamic drag. At the collector the air speed is still fast and the fiber speed decreases back to zero as it is laid down on the collector, resulting in high aerodynamic drag. The magnitude of the drag force at the collector is produced by the speed of the air at the collector. As the DCD is increased the speed of the air arriving at the collector decreases and the resultant drag force is also reduced. This allows the fibers to lay down in a more random pattern and reduce fiber orientation in the MD¹⁴.

1.2.5 Pore Coverage, Pore Size and Pore Shape

Pore coverage is the percentage of the total area of a web that is covered by pores. The total web coverage is the sum of the pore coverage plus the fiber area coverage. Total pore coverage is heavily affected by the air flow rate at any given DCD. When the air flow rate is low the overall pore coverage increases, while when the air flow rate is high, the total pore coverage decreases. The explanation for this change in pore coverage can be attributed to the change in fiber diameter with air flow rate. Low air flow rates produce larger diameter fibers, while high air flow rates generally produce finer fibers. Finer fiber diameters will increase the amount of fiber coverage for the same basis weight and will decrease

both the diameter of each individual pore and overall pore coverage. The effect of varying the DCD on pore coverage is heavily influenced by the air flow rate. At low air flow rates, an increase in DCD results in more fiber entanglement. More fiber entanglement reduces the amount of fiber coverage, which in turn increases the amount of pore coverage. At high airflow rates the reduction in fiber diameter increases the fiber coverage area so much that any effect produced by fiber entanglement is negated and the pore coverage decreases¹⁴.

Pore size follows the general trend of decreasing with an increase in air flow rate at any DCD. DCD has a small influence on pore size in that the pore size increases linearly with increasing DCD, but this effect is overshadowed by the influence of air flow rate. For example, doubling the DCD will cause an increase in pore size by a factor of approximately 1.3, while doubling the air flow rate will cause a decrease in pore size by a factor of 2 at the same DCD. This observation can be related back to fiber cover where it is expected that increasing the DCD will reduce fiber coverage, but increasing the air flow rate will greatly increase the fiber coverage. It should be noted that BW plays a large role in pore size and coverage. At very low BW, the pore size will increase due to the lower amount of fiber coverage necessary to reach the target weight. At high BW, many layers of fibers need to be laid down in order to reach the target weight and the pore size will decrease due to layering.

Pore shape is based on the aspect ratio of length to width. Pore shape and orientation are based on their relationship to the aspect ratio of a circle, which is 1. From the studies investigated, the pore aspect ratio was never less than one, and increased linearly with increasing DCD independently of the air flow rate used. This suggests that individual pores are always elongated regardless of DCD. This can be explained by examining webs on a local basis rather than the entire web as a whole. It has been demonstrated that globally fiber orientation in the MD decreases with increasing DCD, but as DCD increases so does fiber entanglement. Careful microscopic investigation reveals that entangled bundles

of fibers tend to lay down in a linear manner on a local scale, consequently leading to more elongation in pore shape as DCD increases. Coinciding with this, as DCD decreases, the orientation of the pores prefers the MD; while at higher DCDs the pores become disoriented and are laid out in a more random fashion. This data heavily follows the trend of fiber orientation in the MB process¹⁴.

1.3 Poly(lactic acid) (PLA)

1.3.1 Basic Information

PLA is biodegradable aliphatic polyester that can exist in either the syndiotactic (D,L-) or the isotactic (L,L-) forms. The (L,L-) form is a semi-crystalline polymer having around 36% crystallinity, while the (D,L-) form is a very low crystalline or fully amorphous polymer. Figure 9 shows the L- and D- enantiomers of the monomer unit, lactic acid, their dimer ring structures, and the two different mer units of PLA itself. PLA has acquired increasing interest in the last 10-20 years because it is a renewable polymer resource being produced from starch rather than fossil fuels, like conventional hydrocarbon based polymers. Not only is it a biodegradable polymer, but it is also a biocompatible polymer as its degradation products are naturally found and recycled in human biological processes via the Citric Acid Cycle. Due to its unique properties PLA has found many uses in the packaging industry as disposable containers, the agricultural industry as biodegradable mulch, the waste disposal industry as filter material, and the medical industry for biocompatible and bioabsorbable implants.

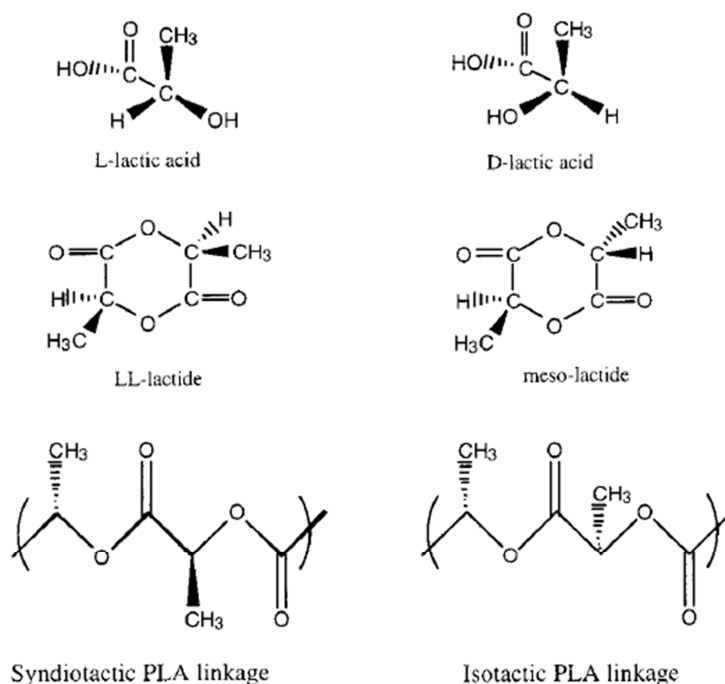


Figure 9. Chemical structures of PLA and its constituent monomers

1.3.2 Production

PLA is produced from 100% renewable starch rich vegetables such as beet roots or potatoes¹⁸. Industrially this process is completed by a microbial carbohydrate fermentation process due to its chemical and economical feasibility compared to pure chemical synthesis. The starch is removed from the plant material and converted to glucose by enzymatic hydrolysis. The glucose is then broken down and used by the microbes for energy with one of the byproducts of cellular respiration being lactic acid. The precursor reagents, lactic acid or lactide, needs to be of high quality in order to prevent enantiomeric impurities from being incorporated into the final product. Impurities affect both optical purity and the rate of degradation. The fermentation vat contains impurities such as organic acids, alcohols, lactates (methyl, ethyl and butyl), amino acids (alanine, serine, glutamine and glycine), protein fragments from bacteria and metabolic intermediates¹⁹.

PLA can be produced via step-growth polymerization from lactic acid, or by ring opening polymerization (ROP) using the dimer lactide. Both methods lead to the production of high molecular weight and high purity PLA. In step growth polymerization of PLA, the total amount of impurities in the lactic acid precursor should be <.05 mol%. The optical purity of lactide used in ROP significantly affects the properties of the PLA produced. Enantiomeric impurities in lactide can originate from the lactic acid used in its creation, or they can be created during the melt condensation reaction used to create the lactide. Fortunately, ROP can yield long isotactic PLA even in the ROP of racemic lactide. Impurities present in PLA will show up as dark char during melt processing.

1.3.3 Processability

PLA is a thermoplastic that can be melt processed into a variety of shapes via injection moulding, film blowing, or melt spinning. The T_m^0 of PLA is 207°C, but for high molecular weight PLA the T_m is between 170-180°C. The lower melting temperature is due to enantiomeric and other impurities that lead to imperfect crystals. Obviously these processing temperatures are well above the degradation temperature of PLA in air, so care must be taken to remove air from the processing environment. Researchers have found that under an N₂ atmosphere PLA can resist any significant volatilization during dynamic heating up to almost 250°C²⁰. Isothermal weight loss studies have shown that under N₂ gas PLA has a small processing time window above its T_m where little to no weight loss will occur. One such study showed that at both 150°C and 200°C PLA did not undergo any significant degradation up to 30 minutes of exposure time, and had a mass loss of 0.4%²¹. The study was conducted under a controlled of moisture level of 0.23%. This translates to PLA having a short residence time for an extrusion processes under normal industrial conditions. Coinciding with this the viscoelastic properties of PLA shift dramatically with temperature. The melt flow rate (MFR) of their PLA increased from 4.2cm³/10minutes at 150°C to 8.6cm³/10minutes at 200°C. Researchers confirmed using size exclusion chromatography

(SEC) that the increase in MFR was due to thermal degradation of PLA as the PDI increased from 1.75 to 1.98²¹. The increase in PDI is most like due to chain scission as discussed in 1.3.6.

Drying PLA prior to processing is a must. Both external studies and those from our PLA's manufacture have shown that even low levels of moisture content (>50ppm) in PLA will increase the rate of degradation during extrusion and lower the amount of allowable residence time^{22, 23}. The molar mass distribution of PLA affects its processability, mechanical properties and degradation characteristics, glass transition and melting temperature¹⁹. It is important to avoid degradation during processing as it causes a decrease in the plastic flow ability and an increase in brittleness of the material.

Understanding the rheological properties of PLA is essential for properly processing it. PLA has very poor thermal stability compared to fossil fuel based polymers. Also, PLA has a poor melt elasticity compare to polyolefins which means that there is a very narrow temperature range over which it may be processed. This is caused by its low entanglement density. The critical entanglement molecular weight for PLA is 16000g/mol at an entanglement density of .16mmol/cm³ at 25°C. Rheological work previously conducted has shown that G' and G'' do not cross in dynamic shear experiments conducted at a controlled strain of 5% up to 1000rads/s²⁴. One researcher used a high M_w isotactic PLA (750Kg/mol) and did not observe a crossover point between G' and G'' at shear rates over 1000rad/s²⁵. The strain rate was set at 5% to insure that the experiments were run in the linear viscoelastic region of PLA. The viscoelastic properties of PLA are also affected by processing condition. In general, PLA melts behave as non-Newtonian fluids and their changes in viscosity with shear rate follow a power law;

$$\eta = m\dot{\gamma}^{n-1} \quad (3)$$

where η is the flow index, m is the consistency parameter and $\dot{\gamma}$ is the shear rate. For most PLA melts when $n < 1$ the viscosity decreases as a function of shear rate¹⁹. It is important to note that multiple studies have shown that PLA will exhibit Newtonian viscosity up to a shear rate of 10rads/s^{24, 26}, but will

exhibit a sudden and sharp decrease in viscosity of more than one order of magnitude as shear rate increases²⁵.

The elongational viscosity of PLA increases two orders of magnitude from 10^4 to 10^6 when subjected to a strain rate of .1/s. Extension thickening occurs when the rate of deformation is considerably larger than the time necessary for molecular relaxation. In the case of PLA, researchers found that the logarithmic strain exceeded a value of 10 for PLA before break²⁵.

1.3.4 Other Properties of PLA

- The enthalpy of melting for 100% crystalline PLA is 93.1J/g. 100% crystalline stereocomplexes may have enthalpies of melting as high as 140J/g due to different crystal structures^{19, 27}.
- The plateau modulus of PLA is around $5-5.5 \times 10^5 \text{ Pa}$ ^{25, 24, 26}.
- The melt viscosity of high molar mass PLA is 5000 to 10,000 poise ($500-1000 \text{ Pa}\cdot\text{s}$) at shear rates of 10 to 50 s^{-1} ¹⁹.
- The zero shear viscosity increases with $M_w^{3.7-4.0}$ due to the steric hindrance between PLA chains^{19, 25}
- The melt density of PLA decreases from 1.090g/cc at 220°C to 1.065g/cc at 250°C²³

1.3.5 Hydrolytic Degradation and Degradation Products

The hydrolytic degradation of PLA occurs in two steps, the first is the infiltration of water into the amorphous zones of the polymer where it causes random rupture of the ester bonds. These ruptures convert the long chain molecules into shorter water soluble fragments. The second stage is the erosion of the crystalline domain surfaces once most of the amorphous zones have been depleted¹⁸. The amorphous region degrades faster due to the slower rate of diffusion of water into the crystalline region. In most biodegradable polymers the diffusion rate of water into the bulk of the polymer is much faster than passive hydrolysis. Therefore it can be assumed that as the water is everywhere inside of the polymer and that hydrolysis occurs uniformly throughout the sample.

Hydrolytic degradation in PLA is typically acid catalyzed. The first step is the attack at a random carbonyl carbon contained within the ester group by a hydronium ion. The hydronium ion will temporarily bond to the tertiary carbon creating a transition species that contains a positively charged oxygen radical. As the oxygen molecule does not like to have a positive charge, it will donate one of its two hydrogens to the nearby ester oxygen linkage and reclaim a few electrons to return to a net zero charge. At this point the oxygen associated with the carbonyl group will donate its free pair of electrons back to the carbon atom, reestablishing a double bond and completing the scission of the ester bond. This whole process comes at the addition of one hydron, an alcohol end group, and a carboxyl end group. These molecules are known to reduce the local pH, accelerate further degradation, and cause inflammation at the site of the scaffold implant¹⁹. Ultimately the pH of the solution will become more acidic if the degradation reaction is allowed to continue. Figure 10 contains a schematic cartoon of the process. The rate of degradation was modeled by Farrar and Gilson in 2002 as;

$$\frac{dC}{dt} = kEwC = \mu_m C \quad (4)$$

where E, C and w are the concentrations of ester groups, carboxyl groups and water in the polymer matrix, k is the hydrolysis kinetic constant, and μ_m is the hydrolysis rate²⁸. The hydrolysis kinetic constant is based on the probability of molecular scission and it is employed under the assumption that water is uniformly distributed throughout the specimen, or in other words the scission process is not diffusion controlled. As it is a thermodynamic quantity it is dependent on temperature, pressure and pH. An increase in temperature will increase the amount of initial energy available to the system and consequently the probability of an ester bond scission. Raising the pH will force the hydrolysis reaction to the right as its product will work to neutralize the basic environment. Applying load the piece of

material will also increase the rate of degradation as less volume to occupy will increase molecular collisions.

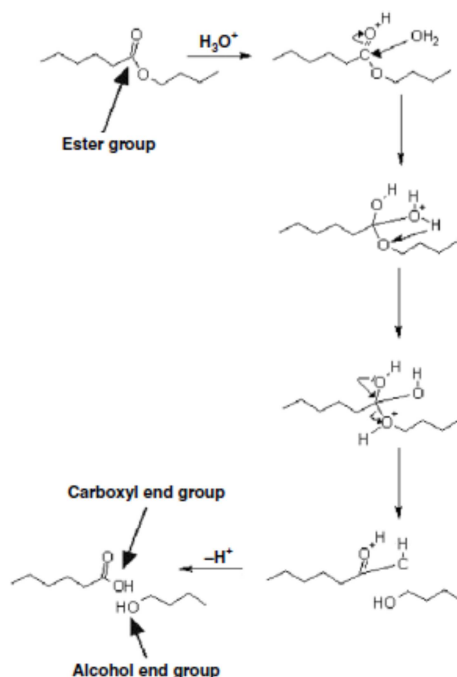


Figure 10. Schematic cartoon representing the hydrolytic degradation of one ester bond in a PLA chain

1.3.6 Thermal Degradation and Products

Studies have shown that in air PLA undergoes thermal oxidative degradation in a single stage process that produces lactide as the decomposition product. PLA will begin to degrade in air at temperatures as low as 70°C as the activation energy for degradation has been calculated to be 22.25kcal/mol in air²⁹. The degradation begins with rapid decrease in molecular weight which then levels off to a more controlled rate of decay. The chemistry of this degradation is similar to that seen in figure 10, but instead of being catalyzed by an acid or base, heat encourages the rupture of the unstable ester bonds. The rupture occurs between the carbonyl-carbon and the oxygen molecule in the ester bond. Observations of this proposed degradation have been seen using UV spectroscopy to look at the change in electron charge distribution, and the increase in melting temperature of thermally oxidized PLA

caused by the increase on -COOH end groups³⁰. The proposed mechanism for this display of sudden molecular weight decrease is the presence of weak bonds within the material capable of undergoing scission first³⁰. After scission occurs at the weak bonds there is an equal probability of scission occurring at that any of the remaining ester bonds. The decrease in rate of weight loss substantiates this claim as the mechanism of degradation changes from random weak bond cleavage to chain end depropagation. This bond cleavage decreases the number of ester linkages in the polymer and increases the number of carboxyl chain end groups, both of which raise the melting temperature of the degraded polymer and cause a decrease in flexibility.

PLA degradation temperature is decreased by residual catalyst, monomers and oligomers. These leftovers also increase the rate of thermal degradation. The degradation products of PLA are lactide and other ring formed oligomers of different sizes, carbon dioxide, carbon monoxide, acrylic acid, methane, ethylene, propylene, methylketene, acetaldehyde and butane-2,3-dione¹⁹.

2. Materials and Methods

2.1 Materials

2.1.1 PLA

The PLA used in this study was Inego™ biopolymer 6252D, purchased from NatureWorks® (Minnesota, USA). The physical properties of this polymer are listed in table 1.

Table 1. Physical Properties of NatureWorks® PLA 6252D

Physical Properties	PLA 6252D
Specific Gravity	1.24
Relative Viscosity	2.5
Melt Index, g/10min (210°C)	70-85
Melt Density (230°C)	1.08
Crystalline Melt Temperature (°C)	160-170
Glass Transition Temperature (°C)	55-60
M _n (g/mol)	25000-28000

2.2 Melt Blowing

2.2.1 Micro Die

Melt blowing was conducted on a 6" line using an "Exxon" style die. The properties of the die as well as the processing conditions used are listed in table 2. Prior to MB, the PLA pellets were dried at 80°C for 4hrs under vacuum to remove any moisture that was present. Extrusion of the PLA was performed using a twin screw extruder (Haake Inc. Saddle Brook New Jersey, USA) under N₂ gas flow in order to prevent thermal oxidative degradation of the polymer during processing. A twin screw extruder was used in order to reduce the residence time of the PLA melt. Webs were collected at a basis weight of 20g/m² for all parameters tested. Webs were created by varying air flow rates between **1 and 35 cubic meters per minute (cmm)** and collected at DCDs of 10, 30 and 50cm for each air flow rate. Air flow rates were

changed by varying the primary air flow pressure. The pump displacement was calculated by collecting the melt blown web for 1 minute and then dividing that value by the density of the material divided by the collector speed. The throughput was calculated by multiplying the pump displacement by the density of the material multiplied by the collector speed. The basis weight was calculated according to ASTM D-3776. In brief, the web was collected for 1 minute and 10 circular discs were cut out of the center of the material and the mass of the material was recorded. The collector speed was determined by dividing the basis weight by the target basis weight and then adjusting the collector speed accordingly. Twenty meters (20m) of material were collected for each MB sample.

2.2.2 Nano Die

The same processing equipment was used to melt blow nano-sized fibers except an “Arthur G. Russel” (AGR) style die was used instead of the “Exxon” style die. The die properties and processing parameters used are listed in table 3. Due to the proprietary nature of its design, the values for the air gap, set back and die angel are not available for report. Prior to MB, the PLA pellets were dried at 80°C for 4hrs under vacuum to remove any moisture that was present. The webs were collected at 20g/m² and were blown at varying air flow rates between 1 and 5cm. Webs were collected at DCDs of 10, 30 and 50cm. 20m of material was collected for each MB sample.

Table 2. Die properties and processing parameters used for the micron hole size "Exxon" style die

Die Properties	Value
Hole Diameter (μm)	254
Holes Per Inch	20
Throughput (g/hole/min)	0.3
Die Angle (degrees)	30
Die Set Back (mm)	2.032
Die Air Gap (mm)	1.52
Processing Parameters	
Extruder Zone 1 Temp ($^{\circ}\text{C}$)	162
Extruder Zone 2 Temp ($^{\circ}\text{C}$)	210
Extruder/Die Connector Temp ($^{\circ}\text{C}$)	225
Top Face of Die Temperature ($^{\circ}\text{C}$)	220
Bottom Face of Die Temp ($^{\circ}\text{C}$)	220
Extruder Screw Speed (RPM)	30
Metering Pump Speed (RPM)	30
Collector Speed (m/min)	12

Table 3. Die properties and processing parameters used for the nano-hole size "AGR" style die

Die Properties	Value
Hole Diameter (μm)	25.4
Holes Per Inch	100
Throughput (g/hole/min)	0.025
Die Angle (degrees)	NA
Die Set Back (mm)	NA
Die Air Gap (mm)	NA
Processing Parameters	
Extruder Zone 1 Temp ($^{\circ}\text{C}$)	164
Extruder Zone 2 Temp ($^{\circ}\text{C}$)	215
Extruder/Die Connector Temp ($^{\circ}\text{C}$)	230
Top Face of Die Temperature ($^{\circ}\text{C}$)	242
Bottom Face of Die Temp ($^{\circ}\text{C}$)	244
Extruder Screw Speed (RPM)	20
Metering Pump Speed (RPM)	6
Collector Speed (m/min)	3

2.3 Characterization of MB Webs

2.3.1 Sampling Method for Determining the Physical Properties of Each MB Web

Each physical characteristic of the MB webs was determined by selecting random sections of material from the middle of the 15cm x 20m webs collected. Figure 11 provides a schematic of the three sections selected for testing. For testing that required more than three samples, subsequent samples were taken from further down the length of the web.

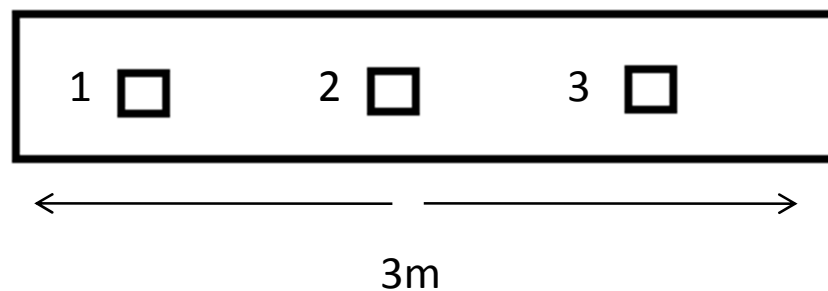


Figure 11. Example of sample selection for testing physical properties of the MB webs

2.3.2 Thickness Testing

Web thickness was determined using a Testing Machines Inc. Model 49-70 thickness tester according to ASTM D5729-97. In brief, 10 sections of the 20m MB webs were sampled for accurate determination of the webs' thicknesses and the average value was taken to as the overall thickness of the webs.

2.3.3 Air Permeability

Air permeability for each web was determined using a Textest FX3300 Air Permeability Tester according to ASTM D737-04. In brief, 10 sections of the 20m MB webs were sampled in order to determine the air

permeability of each web. The average of the 10 measurements was recorded as the air permeability for each sample.

2.3.4 Porosity and Pore Size Distribution

Porosity and pore size distribution for each web was determined using a Capillary Flow Porometer, model CFP-1100-AEX (Porous Materials Inc). As there is no ASTM standard associated with this test, 3 samples of each web were taken from the 20m produced and tested for porosity. The mean pore size and pore size distribution for each web was determined by combining the values of all recorded pore sizes from the 3 different locations and creating a histogram using JMP Pro 10 software. JMP® Pro 10.0.0 is copyrighted analytical software belonging to SAS Institute Inc.

2.3.5 Degradation Study

A degradation study for each of the MB webs was carried out over a 6 week period. Mass loss was recorded for weeks 1, 3 and 6. For each week, 3 samples from each web were taken and placed into 10mL vials in 1mol phosphate buffered saline (PBS) buffer solution at a pH of 7.4. The PBS was heated to 100°C prior to pipetting into the vials in order to ensure that no microbial contamination during the 6 week study would occur. The samples were incubated at 37°C in a drying oven throughout the entire study. Prior to loading, the dry weight of each sample was recorded using a Mettler AE240 microbalance. Upon removal from the vials for mass collection each sample and vial was washed separately 3 times with DI water. The pH of the remaining PBS solution was monitored at the 3 and 6 week intervals using Litmus Paper to qualitatively monitor any changes in pH value caused by the degradation products. The samples were dried at 37°C until the final weight reading stabilized. Weight loss was recorded according to equation 5,

$$Weight\ Loss\ (\%) = \left(1 - \frac{W_f}{W_o}\right) * 100 \quad (5)$$

where; W_f was the final weight and W_o was the initial weight.

2.3.6 Differential Scanning Calorimetry (DSC)

DSC was conducted using a Mettler Toledo Star System DSC according to ASTM D3418-08. This technique was used to follow the crystallization kinetics as a function of processing parameters and to study any effect on the degree of crystallinity of the PLA. In brief, one sample of each MB web with a mass between 5-10mg was placed in a DSC pan and heated from 30-200°C at 10°C/min. The sample was then held at 200°C for 2 minutes and cooled back to room temperature at 5°C/min. The cooling curve for several samples was also constructed by cooling from 190-100°C at 1°C/min to determine if the PLA would form a monodisperse size distribution of crystals. During all DSC experiments, 200cc/min of N_2 gas was used to purge the system. The degree of crystallinity can be calculated by;

$$\%Crystallinity = X_c = 100 * \frac{\Delta H_m - \Delta H_c}{\Delta H_m^\infty} \quad (6)$$

where ΔH_m is the measured endothermic enthalpy of melting, ΔH_c is the exothermic enthalpy given off by the formation of crystals during DSC heating, and ΔH_m^∞ is the theoretical melting enthalpy of 100% crystalline PLA. One MB sample was annealed overnight at 80°C to obtain the maximum amount of crystallinity²⁷.

2.3.7 Thermal Gravimetric Analysis (TGA)

TGA was performed on the as received material under the flow of N_2 gas in order to determine the temperature at which thermal oxidative degradation takes place in the PLA. Analysis was performed on a Mettler Toledo Star System TGA from 25-600°C at a heating rate of 10°C/min. The test was run under a flow of 40cc/min of N_2 gas. Prior to analysis the PLA pellets were dried at 80°C for 4hrs under vacuum to remove any moisture that was present.

2.3.8 Tensile Strength Testing

The tensile moduli of each sample in the parallel and transverse directions were determined according to D5035-06. A United Model# SSTM-1-E-PC Tensile Testing machine equipped with a 10lb load cell was used to conduct the tests. The load cell had a precision range of $\pm 1\%$. In brief, eight samples were cut to 5x1" from the transverse direction and 5 samples were cut in the machine direction for testing. Each sample was placed one at a time between the tensile grips maintaining a 3" gauge length. The cross head speed was set to 12inches/min.

2.3.9 Fiber Diameter Measurements

Scanning Electron Microscopy (SEM) was used to determine the diameter of the MB fibers. A Leo1525 SEM, equipped with Zeiss SmartSEM software, was used to acquire images of the MB webs for fiber diameter measurement. Four areas from each of the 20m in length MB webs were sampled to acquire approximately 100 fiber diameters for each web. ImageJ version 1.45s (Wayne Rasband National Institute of Health, USA) was used to measure the diameter of each fiber in the SEM images. JMP software was used to create a histogram of the fiber diameters and determine the mean fiber diameter and fiber diameter distribution for each web.

2.3.10 Viscosity Average Molecular Weight (M_v)

The M_v of NatureWorks 6252D PLA was determined via the measurement of the materials intrinsic viscosity. Three different concentrations of PLA were dissolved into chloroform, 1.5g/dL, 1.0g/dL and .75g/dL. A Ubbelohde viscometer was placed in a water bath at 25°C and separately each of the three concentrations was transferred to the viscometer and allowed to equilibrate to the bath temperature for 5 minutes. The intrinsic viscosity of the PLA was determined by creating a Huggins-Kraemer plot of reduced viscosity versus concentration. The Mark-Houwink-Sakurada relationship, as seen in equation 7,

was then used to determine the M_v . The K and a values for PLA were taken as 5.45×10^{-4} and 0.73 respectively³¹.

$$[\eta] = KM^a \quad (7)$$

2.4 Scaffold Selection Process for Cell Seeding

The non-woven PLA webs chosen for scaffold use were selected based on their mechanical properties. The properties of interest are average pore size and pore size distribution, the tensile modulus of the fibers, and the average fiber diameter.

The migration of epithelial cells has been studied both individually and as sheets of 10-20 cells^{5, 32}. Individual cells have the capability to produce $30.8 \pm 1.1 \text{ nN}/\mu\text{m}^2$ of tractional force, while sheets of epithelial cells can produce up to $12 \text{ nN}/\mu\text{m}^2$ at the proximal edge of the migrating cluster when placed on a substrate with a modulus of at least 150kPa. As this investigation of cell migration will focus on both individual epithelial cells and sheets of cells, the tensile modulus of the substrate chosen should be $\leq 150 \text{ kPa}$.

Nanofiber scaffolds typically produce pore sizes on the range of a few to tens of microns in diameter³³. Researchers have found that cell attachment on these types of scaffolds works well on the surface, but that infiltration into the bulk of the scaffold is not observed³⁴. On the contrary, most cells cannot cross pore gaps larger than 300 microns⁴. Therefore, scaffolds were chosen with mean pore sizes greater than 20 microns (double the average cell size), but not larger than 50 microns in the hopes that the cells could attach to both the surface and interior fibers of the scaffolds, and once attached spread as sheets across the pores.

As mentioned in the introduction, fiber diameter will affect the way in which seeded cells spread on the scaffold. Micron fiber sized scaffolds were chosen with an average fiber diameter of about $7 \mu\text{m}$, while

nano-sized fiber scaffolds were selected with an average fiber size diameter of about 600nm. The variance in fiber diameter between the micro and nano-sized fibers was purposely chosen to be about one order of magnitude to provide a stark comparison between the effect of fiber size on cellular attachment and spreading.

In all, four scaffolds were chosen to investigate the potential for cellular adhesion, migration and penetration. Two micron sized fiber scaffolds, 1.5cmm collected at 30cm and 1.5cmm feet collected at 50cm, and two nano-sized fiber scaffolds, 0.8cmm at 30cm and 0.8cmm at 50cm were the selected bunch.

2.5 Scaffold Cell Seeding

The four selected scaffolds were seeded using A375 Human Malignant Melanoma cells, generously provided by John Biggerstaff's group (Center for Environmental Biotechnology, The University of Tennessee). Prior to seeding, the scaffolds were soaked in RPMI 1640 medium containing 1% fetal bovine serum and 10% penicillin streptomycin for 48hours to ensure full saturation. A Neubauer hemocytometer was used to determine the cell density for seeding. Prior to obtaining the cell count the cells were stained with tripan blue. The scaffolds were seeded in a 24 well plate and were cut to fit snugly in the well plates in order to ensure that the scaffold would not float during incubation. Duplicates of each scaffold were seeded with 250,000 cells per scaffold. The scaffolds were incubated at 37°C in a 5% CO₂ atmosphere for 24hrs. After the incubation cycle was complete, the cells for SEM analysis were fixed with 4% paraformaldehyde for 10-15 minutes each and then washed three times with 1mol PBS solution. Scaffolds used for confocal microscopy were not treated with paraformaldehyde, but instead were stained with 2mL Calcein AM at a concentration of 0.5μL/mL for 30 minutes. After staining the scaffolds were transferred to optical microscope slides and fixed with 3 drops of prolong gold microscope slide mounting media in order to remove any residual air bubbles.

2.6 Scaffold Drying Procedure for SEM Analysis

The scaffolds were prepared for SEM analysis using an ethanol drying gradient. The scaffolds were dried in sequential order for 10 minutes at a time in a 25% ethanol-water mixture, followed by a 50% ethanol-water mixture, proceeded by a 70% ethanol-water mixture, followed by a 95% ethanol-water mixture and finally by a 100% ethanol solution. After the ethanol drying gradient was complete, the scaffolds were transferred to 100% hexamethyldisilazane (HMDS) mixture for overnight drying through a gradient process. The transfer was performed by saturating the scaffolds for 10 minutes each in 1:2 ethanol-HMDS, followed by 1:1 ethanol-HMDS, followed by 1:2 ethanol-HMDS and finally immersed in 100% HMDS.

2.7 SEM Analysis

SEM analysis was performed to investigate cellular adhesion and spreading on the PLA scaffolds. A Leo 1525 electron microscope equipped with Zeiss SmartSEM Software was used to perform the analysis mode. Prior to analysis, the scaffolds were fixed to aluminum studs using carbon tape and were coated with gold for 20 seconds, using two 10 second bursts. The SEM was run between 2-5KeV and images were taken using both the SE2 and inlense detectors.

2.8 Confocal Microscopy

Confocal Microscopy was performed to observe the distance of cellular penetration into the bulk of the scaffolds. A Leica TCS SP2 laser scanning confocal microscope (Leica Microsystems) equipped with Leica Confocal Software was used to perform the study. Z-mapping was performed on the seeded scaffolds from the top surface into the bulk of the material, and from the bottom scaffold surface into the bulk of

the material. The stopping point for both scans was chosen as the point where no more fluorescence could be visually detected.

3.0 Results

3.0.1 Intrinsic Viscosity and TGA

The Huggins-Kraemer plot used to calculate the M_n of NatureWorks 6252D PLA is presented in figure 12. It is evident that the reduced viscosities for the three concentrations used during the experiment are not collinear. The mass loss versus temperature curve for NatureWorks 6252D PLA is displayed in figure 13. Examination of the curve shows that when placed in an atmosphere containing 100% N_2 PLA does not undergo severe thermal oxidative degradation until temperatures greater than 300°C. After 300°C the degradation process occurs rapidly until almost no material remains around 375°C.

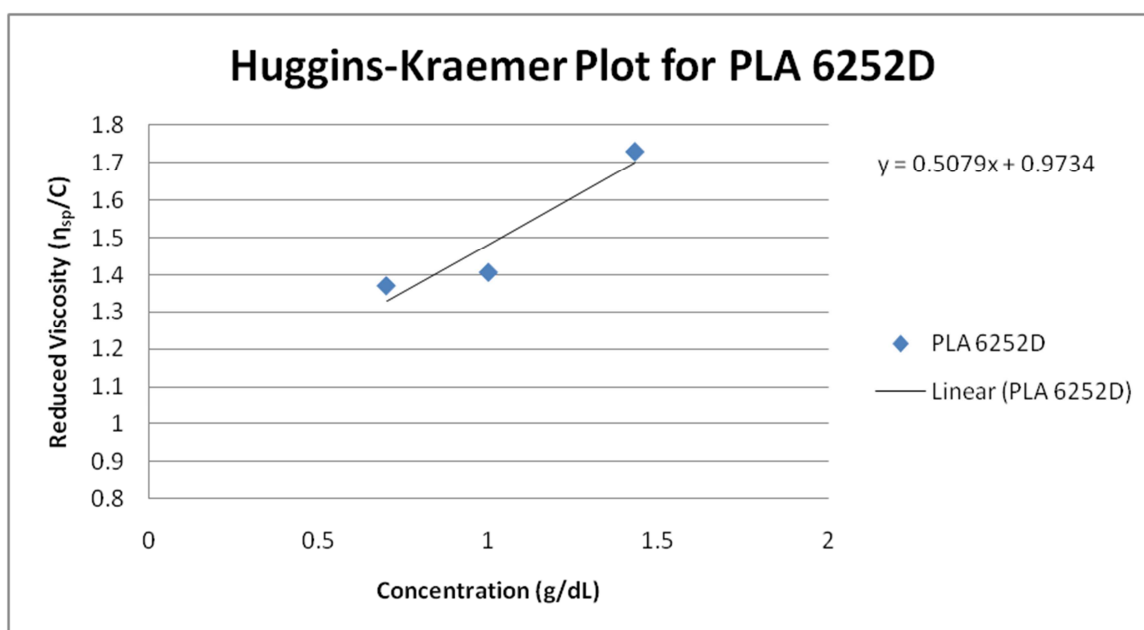


Figure 12. Huggins-Kraemer plot used to determine the intrinsic viscosity and M_n of PLA listed in section 2.1.1

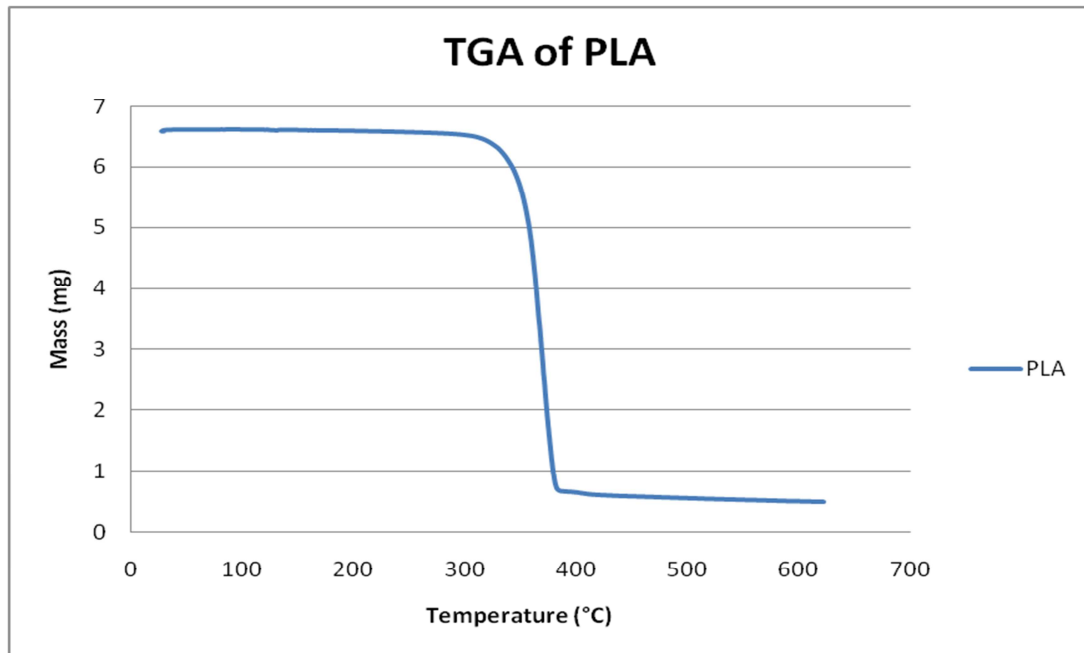


Figure 13. Mass loss versus temperature of NatureWorks 6252D PLA

3.1 Effects of Processing Conditions on the Mechanical Properties of MB Webs

3.1.1 Fiber Size Distributions

The mean fiber diameters for micro sized webs with standard error bars are presented in figure 14. The mean is represented by a red X. Figure 14 shows that there is no statistically significant difference between fiber diameters within the same air flow setting at any DCD collected. However, it is evident that there is a substantial decrease in fiber diameter with increase in primary air flow rate. The fiber diameter decreases one order of magnitude over an air flow increase from 0.8cm to 35cm, and does so close to a linear manner with the regression coefficient being .9266.

The raw fiber count data for the micron sized melt blown webs is presented in tables 4-7. Each table contains fiber size distribution histograms and associated quartile data for one primary air flow rate at the three DCDs collected, 10, 30 and 50cm. The red bar in the box and whisker plot above each

histogram represents the range where greater than or equal to 50% of the fiber counts lie for each MB condition.

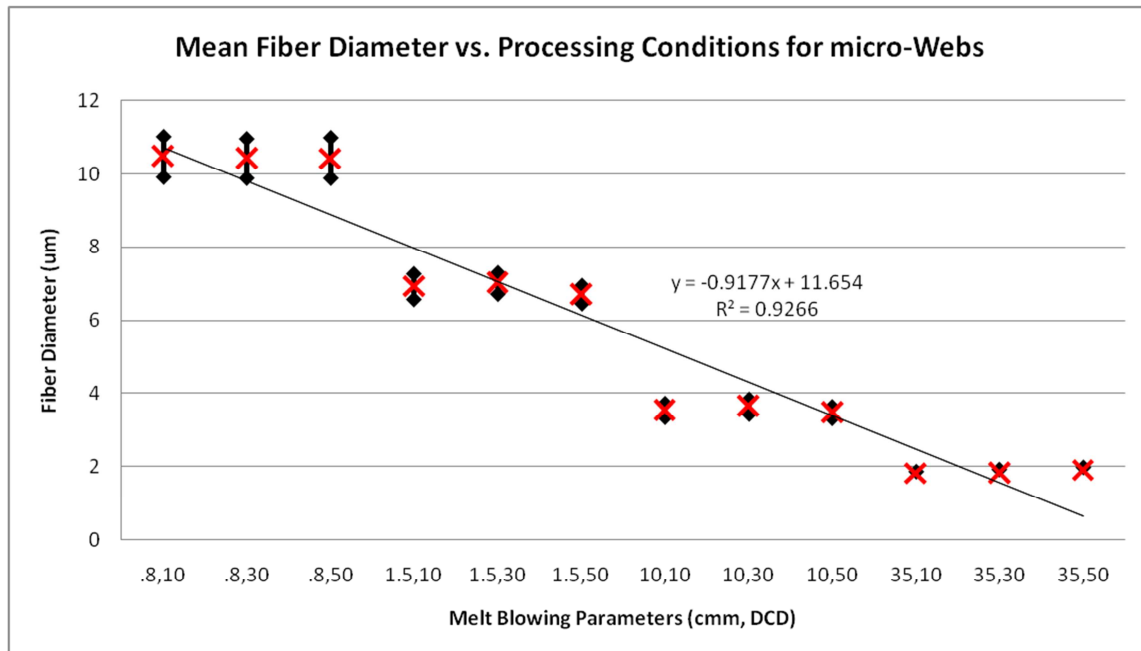


Figure 14. Mean fiber diameter versus processing conditions for MB micro-webs

The mean fiber diameter is represented by a diamond in each plot. Values considered to be outliers are represented by black dots above the histograms. Close examination of the four tables uncovers several trends within the fiber diameter data. The most obvious point of interest is that the fiber diameter decreases as primary air flow increases. This trend is clearly represented in figure 14. More difficult to notice is that both the interquartile ranges and standard error of the means also decrease with increasing primary air flow rate. Figures 15 and 16 provide graphical representations of both these trends.

Figure 17 contains a graph of the mean pore size versus processing conditions for MB nano-sized webs. The mean and standard error are represented by a red X and black error bars, respectively. The figure contains one outlying group, the fibers MB at 0.8cmm and collected at 30cm. Examination of the rest of

the data shows that there is little to no statistically significant difference between the mean fiber diameters and DCD used when the primary airflow rate was the same. The figure also shows that there was little to no statistically significant difference between the mean fiber diameters of different primary air flow rates at any DCD collected. This trend is completely contrary to what was seen for the mean fiber diameter of micron-sized MB webs. For example, the mean fiber diameter collected for a web MB at 5.5cmm collected at 10cm was almost the same at one blown at 0.8cmm collected at 10cm. Tables 8-10 contain the raw fiber count data for the nano-sized melt blown webs. Each table contains fiber size distribution histograms and associated quartile data for one primary air flow rate at the three DCDs collected, 10, 30 and 50cm. Examination of the regressions show that similarly to the mean fiber size there is no correlation between MB processing parameters and interquartile range or the standard error of the mean. Figures 18 and 19 contain graphical layouts of both the interquartile ranges and standard error means for all the processing conditions used to create nano-fiber sized webs.

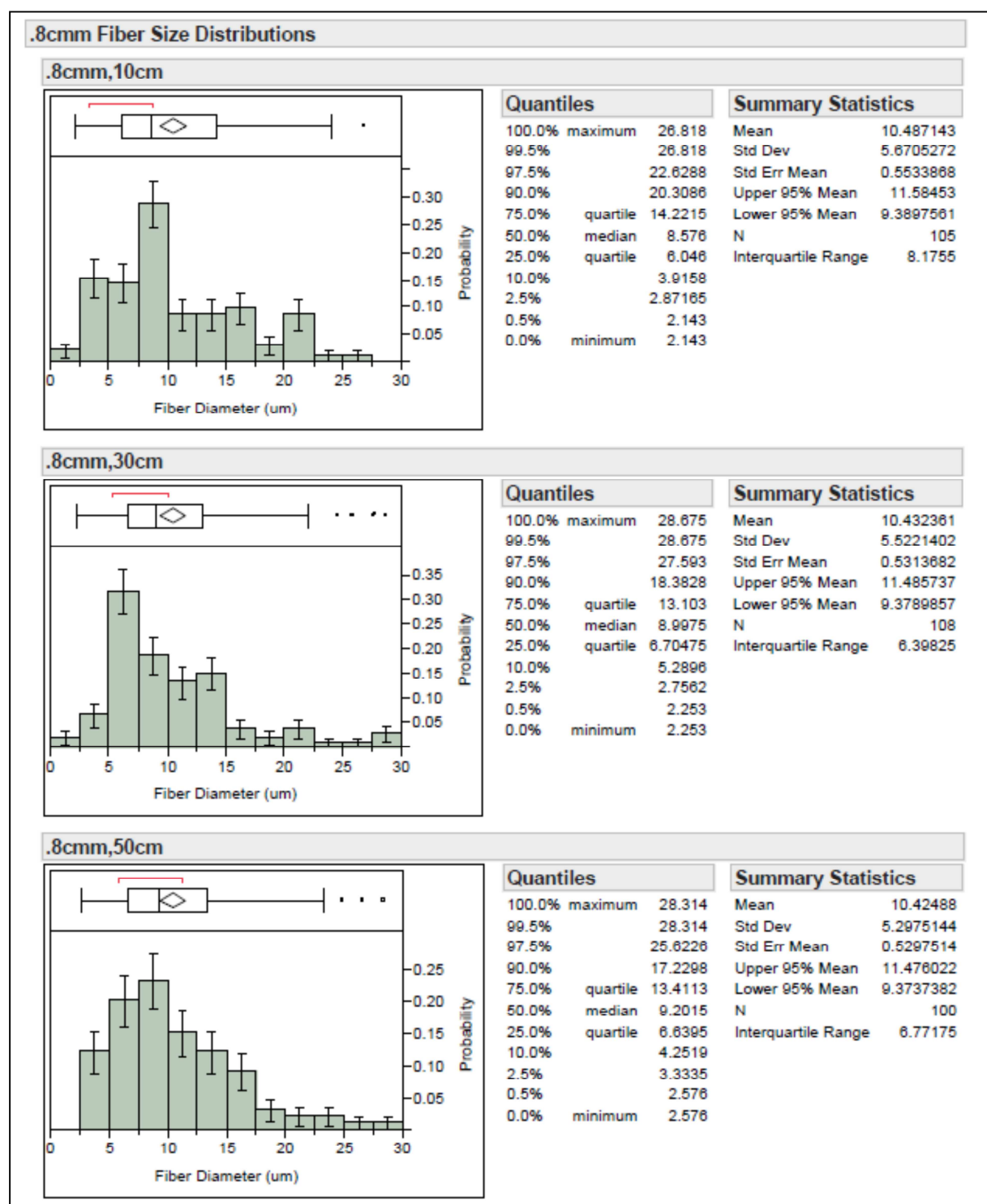


Figure 15. Fiber size distributions and statistics for micron-sized MB webs blown at 0.8cmm and collected at 10, 30 and 50cm

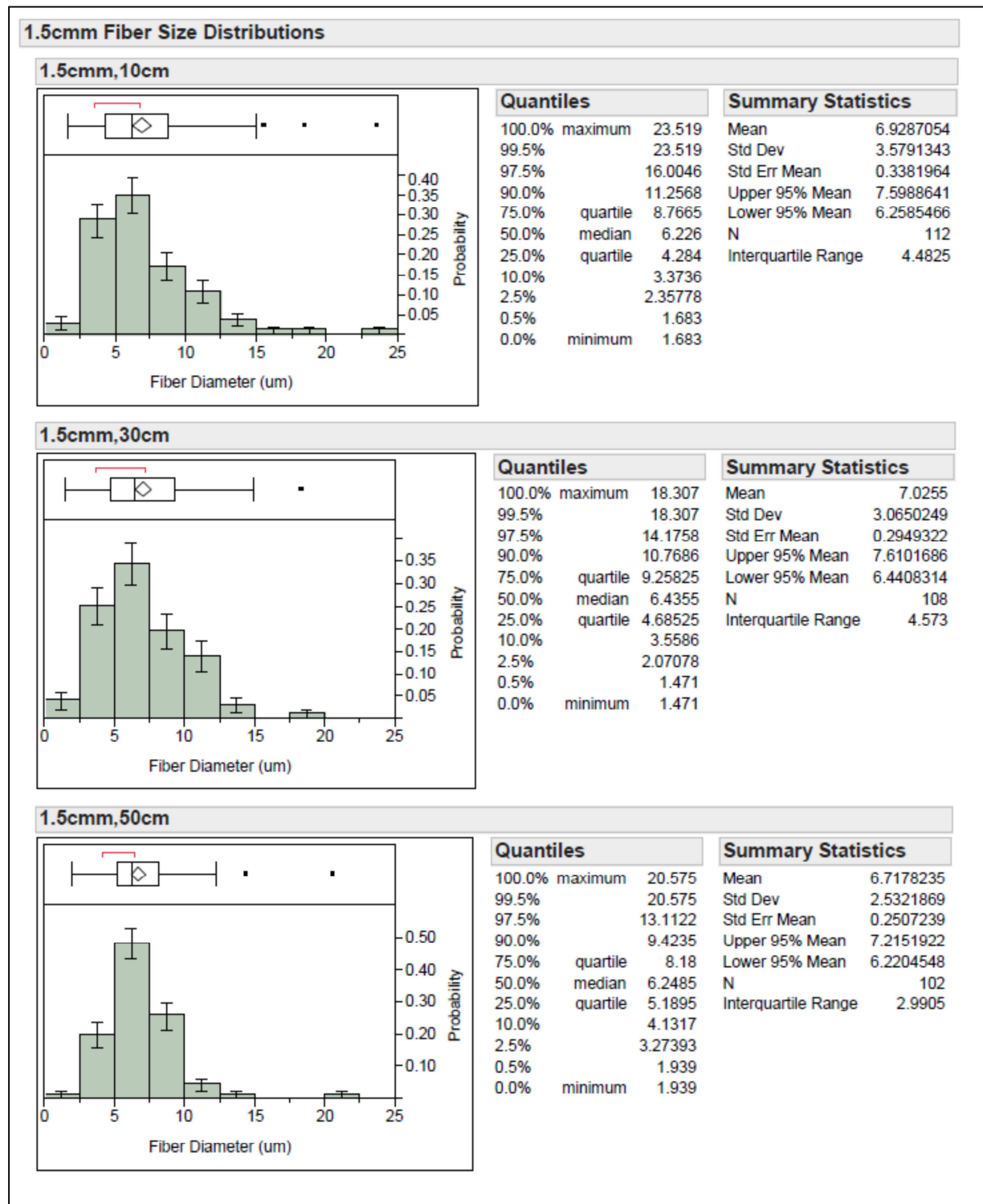


Figure 16. Fiber size distributions and statistics for micron-sized MB webs blown at 1.5cmm and collected at 10, 30 and 50cm

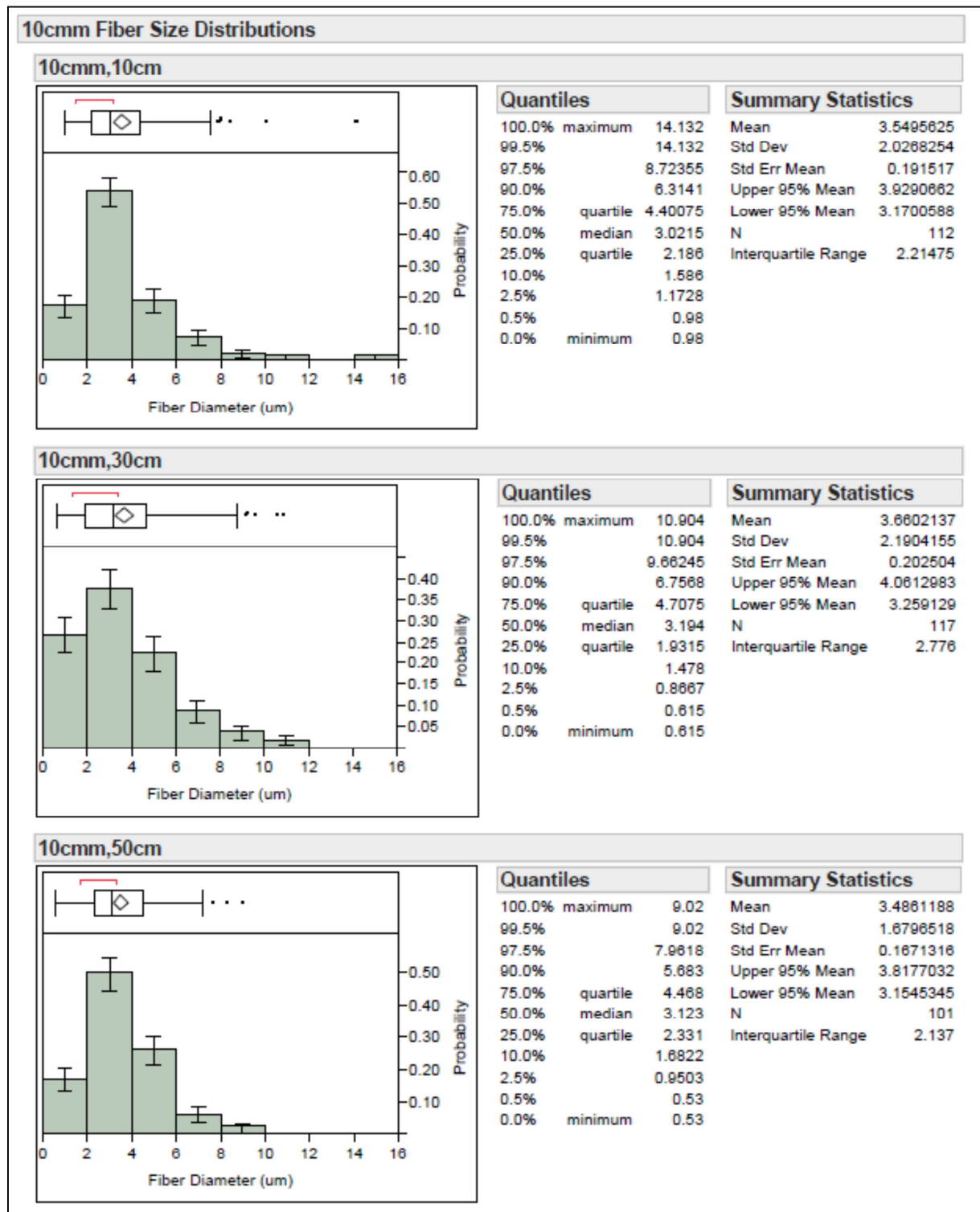
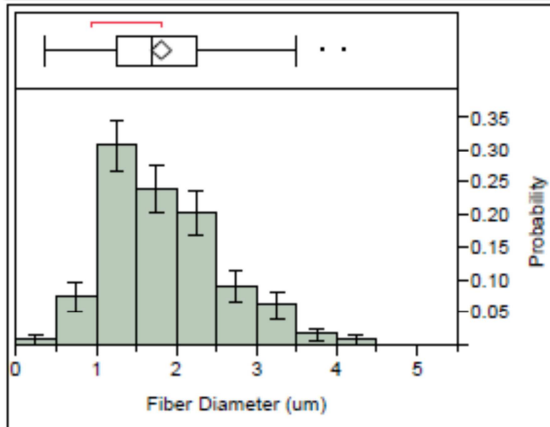


Figure 17. Fiber size distributions and statistics for micron-sized MB webs blown at 10cmm and collected at 10, 30 and 50cm

35cmm Fiber Size Distributions

35cmm,10cm



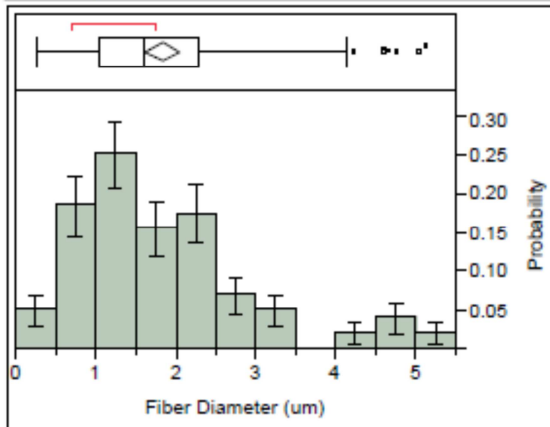
Quantiles

100.0%	maximum	4.087
99.5%		4.087
97.5%		3.49125
90.0%		2.808
75.0%	quartile	2.244
50.0%	median	1.685
25.0%	quartile	1.25475
10.0%		1.02
2.5%		0.74713
0.5%		0.364
0.0%	minimum	0.364

Summary Statistics

Mean	1.8221716
Std Dev	0.7270879
Std Err Mean	0.0628108
Upper 95% Mean	1.946409
Lower 95% Mean	1.6979343
N	134
Interquartile Range	0.98925

35cmm,30cm



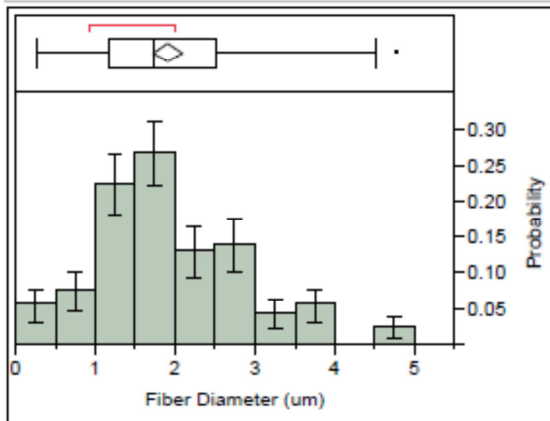
Quantiles

100.0%	maximum	5.13
99.5%		5.13
97.5%		4.87363
90.0%		3.405
75.0%	quartile	2.299
50.0%	median	1.6215
25.0%	quartile	1.03825
10.0%		0.7235
2.5%		0.4195
0.5%		0.276
0.0%	minimum	0.276

Summary Statistics

Mean	1.8340192
Std Dev	1.1053697
Std Err Mean	0.1083904
Upper 95% Mean	2.0489861
Lower 95% Mean	1.6190524
N	104
Interquartile Range	1.26075

35cmm,50cm



Quantiles

100.0%	maximum	4.78
99.5%		4.78
97.5%		4.28113
90.0%		3.2145
75.0%	quartile	2.5165
50.0%	median	1.7515
25.0%	quartile	1.1625
10.0%		0.833
2.5%		0.41
0.5%		0.275
0.0%	minimum	0.275

Summary Statistics

Mean	1.9045426
Std Dev	0.9375632
Std Err Mean	0.0967023
Upper 95% Mean	2.0965741
Lower 95% Mean	1.712511
N	94
Interquartile Range	1.354

Figure 18. Fiber size distributions and statistics for micron-sized MB webs blown at 35cmm and collected at 10, 30 and 50cm

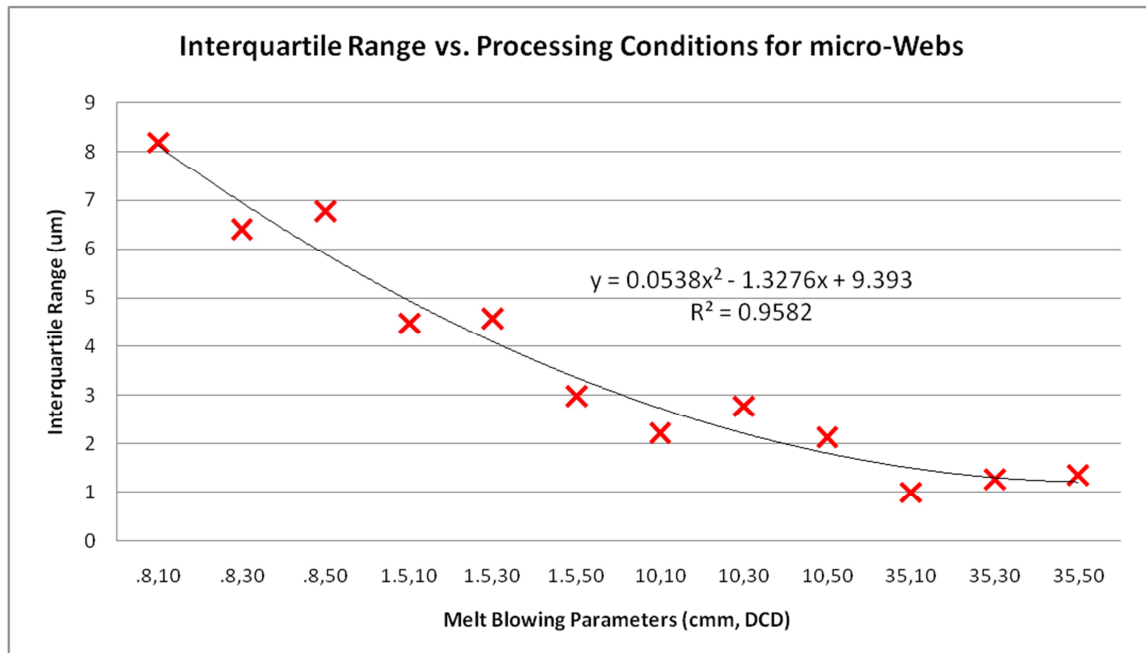


Figure 19. Interquartile ranges for micron-sized MB webs at varying processing conditions

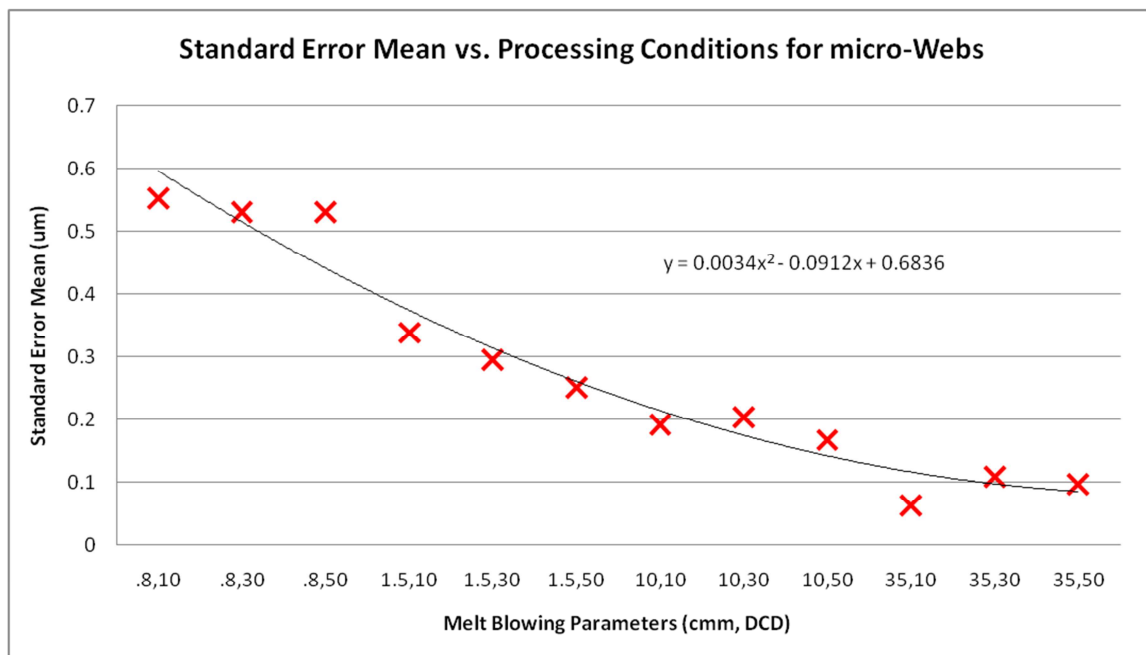


Figure 20. Standard error mean for micron-sized webs produced at different primary air flow rates and collected at varying DCDs

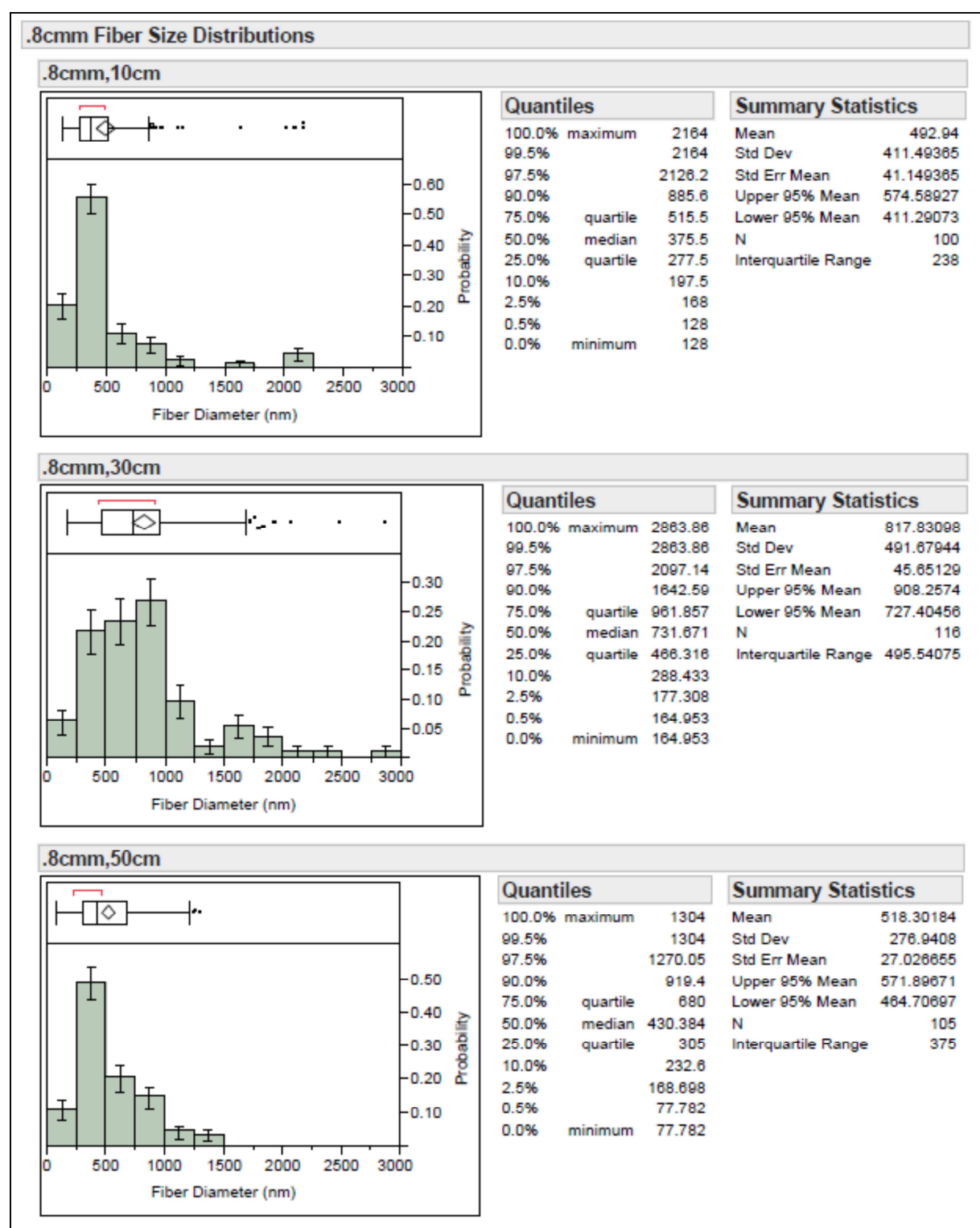
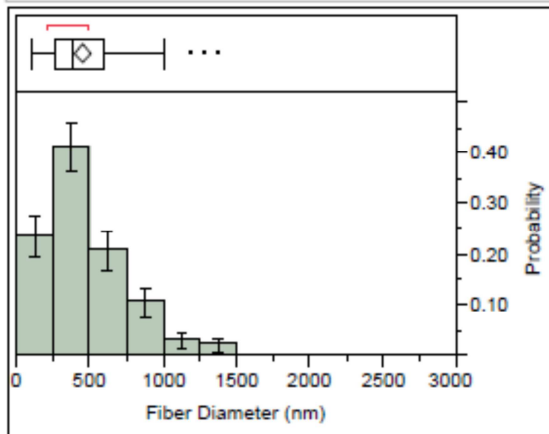


Figure 21. Fiber size distributions and statistics for nano-sized MB webs blown at 0.8cmm and collected at 10, 30 and 50cm

2.3cm Fiber Size Distributions

2.3cm,10cm



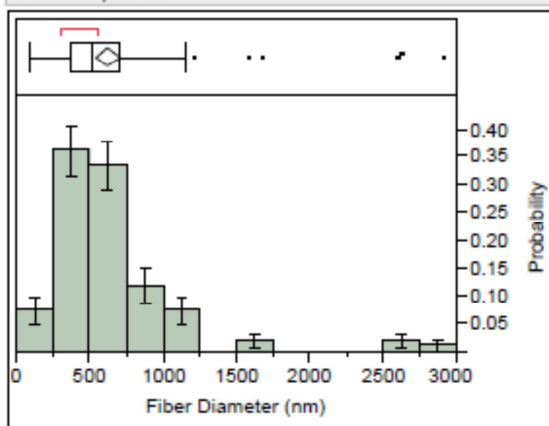
Quantiles

100.0%	maximum	1376
99.5%		1376
97.5%		1215.14
90.0%		834.021
75.0%	quartile	598.512
50.0%	median	380.521
25.0%	quartile	256.589
10.0%		166.659
2.5%		106.783
0.5%		103.55
0.0%	minimum	103.55

Summary Statistics

Mean	452.20964
Std Dev	270.58449
Std Err Mean	26.15839
Upper 95% Mean	504.07119
Lower 95% Mean	400.34808
N	107
Interquartile Range	341.923

2.3cm,30cm



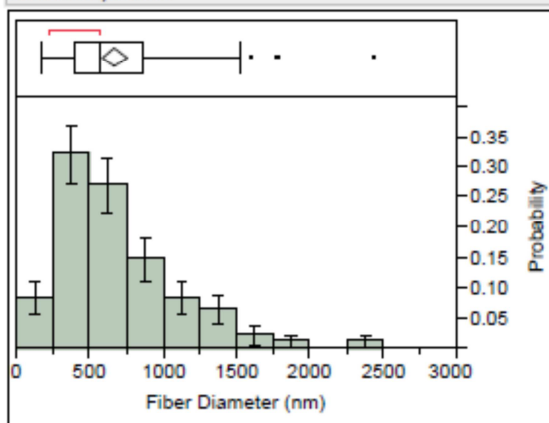
Quantiles

100.0%	maximum	2922
99.5%		2922
97.5%		2612.6
90.0%		1006.4
75.0%	quartile	700
50.0%	median	521
25.0%	quartile	370
10.0%		304.8
2.5%		156.8
0.5%		91
0.0%	minimum	91

Summary Statistics

Mean	621.17117
Std Dev	448.73476
Std Err Mean	42.402187
Upper 95% Mean	705.20235
Lower 95% Mean	537.13999
N	111
Interquartile Range	330

2.3cm,50cm



Quantiles

100.0%	maximum	2438.98
99.5%		2438.98
97.5%		1701.36
90.0%		1267.55
75.0%	quartile	861.075
50.0%	median	567.297
25.0%	quartile	391.874
10.0%		256.464
2.5%		192.396
0.5%		169.673
0.0%	minimum	169.673

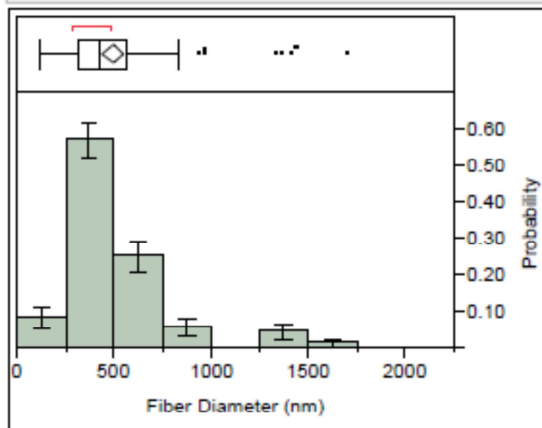
Summary Statistics

Mean	669.77136
Std Dev	400.59713
Std Err Mean	40.674476
Upper 95% Mean	750.50956
Lower 95% Mean	589.03316
N	97
Interquartile Range	469.2005

Figure 22. Fiber size distributions and statistics for nano-sized MB webs blown at 2.3cm and collected at 10, 30 and 50cm

5.5cmm Fiber Size Distributions

5.5cmm,10cm



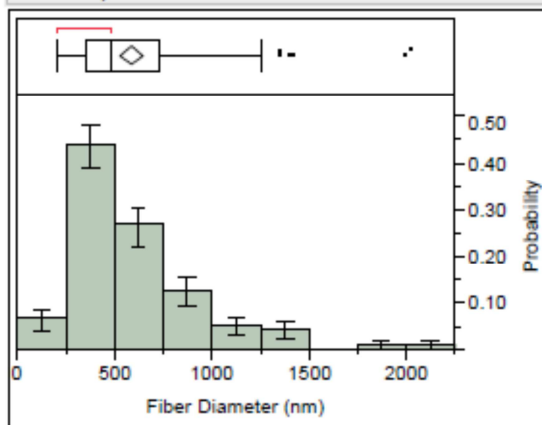
Quantiles

100.0%	maximum	1700.27
99.5%		1700.27
97.5%		1428.06
90.0%		781.165
75.0%	quartile	561.418
50.0%	median	427.533
25.0%	quartile	323.21
10.0%		259.713
2.5%		187.72
0.5%		118.576
0.0%	minimum	118.576

Summary Statistics

Mean	494.94325
Std Dev	282.64953
Std Err Mean	28.698712
Upper 95% Mean	551.90974
Lower 95% Mean	437.97676
N	97
Interquartile Range	238.2075

5.5cmm,30cm



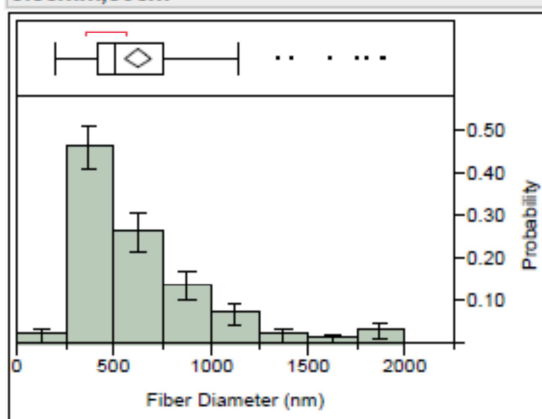
Quantiles

100.0%	maximum	2024
99.5%		2024
97.5%		1418.54
90.0%		1088.49
75.0%	quartile	730.506
50.0%	median	488.035
25.0%	quartile	353.614
10.0%		272.946
2.5%		211.8
0.5%		205
0.0%	minimum	205

Summary Statistics

Mean	587.69818
Std Dev	338.14454
Std Err Mean	30.740413
Upper 95% Mean	648.56203
Lower 95% Mean	526.83428
N	121
Interquartile Range	376.892

5.5cmm,50cm



Quantiles

100.0%	maximum	1885.21
99.5%		1885.21
97.5%		1771.16
90.0%		1055.76
75.0%	quartile	754.194
50.0%	median	505.621
25.0%	quartile	414.973
10.0%		334.917
2.5%		239.499
0.5%		196.958
0.0%	minimum	196.958

Summary Statistics

Mean	625.63496
Std Dev	337.24942
Std Err Mean	33.070027
Upper 95% Mean	691.22156
Lower 95% Mean	560.04836
N	104
Interquartile Range	339.22125

Figure 23. Fiber size distributions and statistics for nano-sized MB webs blown at 5.5cmm and collected at 10, 30 and 50cm

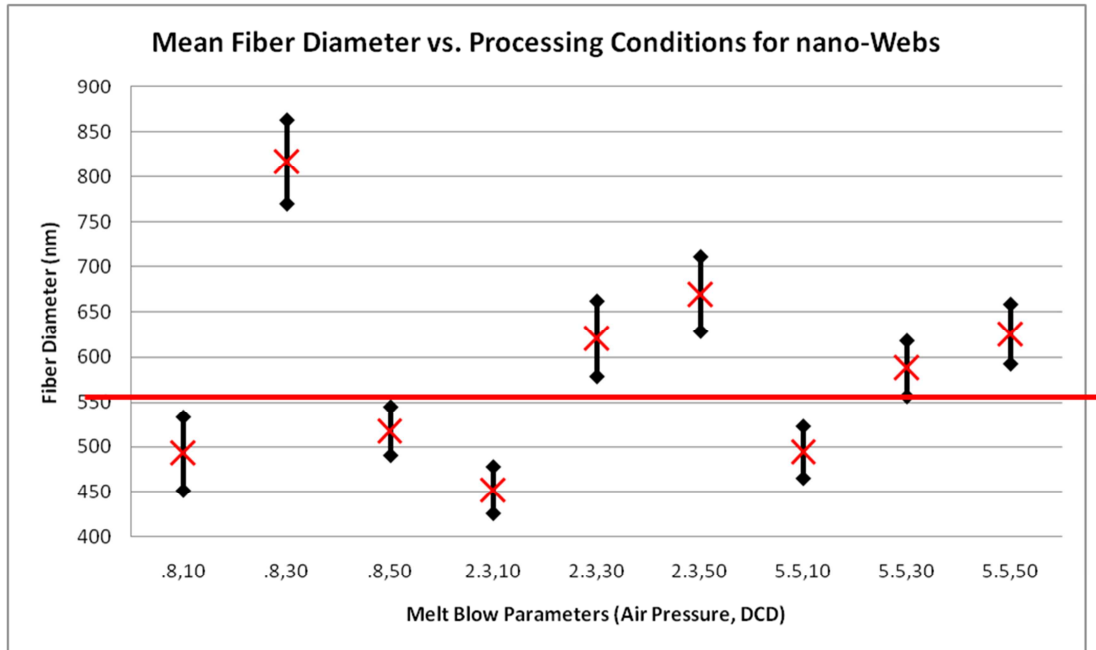


Figure 24. Mean fiber diameter versus processing conditions for MB nano-sized webs. The red line is drawn through the chart to highlight the lack of statistical significance between the mean values

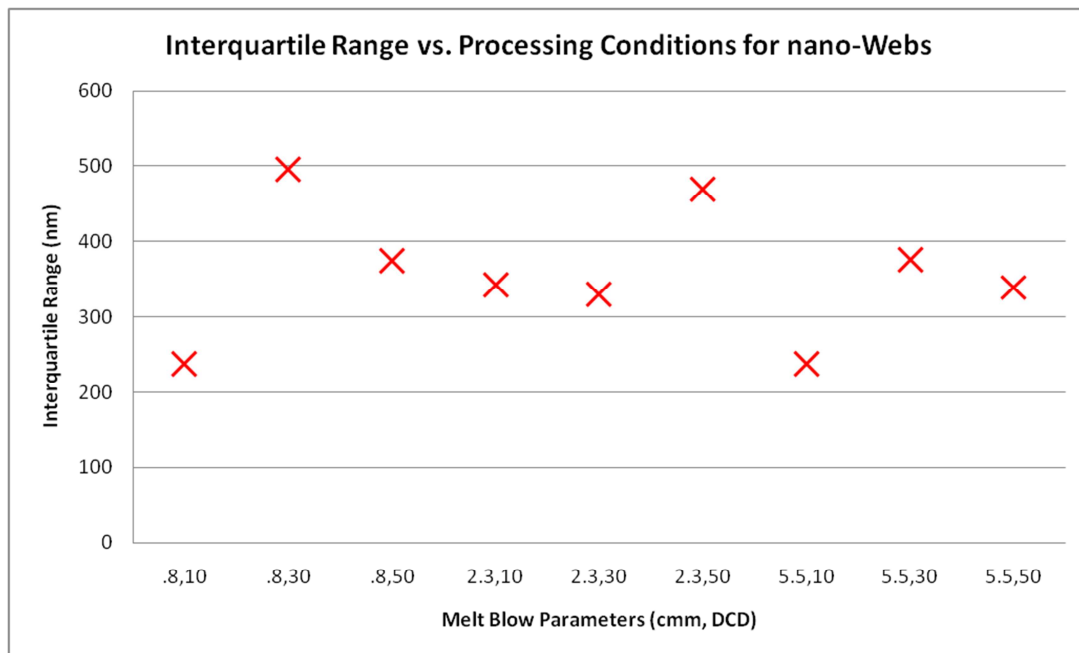


Figure 25. Interquartile range versus processing conditions for MB nano-sized webs

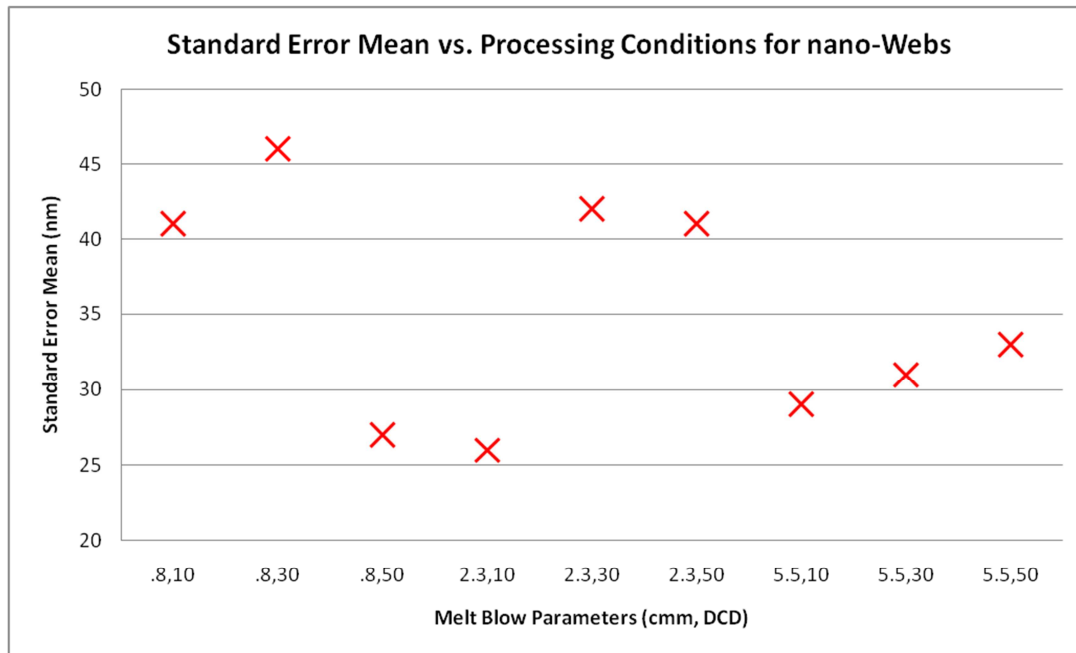


Figure 26. Standard error mean versus processing conditions for MB nano-sized webs

3.1.2 Pore Size Distributions

Graphical representation of the effects of processing conditions on the mean pore size of MB micron-sized webs is presented in figure 21. It is clear that the mean pore size decreases with increasing primary air flow rates for all DCDs. It is also obvious that as the DCD is increased so does the mean pore size for all primary air flow rates used.

The same data presented in figure 21 can be examined by primary air flow rate rather than DCD to observe different trends, and is presented in figure 22. Figure 22 shows that for 3 out of the 4 primary air flow rates used, an increase in DCD caused an increase in mean pore size of about $10\mu\text{m}$ for every 20cm the DCD was increased. Figure 22 also shows that unlike the other webs, an increase in DCD to 50cm caused a decrease in the mean pore size for the web produced at a primary air flow rate of 1.5cmm.

Tables 11-14 contain the raw data, statistical analysis and histograms of the pore size distributions for each of the primary air flow rates and DCDs used. Each table is organized by primary air flow rate and contains all the data for the three DCDs used. Examination of the tables shows that for all primary air flow rates used, an increase in DCD not only increases the mean pore size, but it also increases the width of the pore size distribution (PSD). Examination of trends within a single primary air flow rate show that an increase in DCD of 20cm can be expected to produce an increase in the mean pore size of about 10cm. This is the case in 3 of the 4 primary air flow rates used, except when webs were blown using 1.5cmm.

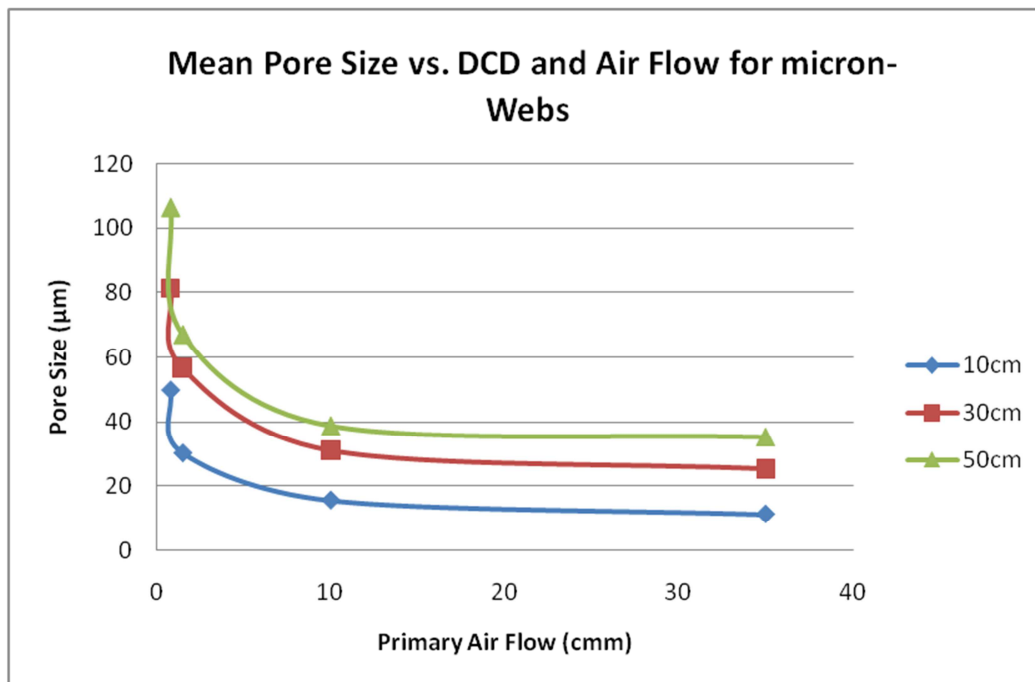


Figure 27. Mean pore size versus processing conditions for MB micron-sized webs

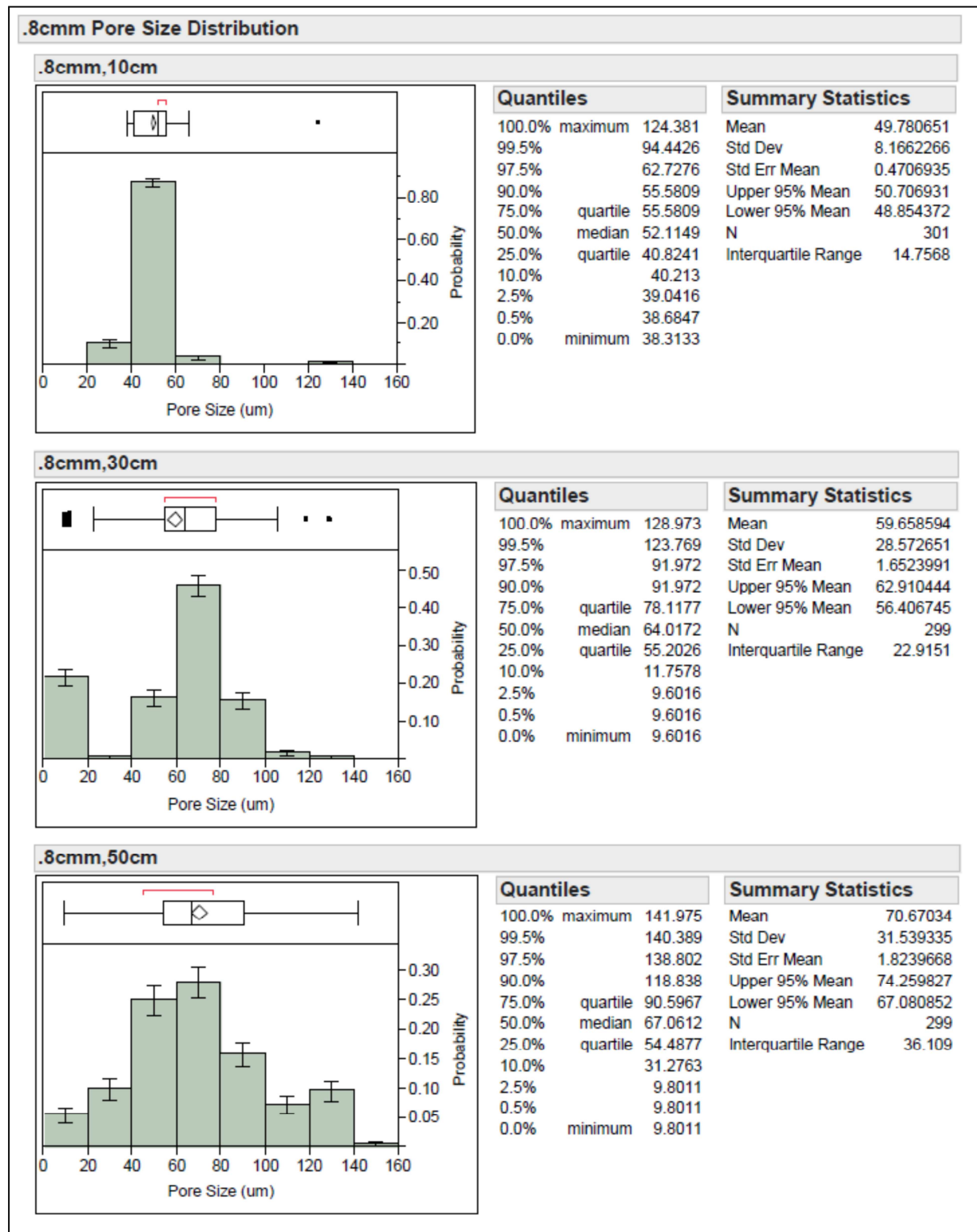


Figure 28. Pore Size distributions and statistics for micron-sized MB webs blown at 0.8cmm and collected at 10, 30 and 50cm

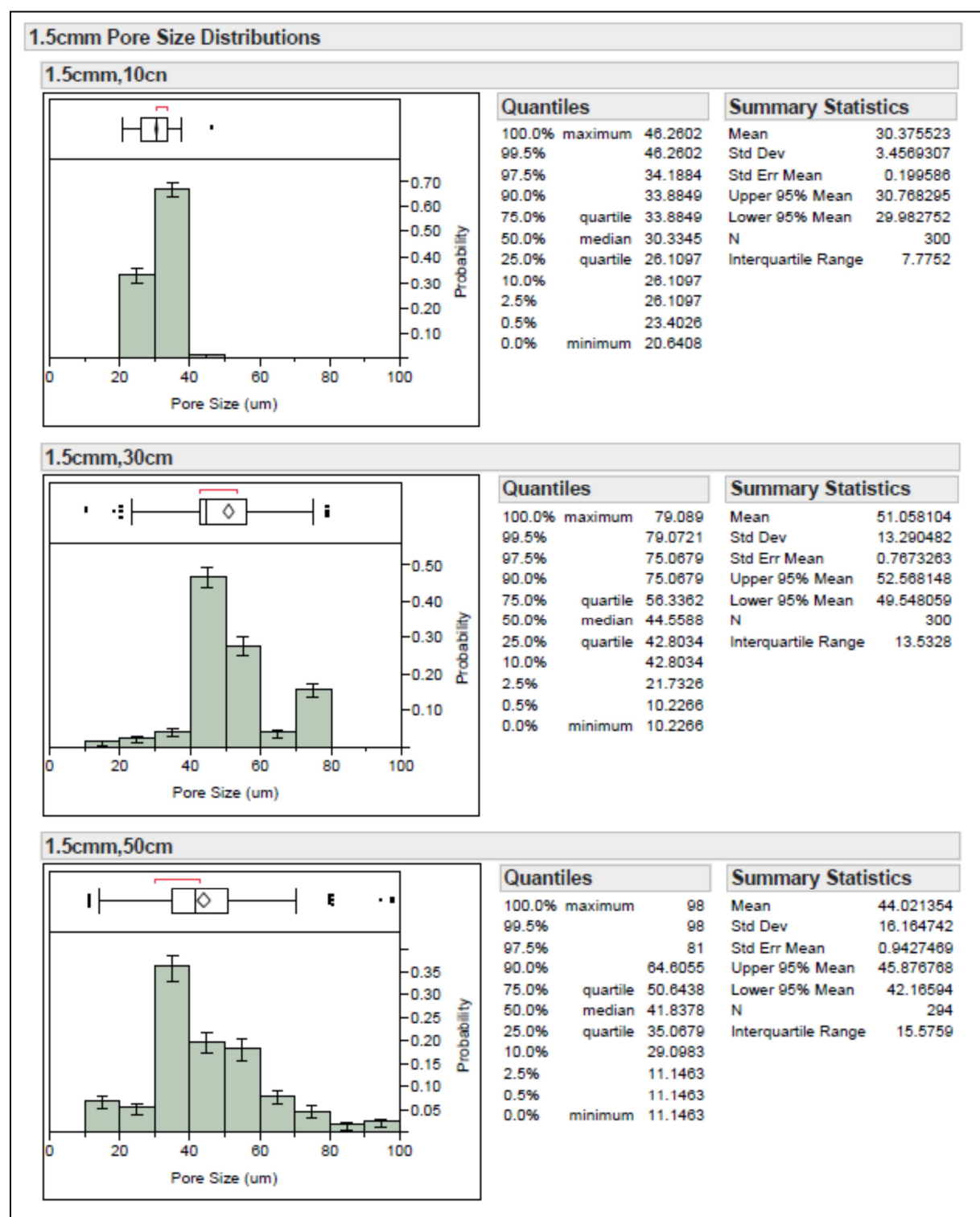


Figure 29. Pore Size distributions and statistics for micron-sized MB webs blown at 1.5cmm and collected at 10, 30 and 50cm

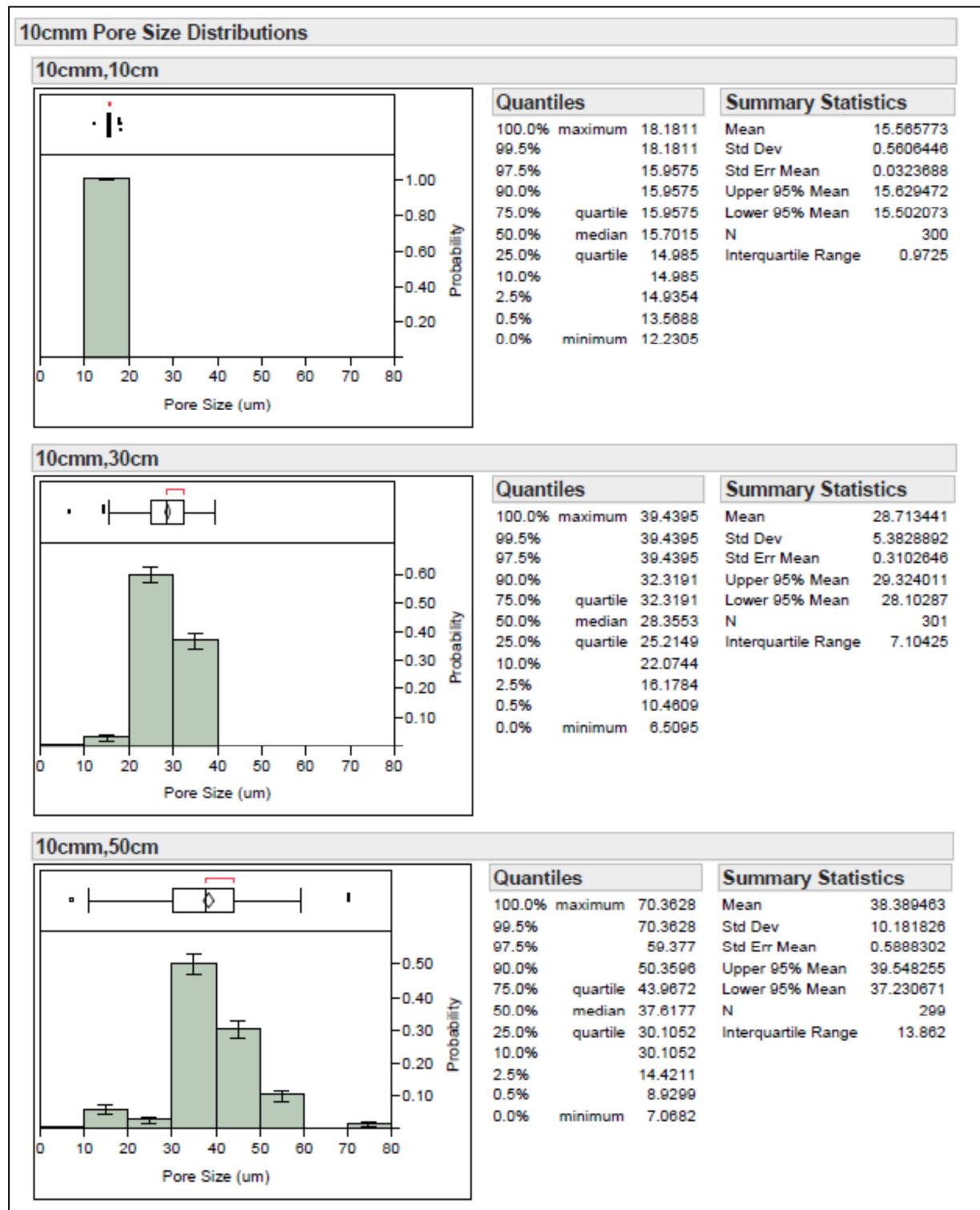


Figure 30. Pore Size distributions and statistics for micron-sized MB webs blown at 10cmm and collected at 10, 30 and 50cm

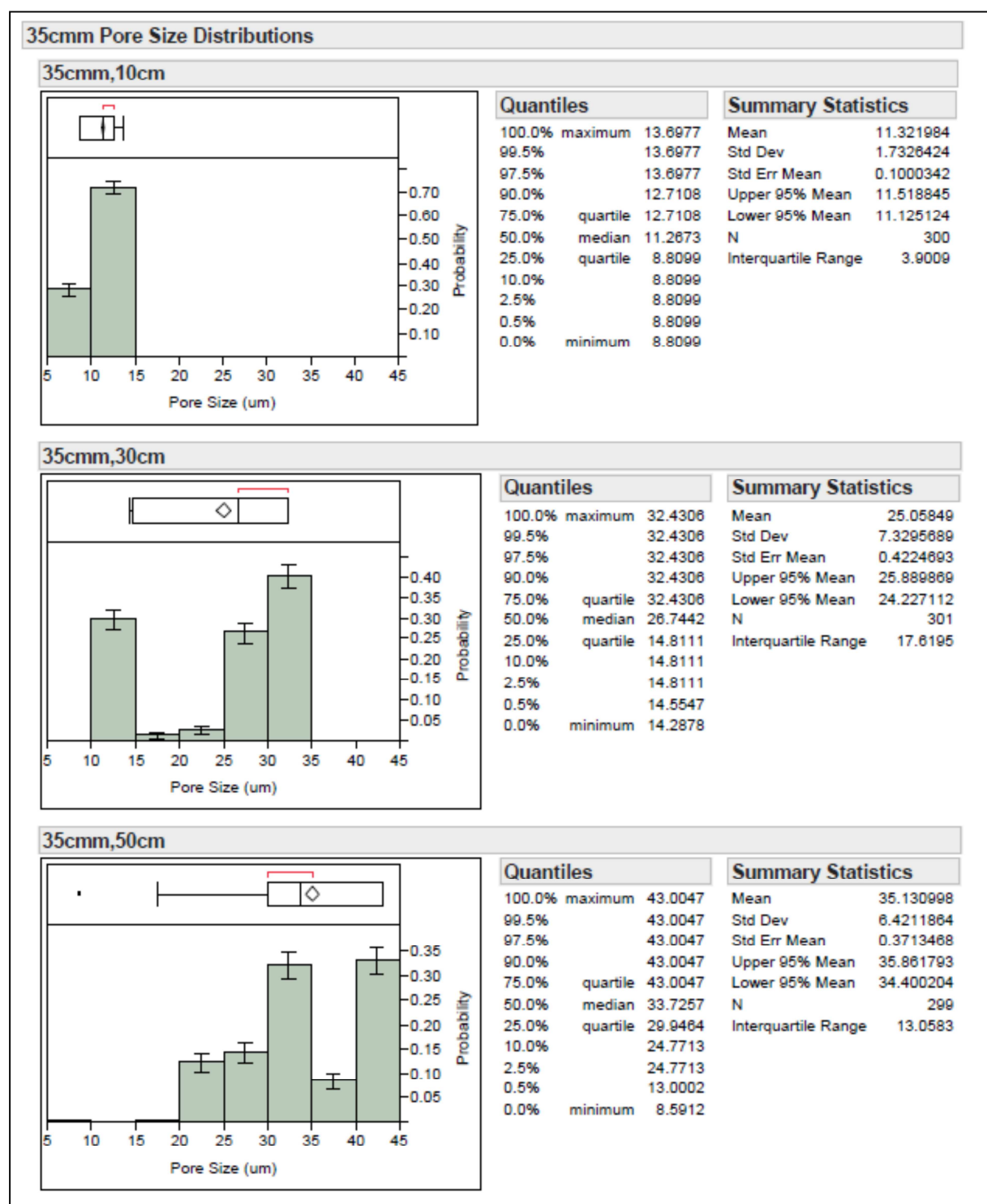


Figure 31. Pore Size distributions and statistics for micron-sized MB webs blown at 35cmm and collected at 10, 30 and 50cm

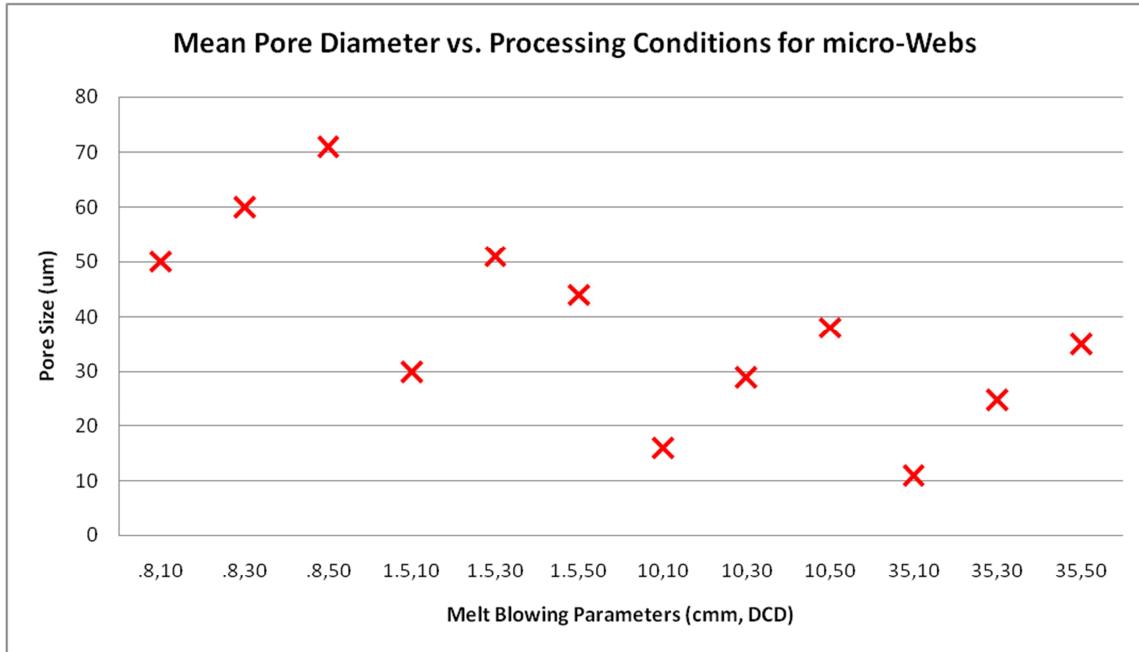


Figure 32. Variation in mean pore size with increase in DCD for each primary air flow rate used and DCD collected. Notice that the trend of positive increase does not hold true for webs MB at primary air flow rates of 1.5cmm

Figure 22 contains a graphical representation of the mean pore size versus MB processing parameters for each of the three primary air flow rates and DCDs used to create nano-fiber sized webs. Examination of the data points shows that mean pore size decreases with increasing primary air flow rate. Coinciding with this, mean pore size increases with an increase in DCD as was seen for micron-sized webs.

Tables 15-17 contain the raw data, statistical analysis and histograms of the pore size distributions for each of the primary air flow rates and DCDs used to create nano-fiber sized webs. Each table is organized by primary air flow rate and contains all the data for the three DCDs used. Examination of the data shows that in all three cases an increase in DCD leads to an increase in both mean pore size and PSD. Primary air flow rate had a much smaller effect on the pore size for the nano-sized webs compared to the micro webs. As can be seen in figure 23, varying the primary air flow rate by 5cmm only caused a decrease in mean pore size of about 5-10um for samples collected at the same DCD. Comparatively,

increasing the primary air flow rate by just 1cm for the MB micro-sized webs cause a decrease of about 20-30um.

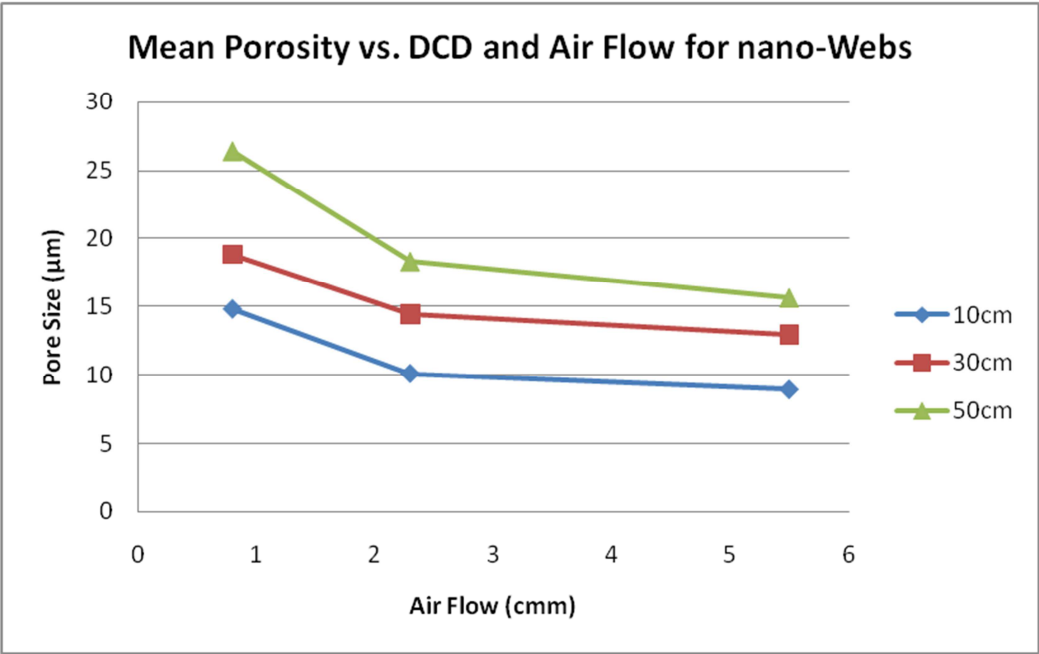


Figure 33. Mean pore sizes versus processing conditions for MB nano-webs

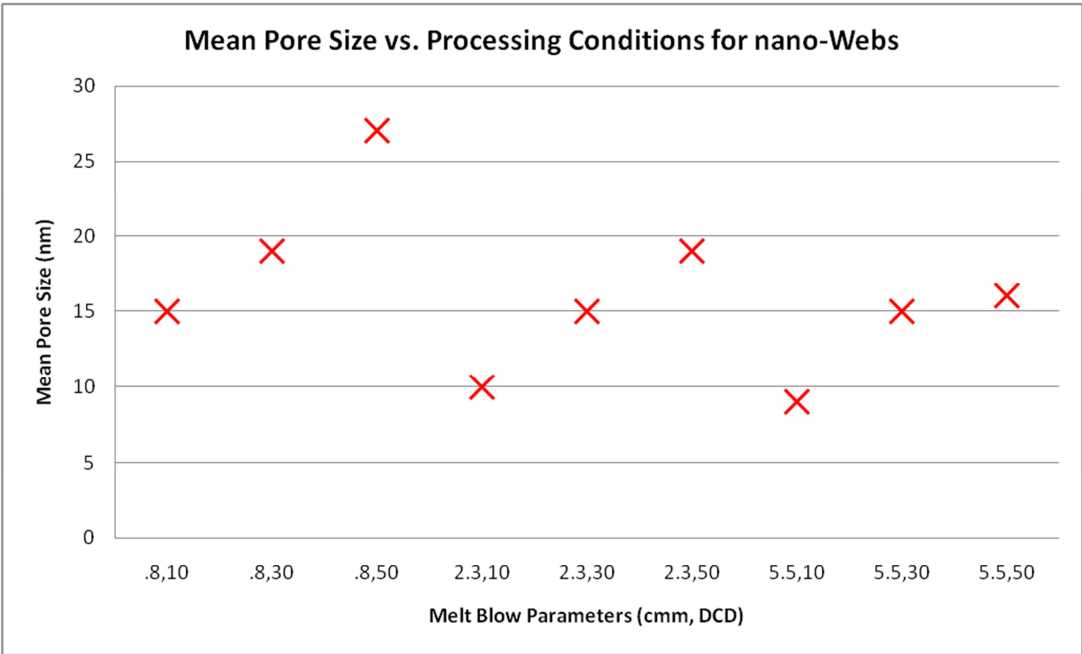


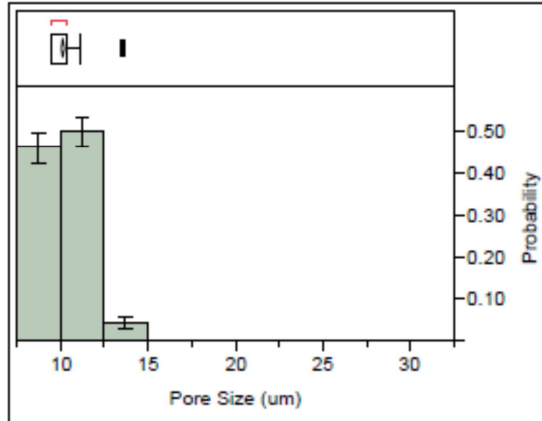
Figure 34. Mean pore size versus processing conditions for MB nano-webs



Figure 35. Pore Size distributions and statistics for nano-sized MB webs blown at 0.8cmm and collected at 10, 30 and 50cm

2.3cmm Pore Size Distributions

2.3cmm,10cm



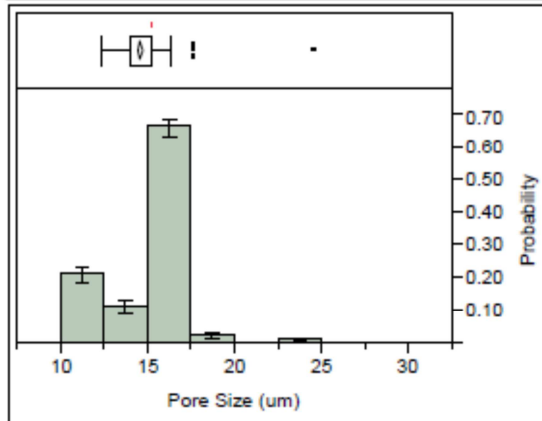
Quantiles

100.0%	maximum	13.5612
99.5%		13.5612
97.5%		13.5612
90.0%		10.3614
75.0%	quartile	10.3614
50.0%	median	10.3614
25.0%	quartile	9.4794
10.0%		9.4794
2.5%		9.4794
0.5%		9.4794
0.0%	minimum	9.4794

Summary Statistics

Mean	10.114524
Std Dev	0.8566865
Std Err Mean	0.0605769
Upper 95% Mean	10.233979
Lower 95% Mean	9.995069
N	200
Interquartile Range	0.882

2.3cmm,30cm



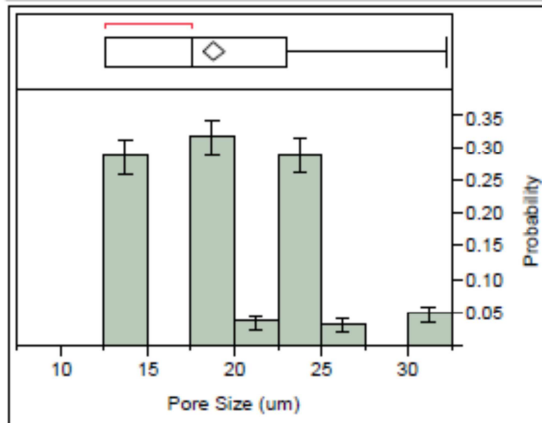
Quantiles

100.0%	maximum	24.5637
99.5%		21.0448
97.5%		16.9502
90.0%		15.2186
75.0%	quartile	15.2186
50.0%	median	15.194
25.0%	quartile	13.9869
10.0%		12.3966
2.5%		12.3966
0.5%		12.3966
0.0%	minimum	12.3966

Summary Statistics

Mean	14.594015
Std Dev	1.3490313
Std Err Mean	0.0778864
Upper 95% Mean	14.74729
Lower 95% Mean	14.44074
N	300
Interquartile Range	1.2317

2.3cmm,50cm



Quantiles

100.0%	maximum	32.2099
99.5%		32.2099
97.5%		32.2099
90.0%		22.9999
75.0%	quartile	22.9999
50.0%	median	17.5358
25.0%	quartile	12.5436
10.0%		12.5436
2.5%		12.5436
0.5%		12.5436
0.0%	minimum	12.5436

Summary Statistics

Mean	18.803783
Std Dev	5.2187891
Std Err Mean	0.3023163
Upper 95% Mean	19.398737
Lower 95% Mean	18.20883
N	298
Interquartile Range	10.4563

Figure 36. Pore Size distributions and statistics for nano-sized MB webs blown at 2.3cmm and collected at 10, 30 and 50cm



Figure 37. Pore Size distributions and statistics for nano-sized MB webs blown at 5.5cmm and collected at 10, 30 and 50cm

3.1.3 Thickness

Mat thickness for all the webs produced varied between .2-.6mm, regardless of the processing parameters used. For the micron-sized webs produced, mat thickness appears to work its way towards a constant value as both primary air flow rate and DCD are increased. The variation in thickness between different processing parameters decreases as the primary air flow rate is increased. For the nano-sized webs a similar trend is observed for collected at 10 and 50cm, but collection at 30cm shows an outlier when the primary flow rate was 2.3cmm. The MB settings of 2.3cmm and a DCD of 30cm appear to be a consistent outlier. The data for both the micro and nano-webs are presented in figures 24 and 25, respectively.

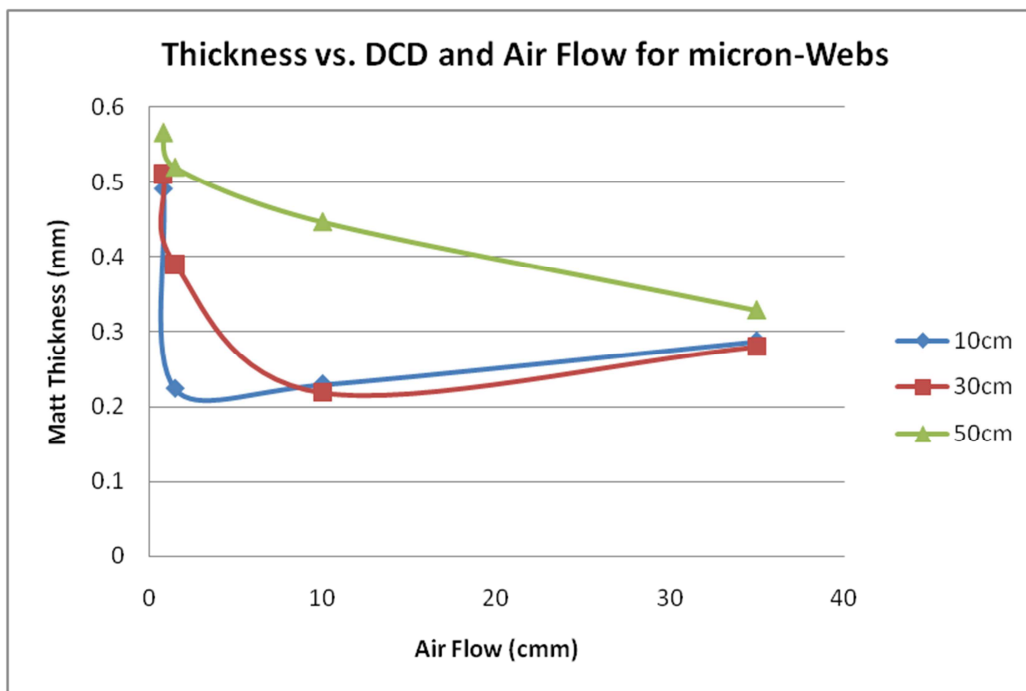


Figure 38. Web thickness versus air flow and DCD for micron-webs

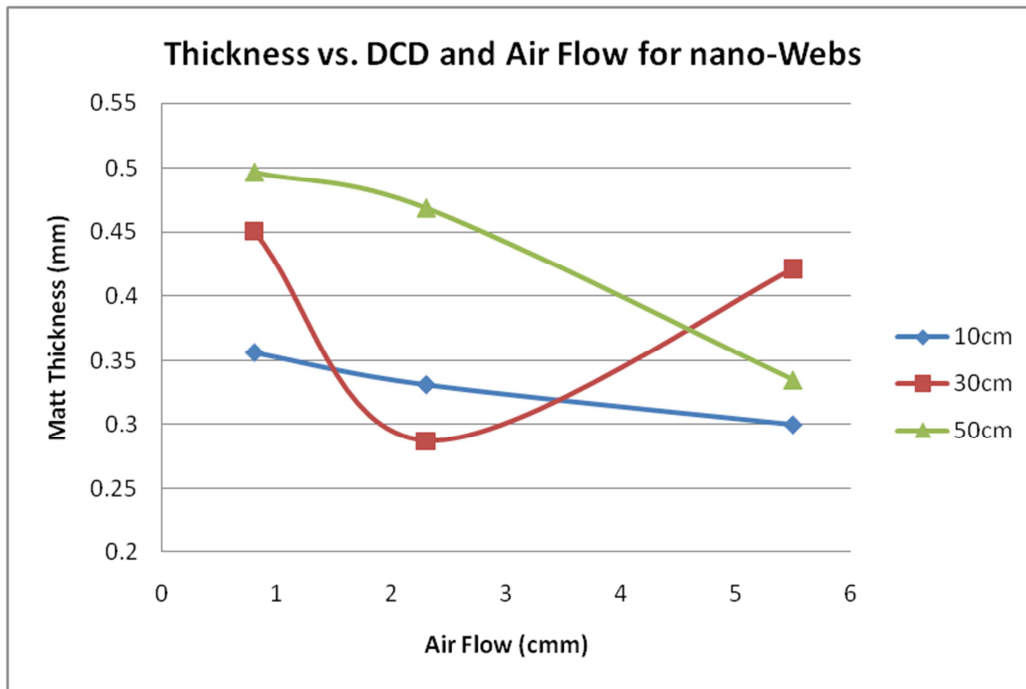


Figure 39. Web thickness versus air flow and DCD for nano-webs

3.1.4 Air Permeability

The results of the air permeability studies performed on the micro and nano-sized webs are presented in figures 26 and 27. For micron-sized webs the primary air flow rate varies from 1cmm to 40cmm depending on the processing conditions used during MB.

Figure 27 shows that the nano-sized webs possessed a much tighter window of air permeability, with a range only going from 1cmm to about 4cmm. Further examination of the charts shows that the air permeability decreases with increasing primary air flow rate and increases with DCD for all parameters used. These trends very closely match the trends seen for mean pore size.

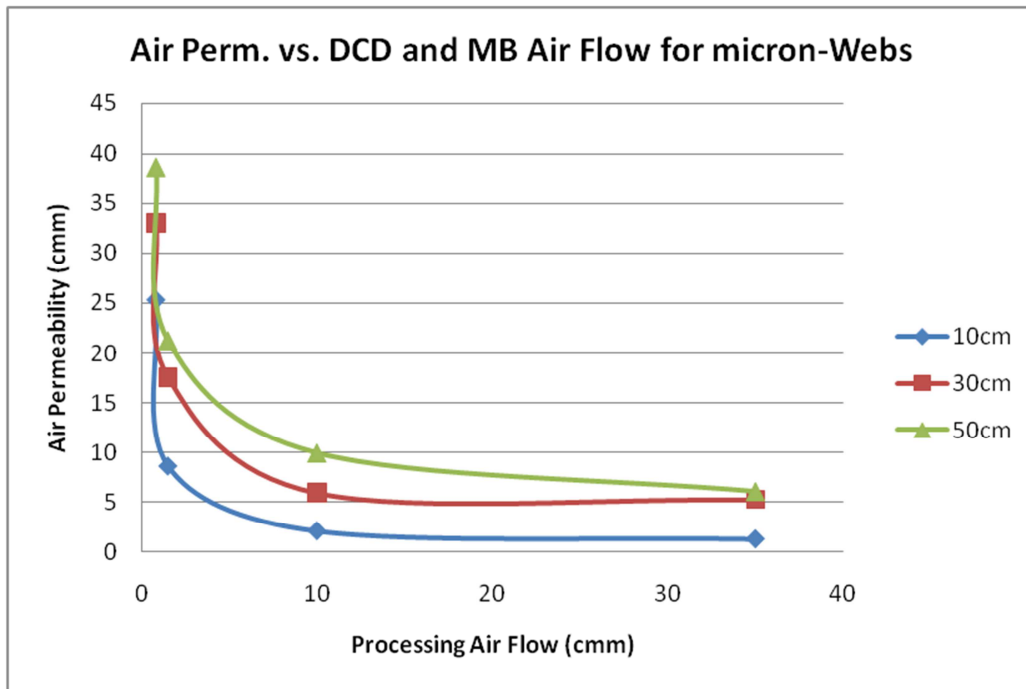


Figure 40. Air permeability versus air flow and DCD for micron-webs

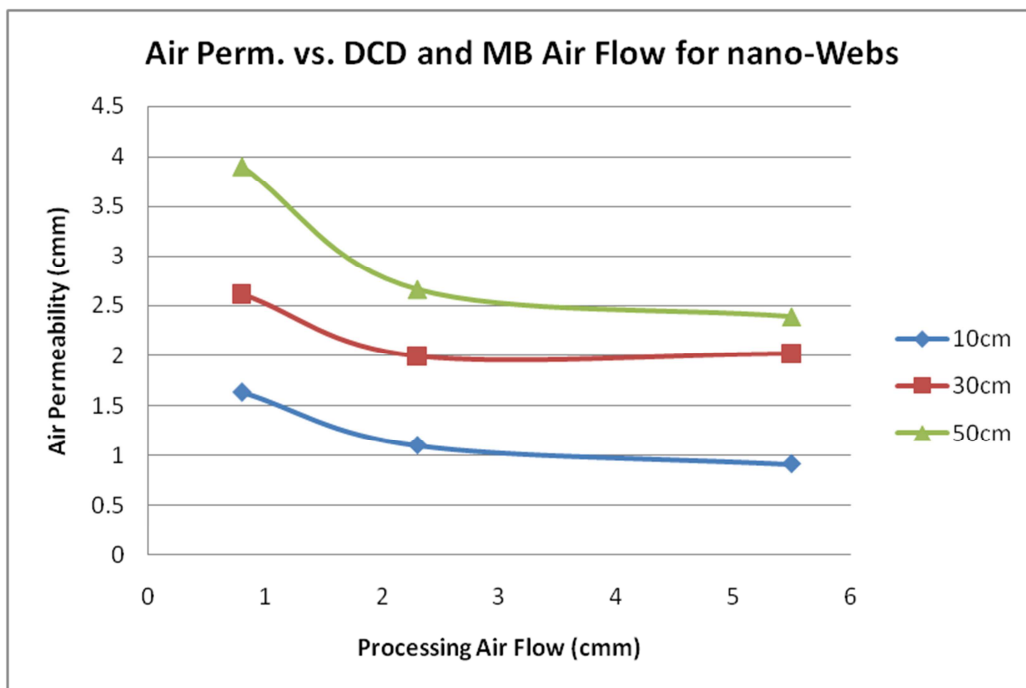


Figure 41. Air permeability versus air flow and DCD for nano-webs

3.1.5 Tensile Modulus

The tensile modulus for micro-sized webbed can be seen in figure 28. The figure contains the tensile modulus for each melt blown web in both the machine direction (MD) and the transverse direction (TD). Two things immediately stick out from the graph, the first is that there is a substantial decrease in tensile modulus as the DCD is increased. A decrease of approximately 2 orders of magnitude can be seen in the MD between webs MB at 10cmm, but collected at 10cm and 50cm. The second remarkable point is that there is an obvious difference between the tensile modulus in the MD and TD for webs collected at a DCD of 10cm blown at primary air flow rates of 10 or 35cmm. Finally, there is a slight increase in tensile modulus with air flow rate for webs collected at 30 and 50cm, but the in effect of increasing the DCD appears to outweigh the effect of increasing the primary air flow rate.

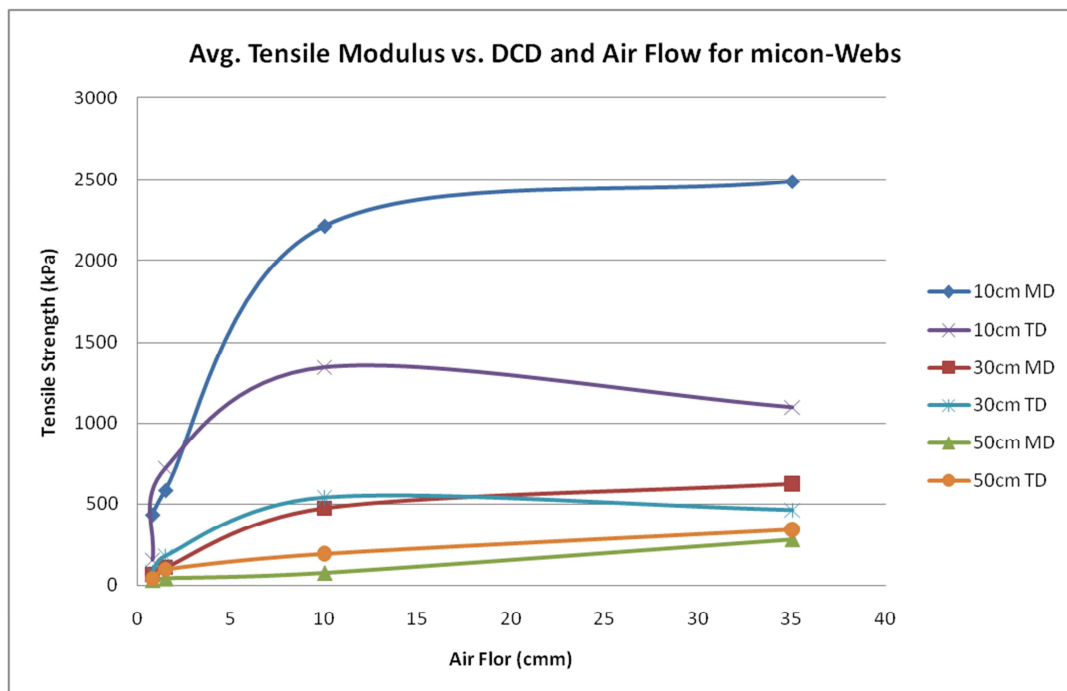


Figure 42. Average tensile modulus verses DCD and air flow for micron-webs

The trends associated in the tensile modulus of nano-sized webs with varying process conditions appear to be similar to those found in the micro-sized webs. The tensile data recorded for the nano-sized webs

in both the MD and TD is presented in figure 29. Examination of the graph shows that at very close DCD a small increase in the primary air flow rate will cause a large increase in the tensile modulus in the machine direction. At further DCDs an increase in primary air flow rate only causes a small increase in tensile strength, and in the case of the webs collected at a DCD of 30cm there was actually a decrease in tensile strength. Similarly to the micro-sized webs, there is a substantial difference in the tensile modulus between the MD and TD of webs collected at 10cm using a primary airflow rate of 2.3cm or greater.

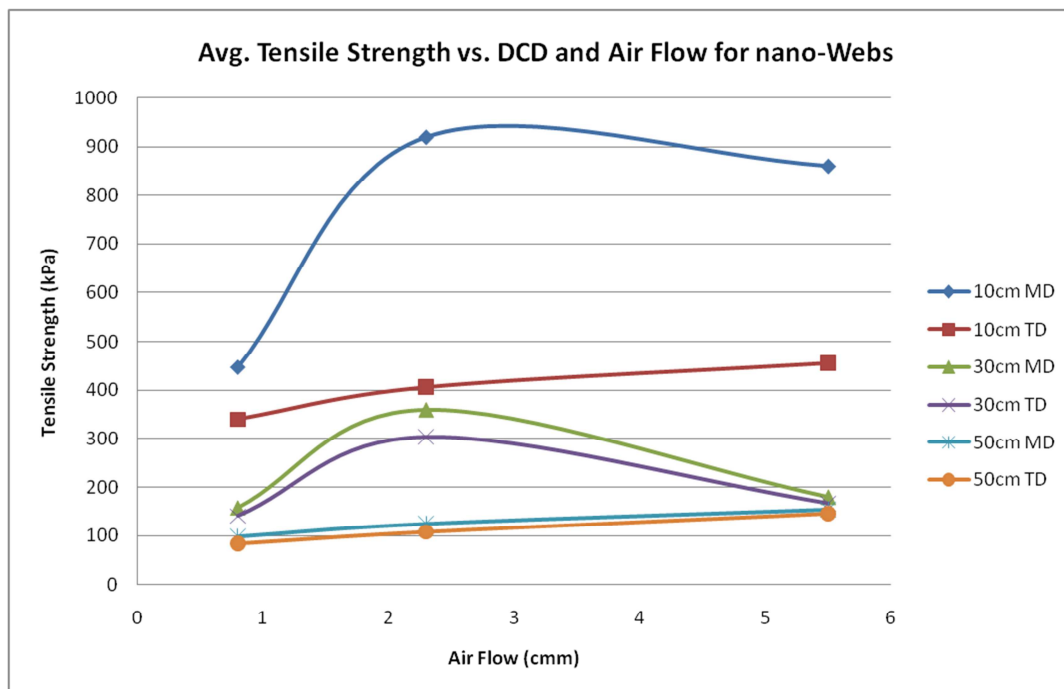


Figure 43. Average tensile modulus verses DCD and air flow for nano-webs

3.1.6 DSC

DSC was performed on native PLA, each of the twenty-one MB webs and one web annealed for 12hrs at 80°C. Table 19 contains sample information as well as the calculated crystallinity for each run performed. The crystallinity of native NatureWorks 6252D PLA was calculated at 38.9%. The annealing

process caused an increase in crystallinity up to almost 55%. Comparatively, none of the twenty-one MB webs reached a degree of crystallinity higher than 37%. Figure 30 shows the DSC heating curve for native PLA. As can be seen, the T_g is around 60°C and the onset of melting occurs near 170°C. The DSC curves for micro-sized MB webs produced at primary air flow rates of 1.5, 10 and 35cm are presented in figures 31-33. Examination of these figures reveals both similarities and differences from the DSC curve of native PLA. The similarities are that all of the T_g s fall at or near 60°C and the T_m s are all in the neighborhood of 170°C. Not seen in the curve for native PLA is the large exothermic peak located near 100°C for all of the MB webs.

Table 4. Values obtained from DSC heating curves and calculated crystallinity for both micron-sized and nano-sized MB webs.
****Refers to a sample that was annealed at 80°C for 12hrs to obtain the maximum amount of crystallinity**

Sample ID	Mass (mg)	ΔH_c (mJ/mg)	ΔH_m (mJ/mg)	Normalized ΔH_c (J/g)	Normalized ΔH_m (J/g)	ΔH_m Infinity (J/g)	%Crystallinity
Micro							
1.5cmm,10cm	7.2	191.79	355	26.6375	49.30555556	93	24.4
1.5cmm,30cm	7.5	208.7	359.23	27.82666667	47.89733333	93	21.6
1.5cmm,50cm	6.9	189.69	346.24	27.49130435	50.17971014	93	24.4
10cmm,10cm	8.5	180.68	374.55	21.25647059	44.06470588	93	24.5
10cmm,30cm	7.3	150.66	378.39	20.63835616	51.83424658	93	33.5
10cmm,50cm	10	230.45	507.84	23.045	50.784	93	29.8
35cmm,10cm	9.6	167.71	497.3	17.46979167	51.80208333	93	36.9
35cmm,30cm	7.4	120.42	338.58	16.27297297	45.75405405	93	31.7
35cmm,50cm	11.5	182.27	540.17	15.84956522	46.97130435	93	33.5
1.5cmm,10cm HT**	7.7	0	391.39		50.82987013	93	54.7
PLA 6252D	10.3	0	372.82		36.1961165	93	38.9
Nano							
.8cmm,10cm	10.6	120.43	437.14	11.36132075	41.23962264	93	32.1
.8cmm,30cm	7.5	103.27	338.32	13.76933333	45.10933333	93	33.7
.8cmm,50cm	9.6	198	445.32	20.625	46.3875	93	27.7
2.3cmm,10cm	9.3	195.7	401.11	21.04301075	43.13010753	93	23.7
2.3cmm,30cm	8.3	117.55	370.85	14.1626506	44.68072289	93	32.8
2.3cmm,50cm	6.9	152.49	317.79	22.1	46.05652174	93	25.8
5.5cmm,10cm	8.2	85.23	344.75	10.39390244	42.04268293	93	34.0
5.5cmm,30cm	10.6	207.99	454.52	19.62169811	42.87924528	93	25.0
5.5cmm,50cm	7.7	150.56	339.5	19.55324675	44.09090909	93	26.4

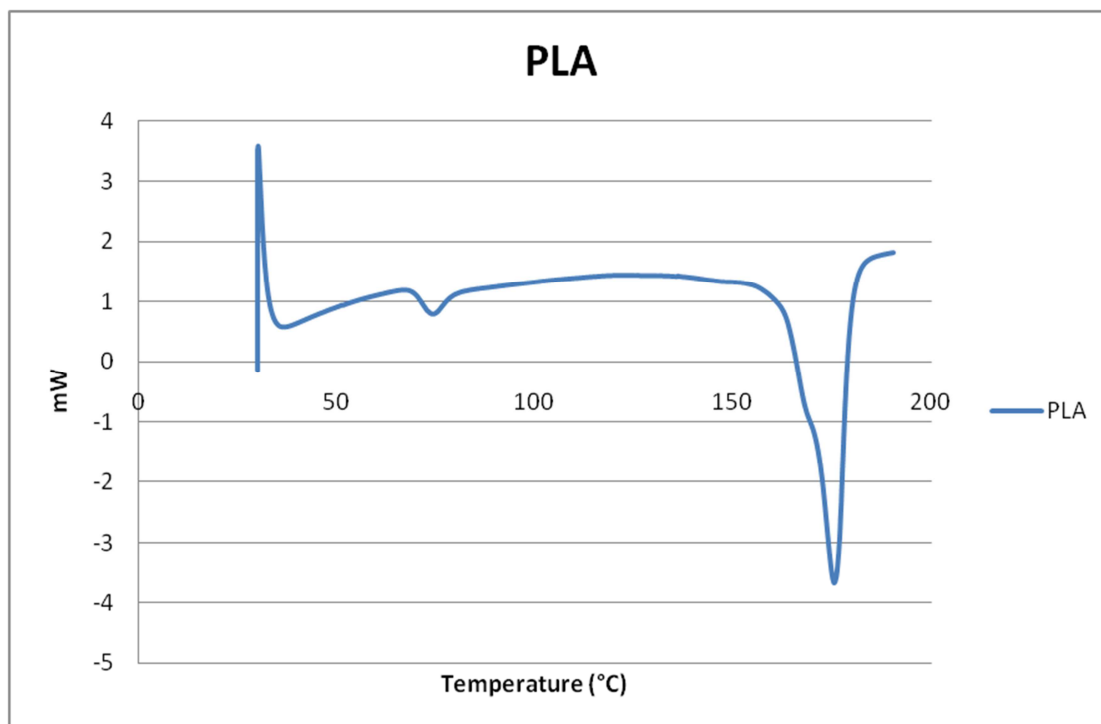


Figure 44. DSC heating curve for native NatureWorks 6252D

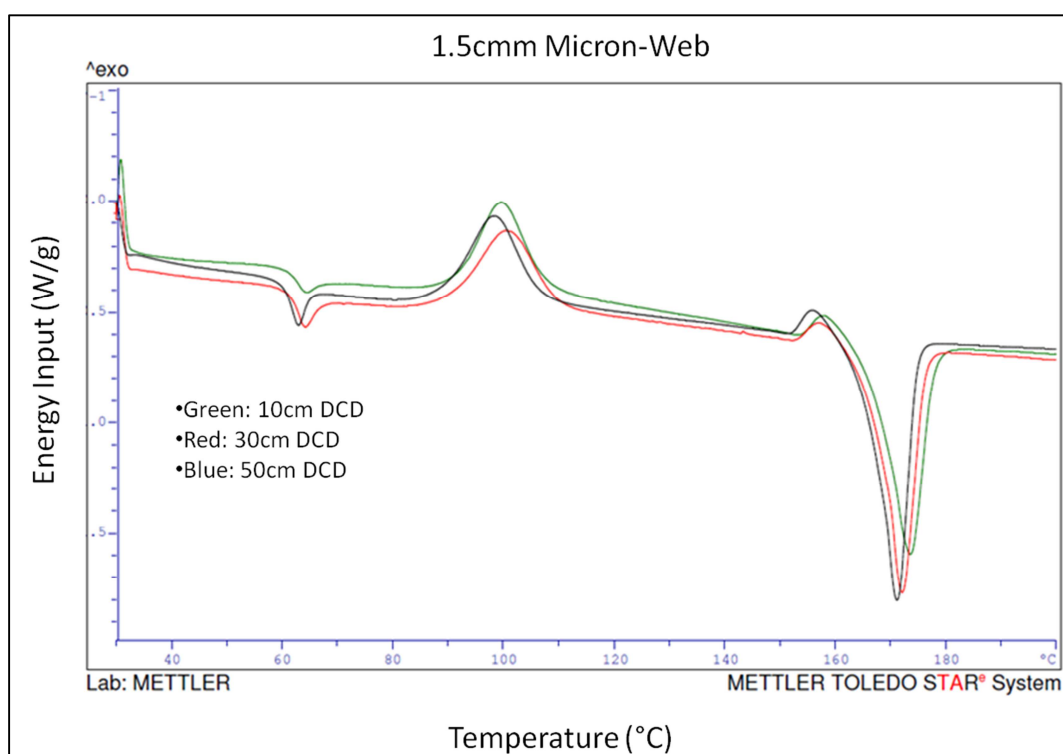


Figure 45. DSC heating curves for micron-sized 1.5mm MB webs

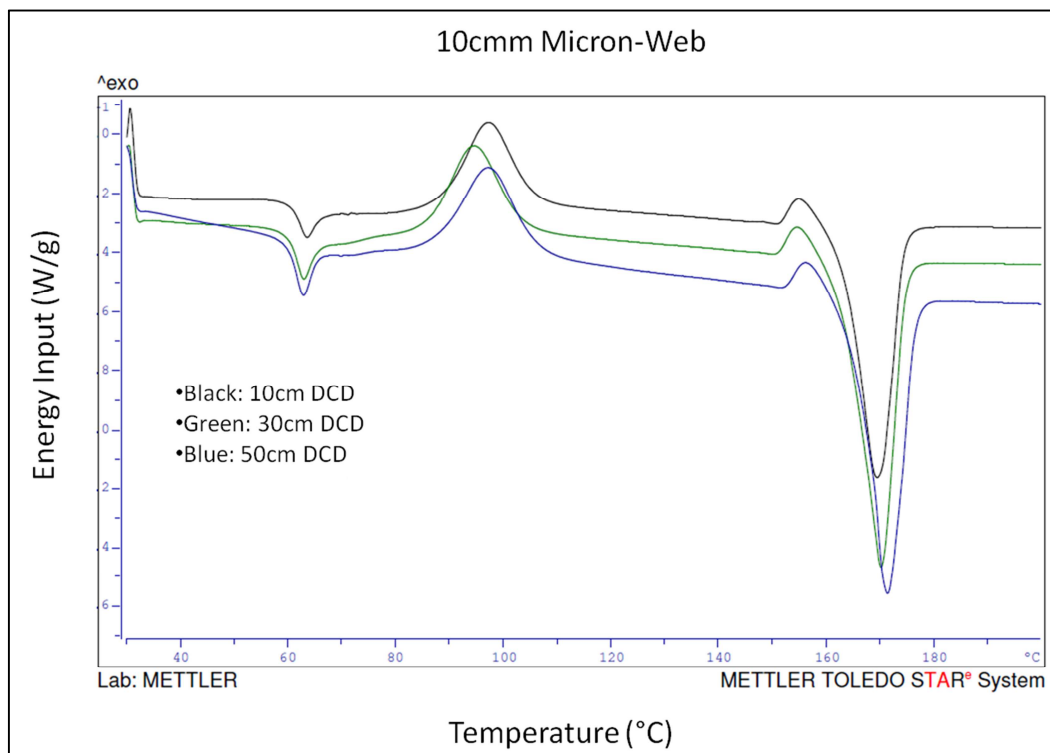


Figure 46. DSC heating curves for micron-sized 10cmm MB webs

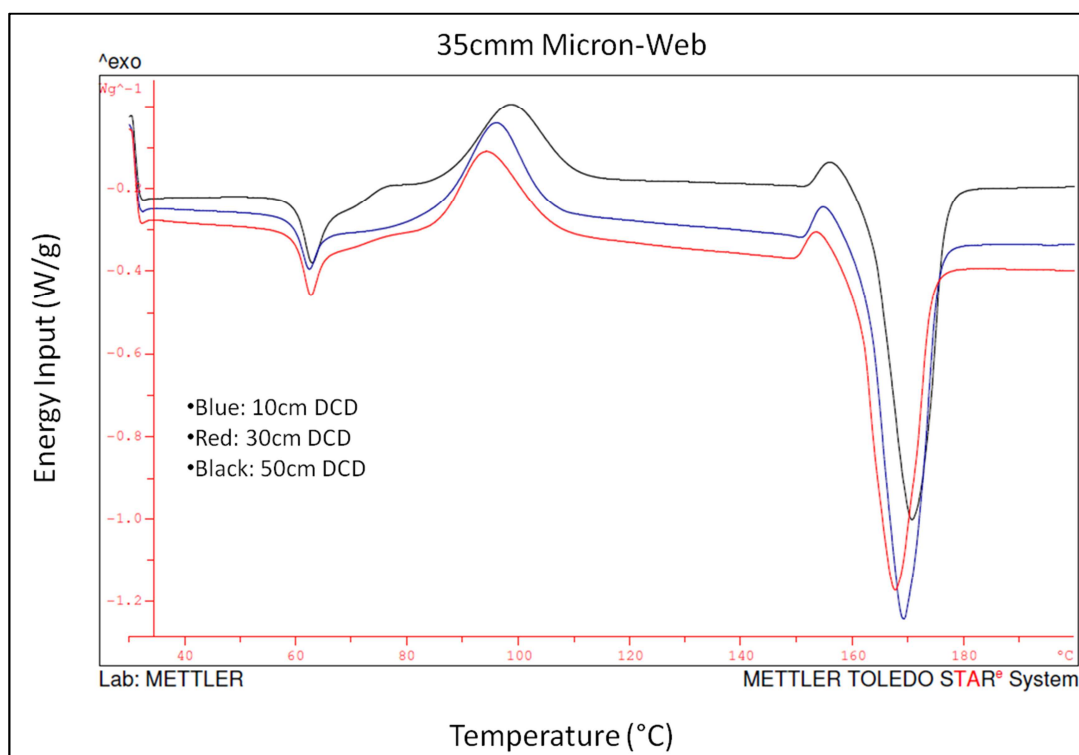


Figure 47. DSC heating curves for micron-sized 35cmm MB webs

Similarly, another, yet smaller, exothermic peak is located near 160°C, just before the PLA begins to melt.

The DSC heating curves of nano-sized webs MB at primary air flow rates of 0.8, 2.3 and 5.5cm are presented in figures 34-36. As seen in native PLA, the webs' T_g occurs near 60°C and they have a T_m of approximately 170°C. Similarly to the micro-sized webs, the nano-sized webs have two exothermic peaks, one near 100°C and the other close to 160°C.

Figure 37 contains both the heating and cooling DSC curves for an annealed micron-sized MB web produced using a primary air flow of 1.5cm. A noticeable difference about this thermogram is the absence of an exothermic peak located near 100°C as in the other MB webs. Also, the T_g endothermic peak is heavily depressed in this thermogram, signifying that the sample may have been at a temperature close to its T_g when the curve was acquired.

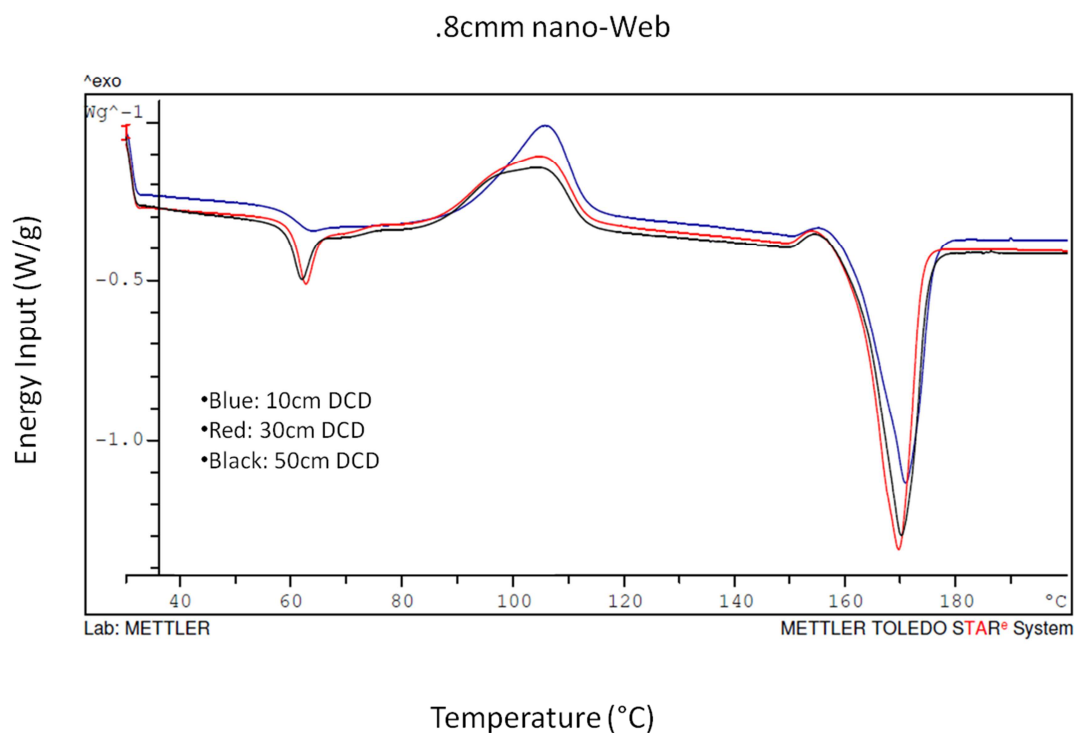
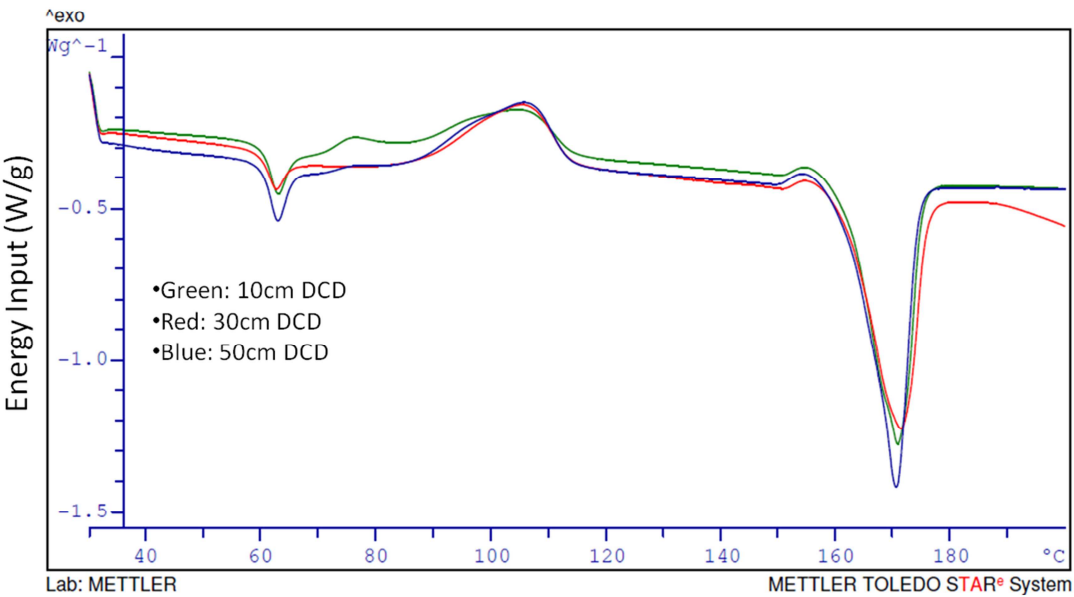


Figure 48. DSC heating curves for nano-sized 0.8cm webs

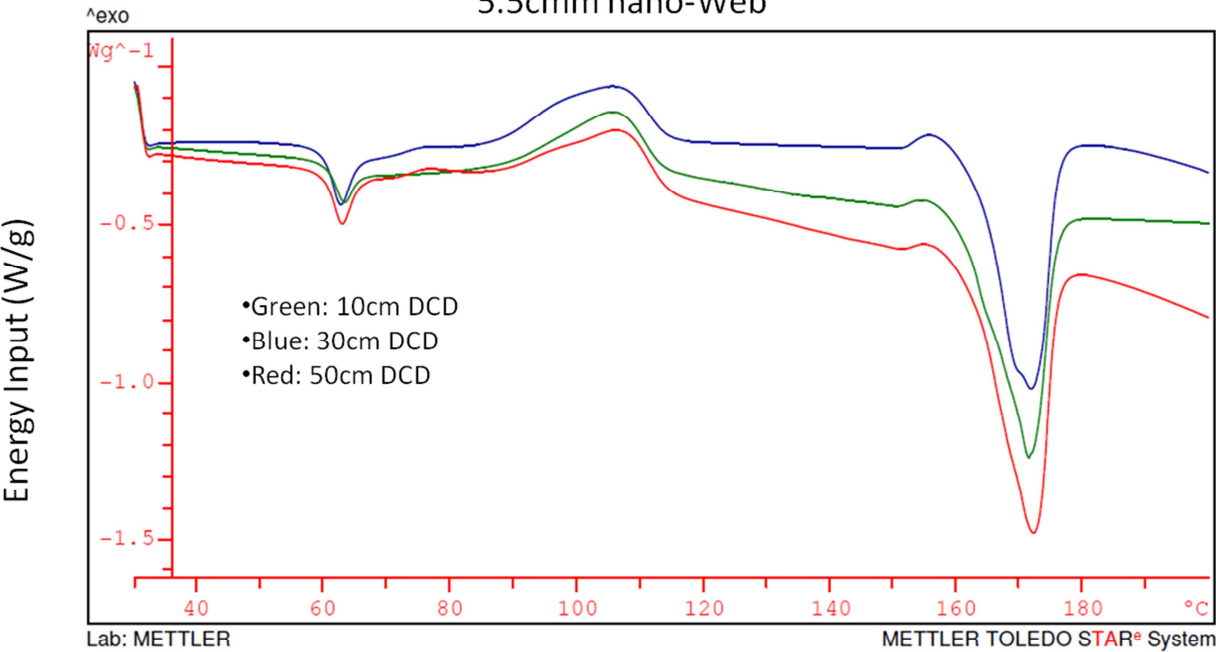
2.3cmm nano-Web



Temperature (°C)

Figure 49. DSC heating curves for nano-sized 2.3cmm webs

5.5cmm nano-Web



Temperature (°C)

Figure 50. DSC heating curves for nano-sized 5.5cmm webs

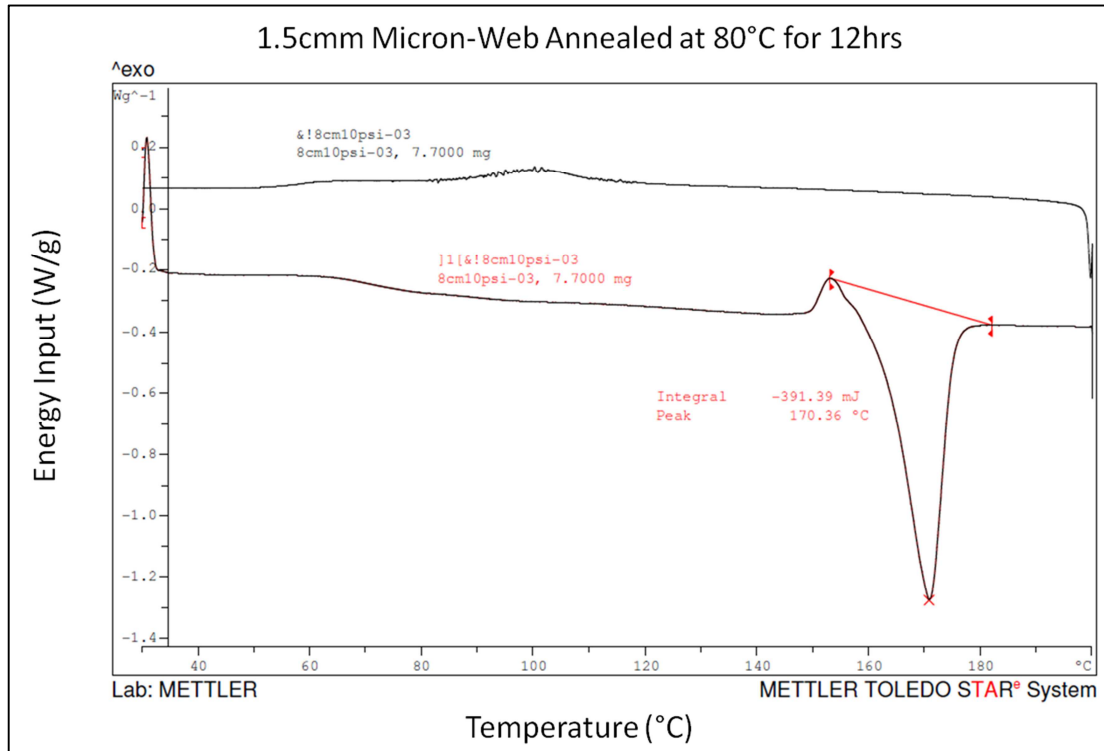


Figure 51. DSC curve for a web that was annealed at 80°C for 12 hours to negate crystallization upon DSC heating

3.1.7 PLA Web Degradation

Figures 38 and 39 contain the weight loss observed in the PLA webs over the 6 week degradation study. All of the samples studied decreased in mass from 1-5%, except the micron-sized webs produced using a primary air flow rate of 1.5cm. Examination of the weight loss for both the micron and nano-sized webs reveals no clear correlation between any processing parameter and degradation rate.

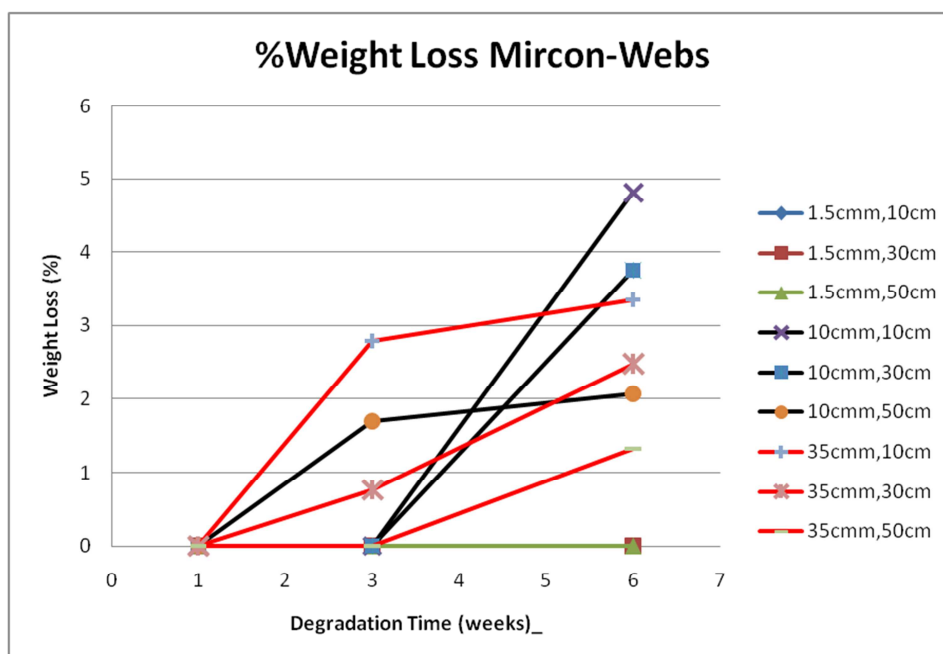


Figure 52. Weight loss versus time for PLA MB micron-webs

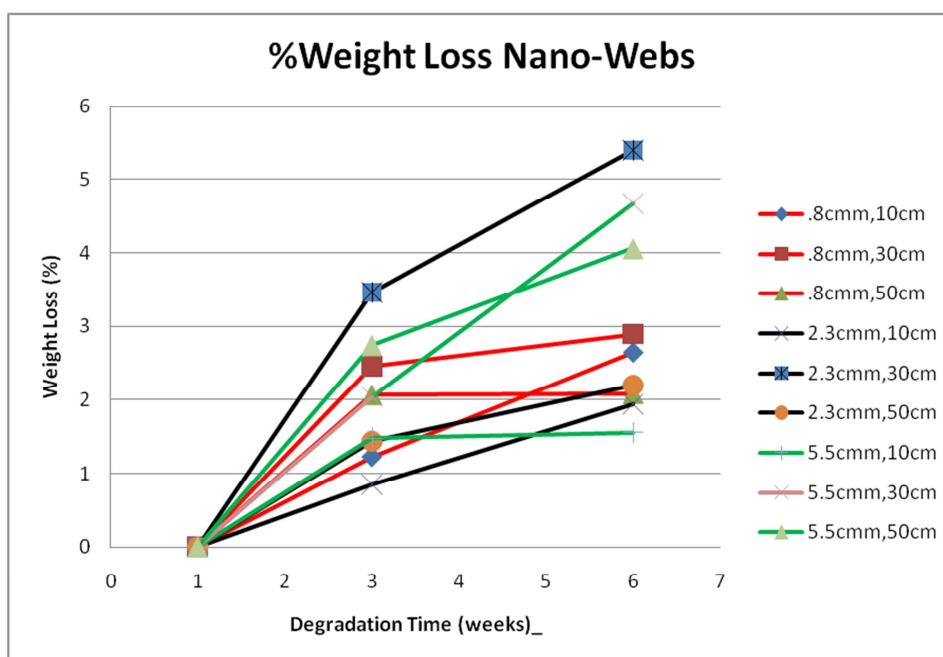


Figure 53. Weight Loss versus time for PLA MB nano-webs

3.1.8 Physical Properties Summary

A summary of the physical properties of the twenty MB webs presented in the results section is located in table 19. The webs are identified by the air flow rate at which they were formed in cubic meters per minute (cmm), followed by the DCD at which they were collected. The four webs that were chosen to be used as scaffolds for cell seeding are highlighted in the table.

Table 5. Summary table of the physical properties of the MB webs. The highlighted samples represent those that were selected for use as tissue engineering scaffolds

Summary of MB Web Physical Properties								
Sample ID	Mean Fiber Diameter (um)	Mean Pore Diameter (um)	Air Permeability (cmm)	Thickness (mm)	Tensile Modulus MD (kPa)	Tensile Modulus TD (kPa)	Weight Loss @6Wks (%)	Crystallinity (%)
Micro								
.8cmm,10cm	10.48	50	25.37	0.492	434	154	NA	NA
.8cmm,30cm	10.43	60	33.01	0.511	65	76	NA	NA
.8cmm,50cm	10.42	71	38.64	0.566	31	41	NA	NA
1.5cmm,10cm	6.93	30	8.61	0.224	585	720	0	24.4
1.5cmm,30cm	7.02	51	17.58	0.39	109	178	0	21.6
1.5cmm,50cm	6.72	44	21.27	0.52	41	99	0	24.4
10cmm,10cm	3.55	16	2.08	0.23	2214	1343	4.8	24.5
10cmm,30cm	3.66	29	5.94	0.219	475	538	3.75	33.5
10cmm,50cm	3.49	38	9.95	0.447	74	193	2.06	29.8
35cmm,10cm	1.82	11	1.28	0.288	2490	1097	3.35	36.9
35cmm,30cm	1.83	25	5.26	0.281	625	461	2.48	31.7
35cmm,50cm	1.9	35	6.08	0.329	277	340	1.32	33.5
Nano	(nm)							
.8cmm,10cm	493	15	1.63	0.356	447	340	2.65	32.1
.8cmm,30cm	818	19	2.61	0.451	157	141	2.89	33.7
.8cmm,50cm	518	27	3.91	0.497	99	84	2.08	27.7
2.3cmm,10cm	452	10	1.1	0.331	919	406	1.94	23.7
2.3cmm,30cm	621	15	1.99	0.287	360	304	5.4	32.8
2.3cmm,50cm	670	19	2.66	0.469	125	108	2.2	25.8
5.5cmm,10cm	495	9	0.91	0.3	860	457	1.55	34.0
5.5cmm,30cm	588	14	2.02	0.421	179	166	4.67	25.0
5.5cmm,50cm	626	16	2.39	0.335	154	145	4.06	26.4

3.2 Scaffold Seeding

3.2.1 Electron Microscopy of Single Cells

SEM was performed on the seeded scaffolds to examine both single cell-fiber interactions as well as cell colony-scaffold interactions after 24 hours of cell culture. Figures 40-43 contain images of single cells and cell colonies attached to micron-sized PLA fibers. Figure 40 contains an image of a single malignant melanoma cell attached to a single isolated PLA micron-sized fiber.

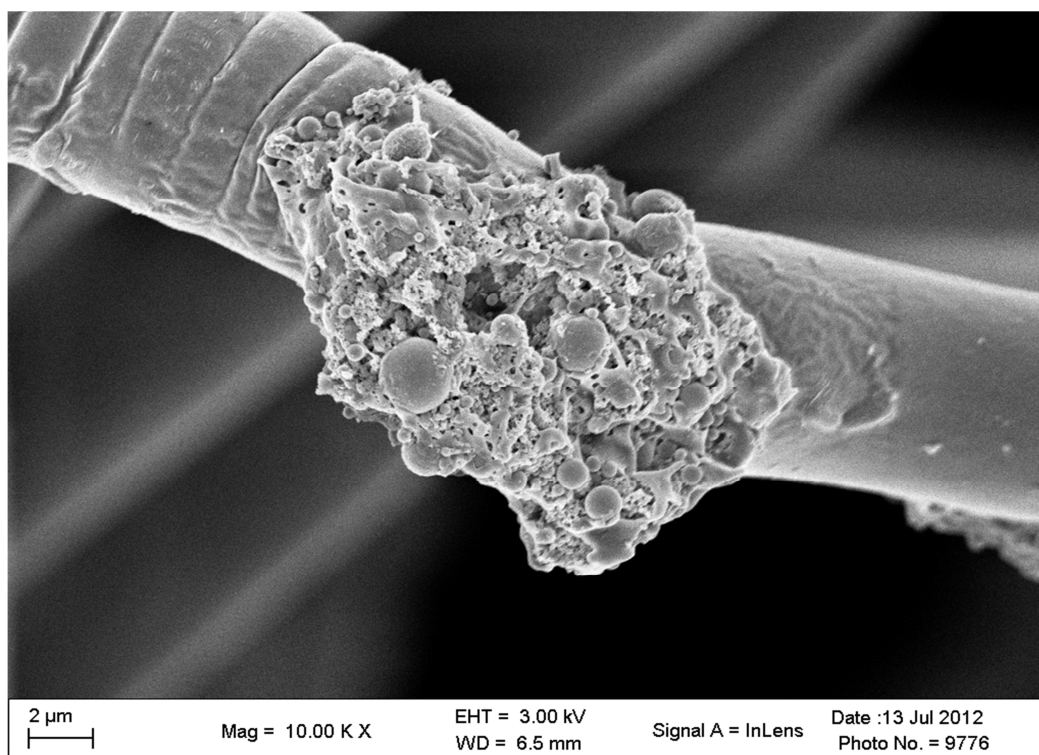


Figure 54. SEM micrograph of a single human malignant melanoma cell attached to a micron-sized fiber

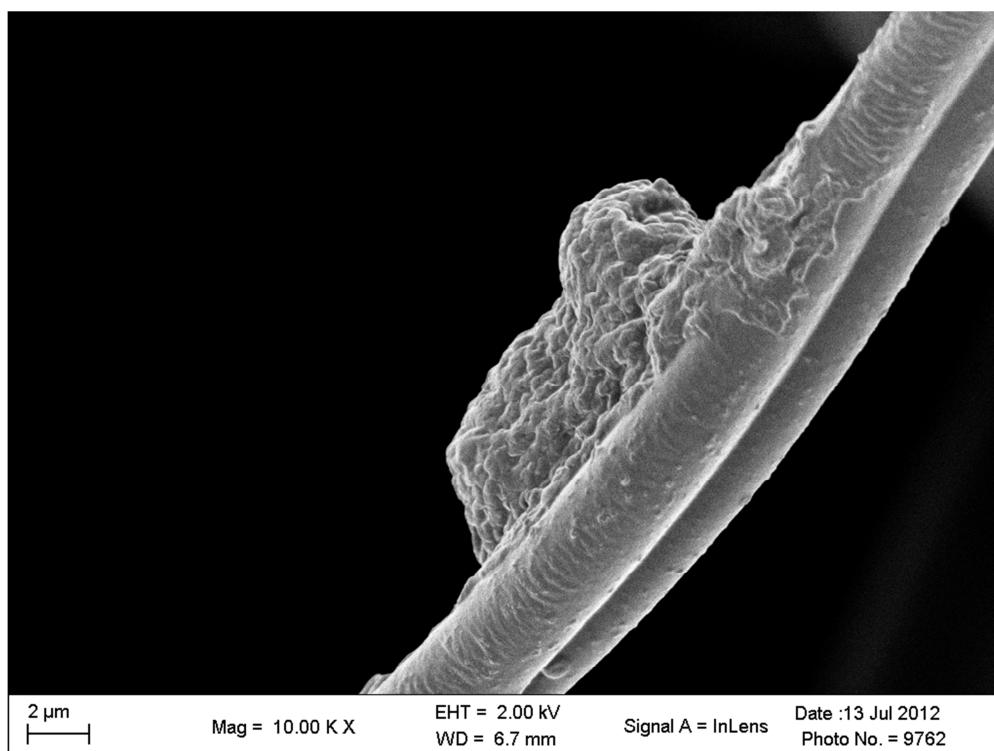


Figure 55. SEM micrograph of a single cell attached to a micron-sized PLA fiber

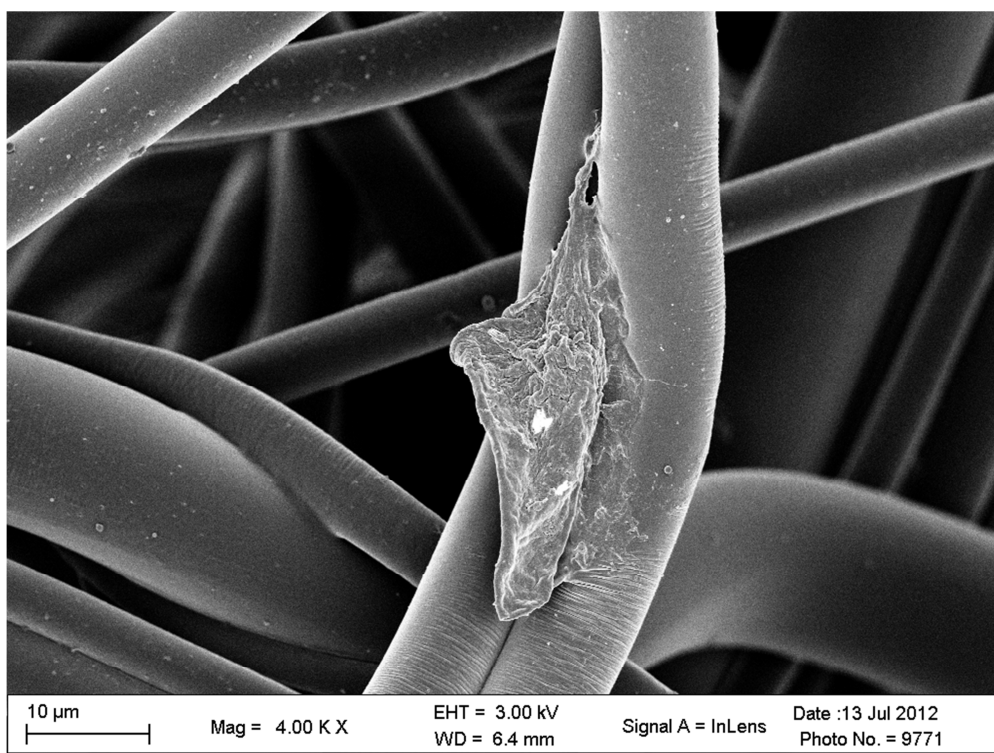


Figure 56. SEM micrograph of a single cell attached to a micron-sized PLA fiber

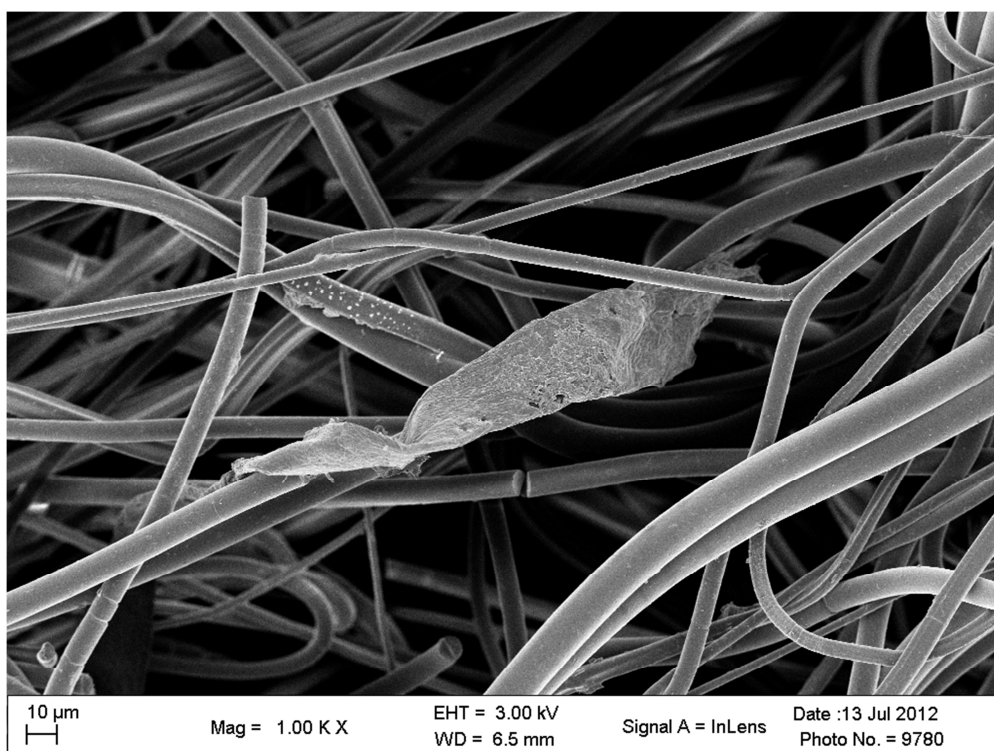


Figure 57. SEM micrograph of a group of cells spreading down two PLA fibers

Notice the cell only assumes a diameter as wide as the fiber it is on, and begins to spread along the long axis of the fiber. Figure 41 is a side view of a single melanoma cell attached to a PLA fiber. Notice the cell remains bunched up and only assumes a width equal to the diameter of the fiber. Propagation begins as the cell wraps around the fiber in its early stages of spreading. Figure 42 is an image of a single melanoma cell attached across two micron-sized fibers. As the cell is in contact with two adjacent fibers it has begun to spread both laterally and along the fibers' long axes. The width of the cell is now greater than the diameter of one fiber. Figure 43 shows multiple cells spreading along two adjacent PLA fibers. The cells have assumed a width approximately equal to the width of the two guiding fibers. The direction of propagation is along the long axes of the fibers in both directions. Adjacent fibers in this image are 20-60 μm away.

Images of single cells seeded on nano-sized PLA fibers are presented in figures 44-47. Figure 44 contains the image of a single cell that is seeded on nano-fibers that are located only a few microns from one another. It is evident that the cell has spread across several fibers evenly in the x and y-directions. In the top right corner of the image it is apparent that once the cell has spread to a fiber that is isolated it begins to propagate along the long axis of the fiber. Coinciding with this, the cell at that point does not spread larger than the diameter of the fiber. Figure 45 shows the effects of cell seeding on an isolated nano-fiber. The cell in the image has clearly attached to a sub-micron sized fiber that does not have any neighbors. Consequently, the cell has balled up and failed to spread. Above and to the right of the balled up cell another cell has seeded itself on a much larger diameter group of bundled/coalesced fibers. This cell has begun to spread along the long axis of the fiber bundle it is resting on. Figure 46 shows the benefits of having nano-fibers adjacent and crossing over micron-sized fibers. It is evident that the cell in the image is propagating along the long axis of several micron sized fibers situated next to one another. At the junctions of many nano-micron fiber interactions it can be seen that the cell has begun to widen and bridge the gaps between the neighboring fibers using the nano-fibers as a bridge. Figure 47 shows a

single cell seeded on the junction of many nano-fibers. The cell has spread in 360° degrees and has begun to propagate along the long axes of the fibers as they move away from the junction.

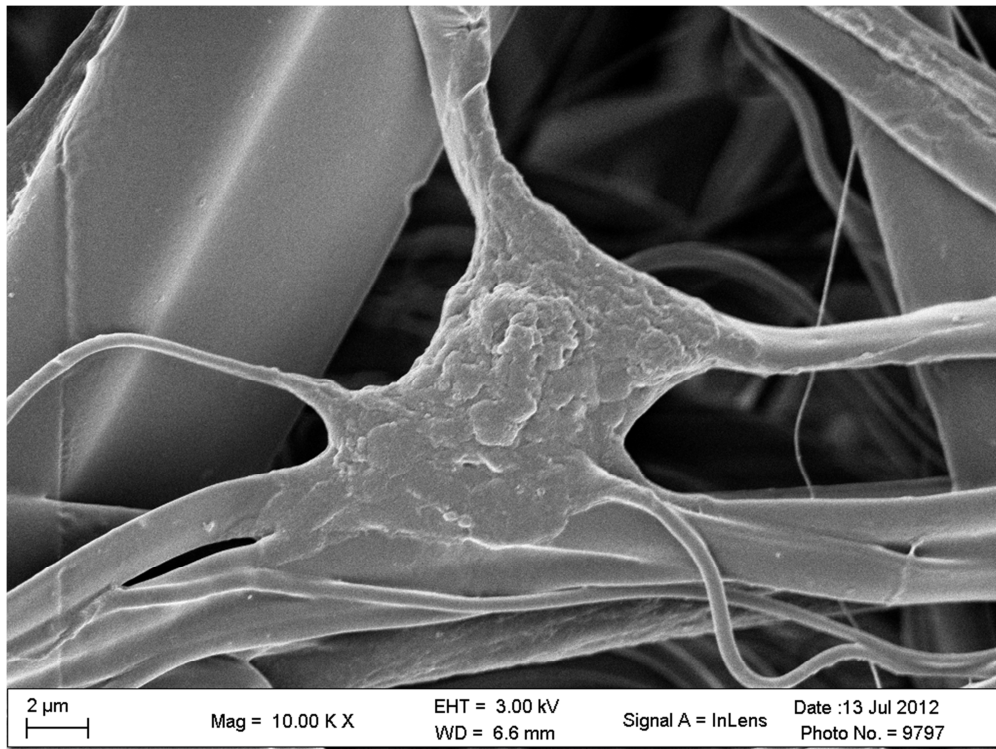


Figure 58. Single melanoma cell seeded on nano-sized PLA fibers

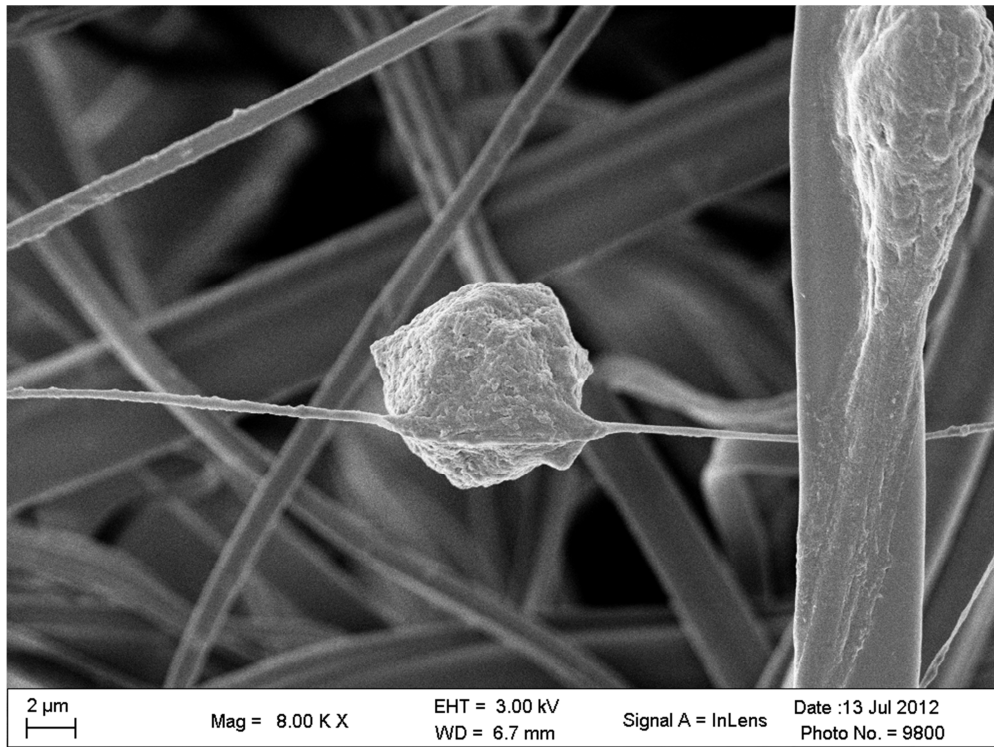


Figure 59. Single cell attached to an nano-sized fiber of PLA that is isolated from adjacent fibers

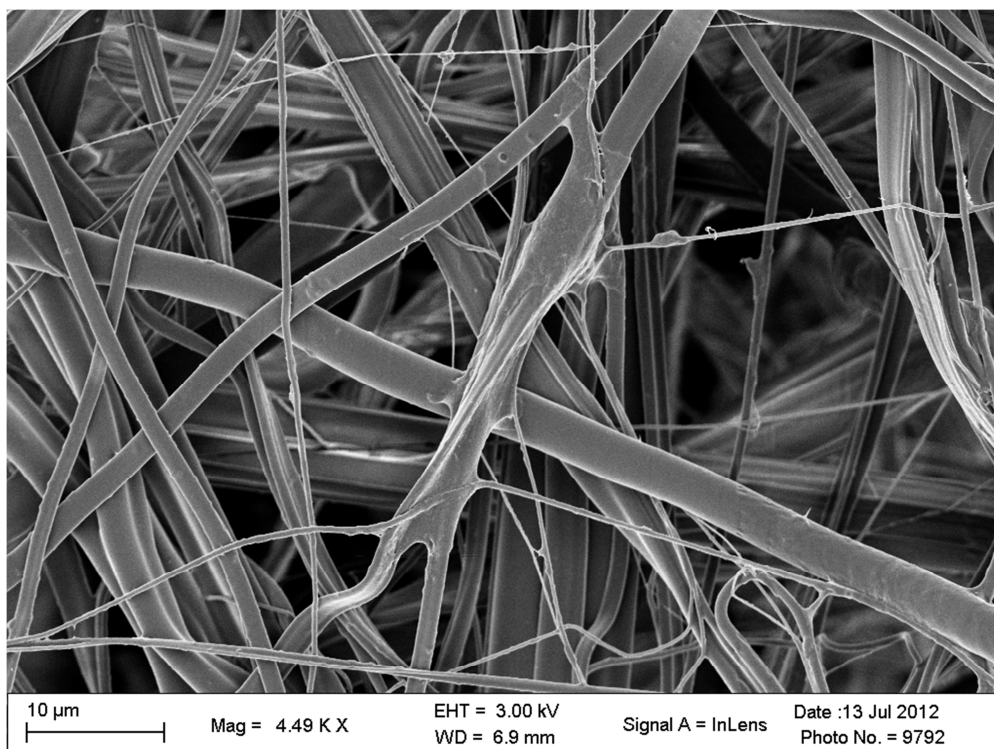


Figure 60. Single cell seeded on nano-sized PLA fibers spreading in multiple directions

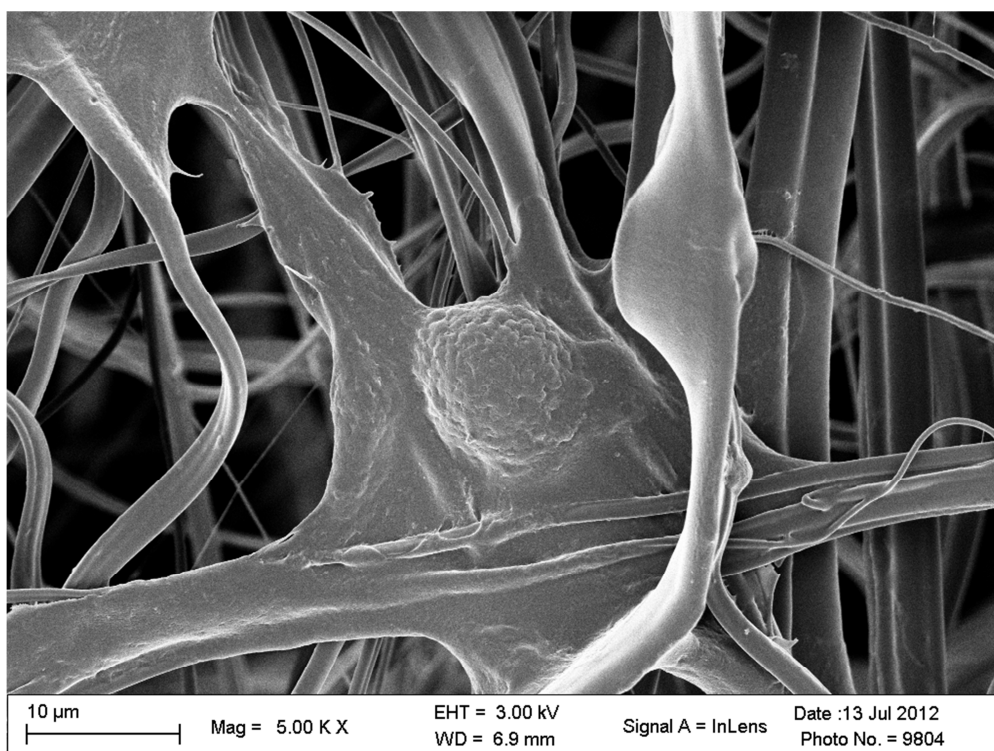


Figure 61. Single fiber seeded on nano-sized PLA fibers propagating in multiple directions

3.2.2 Electron Microscopy of Multiple Cells

3.2.2.1 Micron-Fiber Size Webs Produced using a Primary Air Flow of 1.5cm Collected at 30cm

Clusters of melanoma cells seeded on micro-sized scaffolds MB using a primary air flow rate of 1.5cm, collected at 30cm are presented in figures 48-52. Figure 48 shows that the cells adhered to the scaffold well and began to form sheets in the short 24 hour incubation time period. It can be seen that although the cells formed sheets, they had difficulty spreading across the whole area of the scaffold due to large gaps between adjacent fibers. Areas of spreading difficulty can be seen by the yellow and white arrows in the figure.

Figure 49 shows that the sheets of cells grew preferentially along the long axes of the micron sized fibers. It appears that most of the spreading occurred by across contacting adjacent fibers and at fiber-fiber junctions and cross points. The colonization of a bundle of fibers is presented in figures 50-52.

Figure 50 shows cells propagating along the long axis of the bundle of fibers located in the middle of the image(denoted by a yellow and white arrow)and eventually engulfing the circumference of the fiber. Closer examination of the engulfed fiber reveals a few points of interest. First, significant damage was imparted to the scaffold during sample preparation, noted as the large fissures that penetrate the depth of the cellular sheets. Evidence of damage can also be seen pock marked surface visible in figures 51 and 52. The second piece of information that is revealed is that these in fact are multiple colonies of cells. If the images seen in figures 51 and 52 are compared to that found in figure 40, a clear resemblance can be established between the single cell seen in figure 40 and the colonies of cells seen in figures 51 and 52.

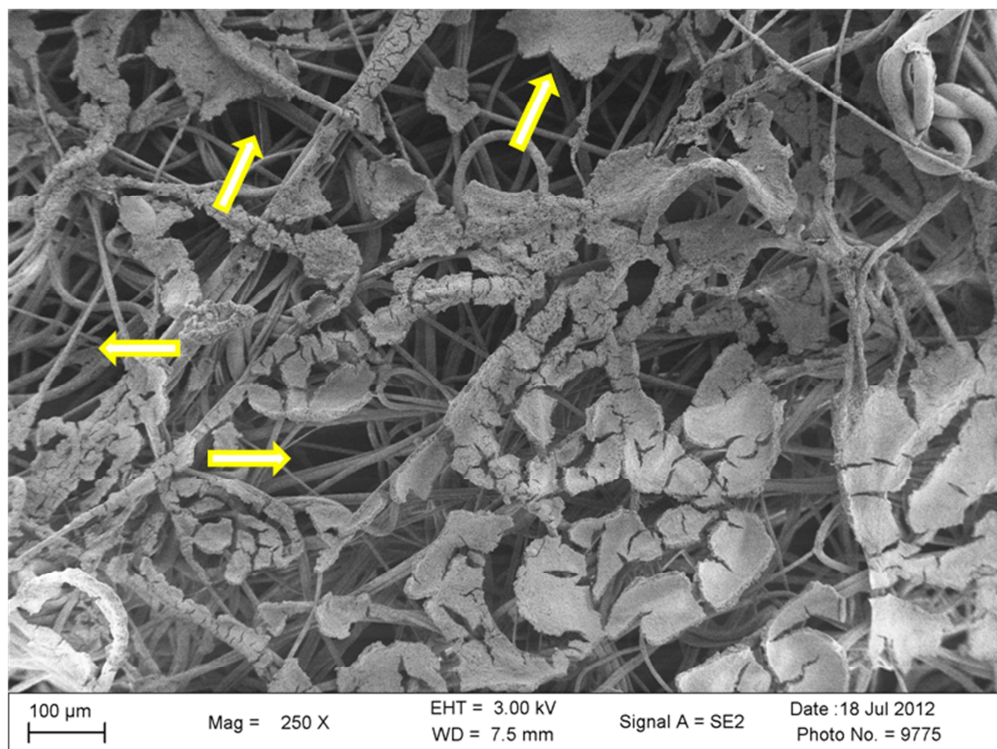


Figure 62. Cell colonies seeded on a micro-fiber sized MB scaffold processed using a primary air flow rate of 1.5cmm, collected at 30cm. The yellow and white arrows represent areas of difficulty for cell sheet spreading

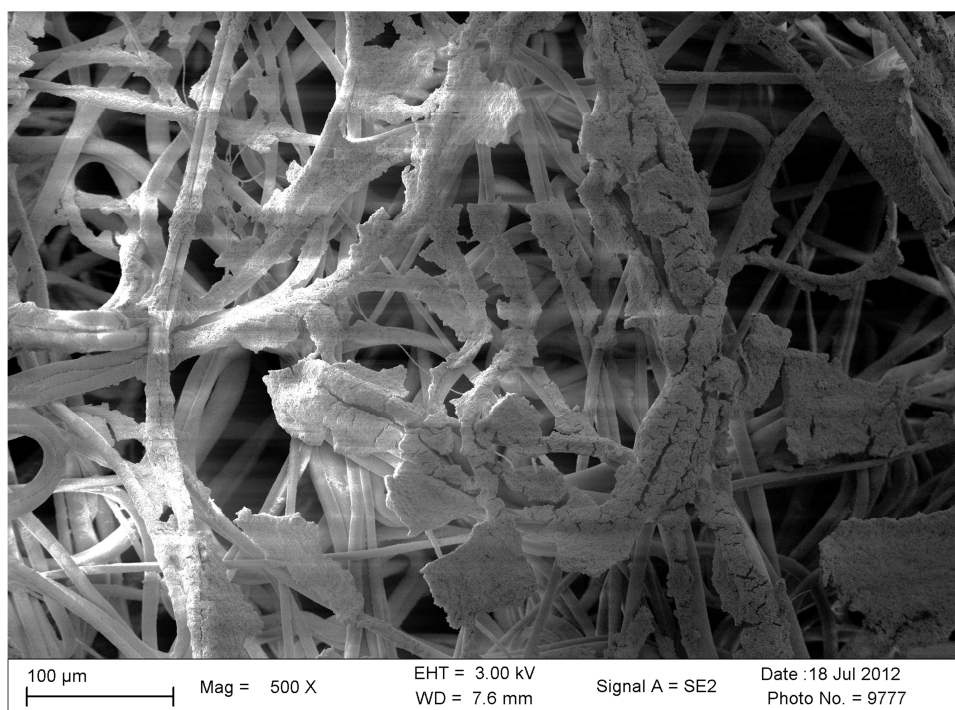


Figure 63. Preferential growth of cell colonies seeded on micro-fiber sized MB scaffold processed using a primary air flow rate of 1.5cm, collected at 30cm

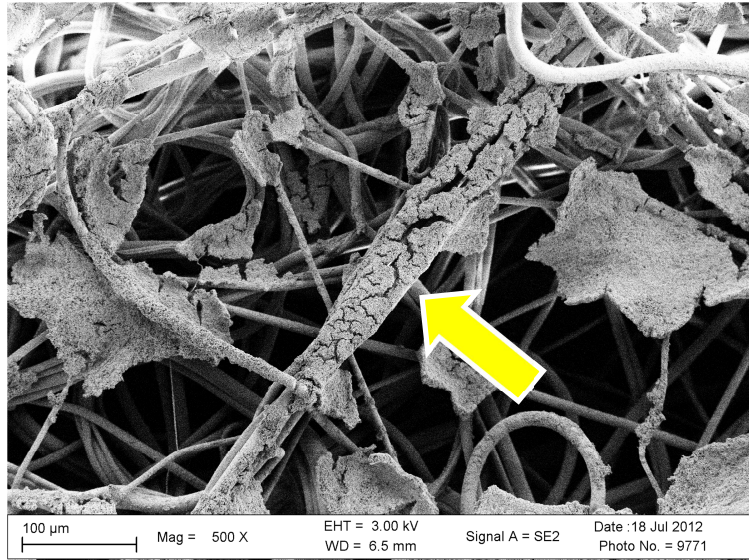


Figure 64. Cell colonies engulfing a cluster of fibers on micro-fiber sized MB scaffold processed using a primary air flow rate of 1.5cm, collected at 30cm

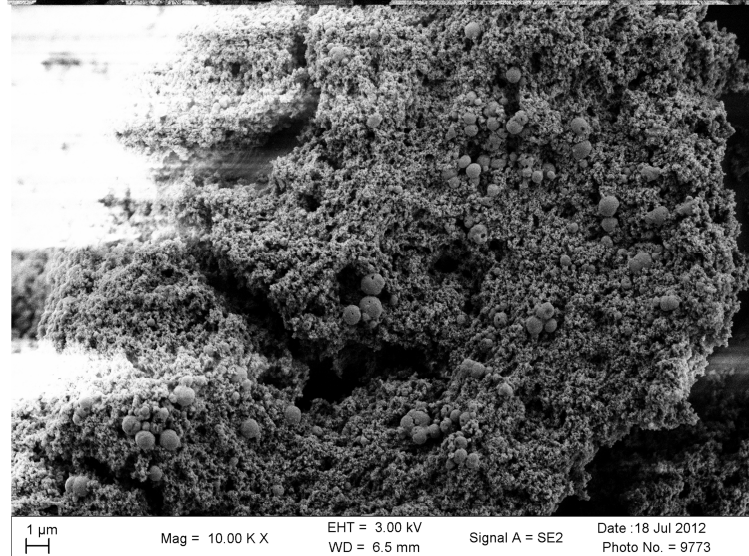


Figure 65. Magnification of cell colonies on a bundle of micro sized fibers

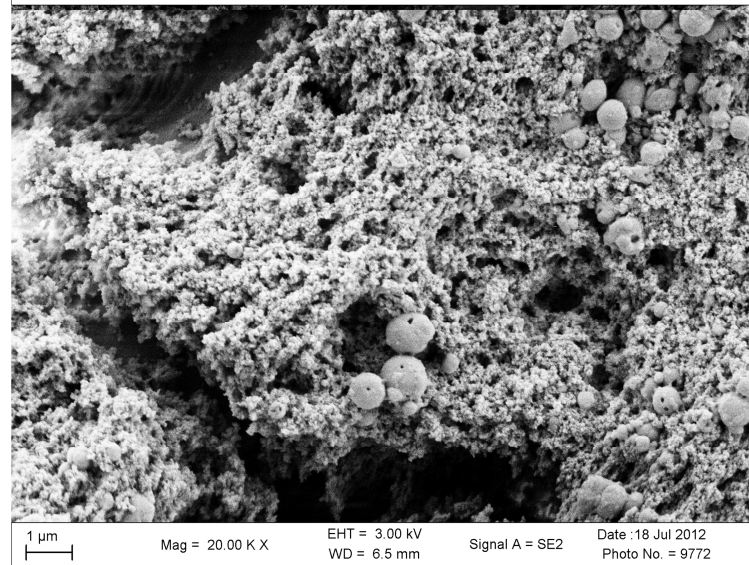


Figure 66. Enlarged image of cells colonizing a bundle of PLA micro-sized fibers on a MB scaffold processed using a primary air flow rate of 1.5cm, collected at 30cm25kX

3.2.2.2 Micron-Fiber Size Webbed Produced using a Primary Air Flow of 1.5cmm Collected at 50cm

The cell colonization on the MB webs produced using a primary air flow rate of 1.5cmm collected at 50cm was similar to the characteristics display on the scaffold produced at a primary air flow rate of 1.5cmm collected at 30cm. Figure 53 shows colonization across the scaffold. It can be seen that colonization proceeded in a similar manner, but spreading appears to be better facilitated by the more random web structure produced at the larger DCD. The frontal boundary of the cell migration encountered the same problem of large gaps between adjacent fibers as was evident in the seeded web collected at 30cm. The yellow and white arrows in figure 53 depict the large gaps encountered by the cells.

One method by which the cells were able to migrate across the scaffold is presented in figure 54. It is evident that the webbed structure provided bridges between parallel fibers for cells to move across the gaps between them. It is also important to note the vacancy of cells exist across the large gaps between parallel fibers where no connecting fibers were present. Once again, damage to the cell sheets due to the sample preparation process can be seen by the large fissures penetrating through the sheets and the appearance of a pock marked surface. Examination of the scaffold surface in figure 51 also shows that cell penetration into the depth of the scaffold appears to be pore.

The cell migration frontal boundary is presented in figure 55, denoted by the yellow and white arrows. Inspection of the image shows that the cells appear to be migrating from the top left of the image to the bottom right of the image with a well defined sheet of cells slowly dissipating into a less dense region. Other evidence to support this claim is the migration of cell below the top layer of fibers. As the cells move from the top left to the bottom right of the image it can be seen that they follow a path dictated

by the original fibers they adhered to. Once again, large pore gaps can be seen where cells were unable to connect with adjacent colonies across from them.

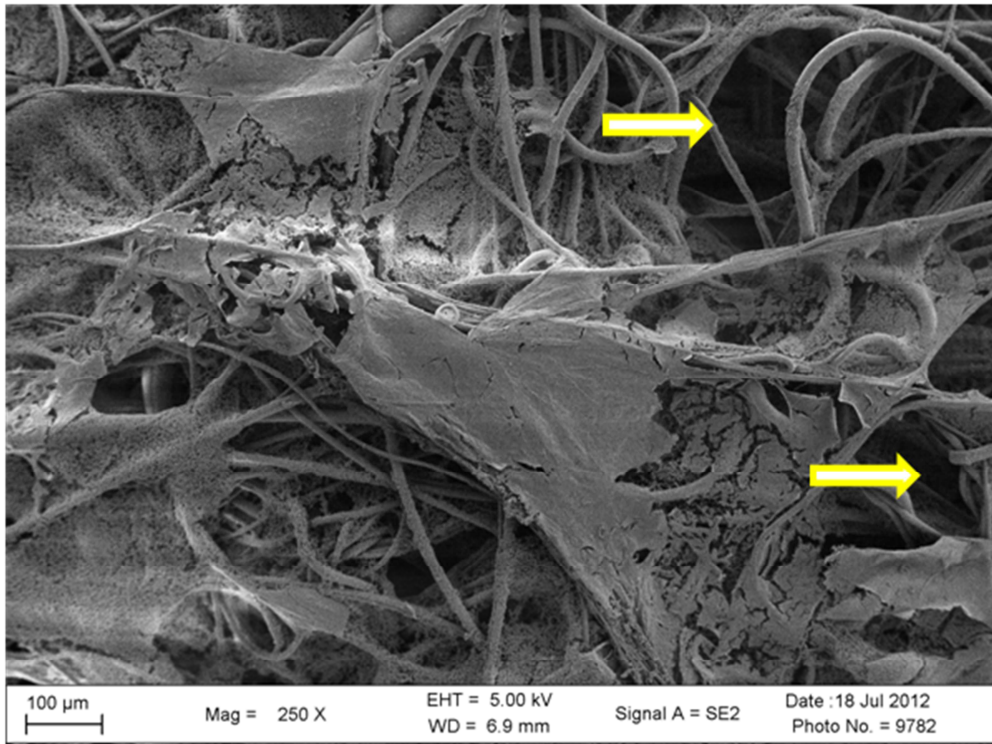


Figure 67. Cell colonies seeded on a micro-fiber sized MB scaffold processed using a primary air flow rate of 1.5cm/s, collected at 50cm. The yellow and white arrows represent areas of difficulty for cell sheet spreading

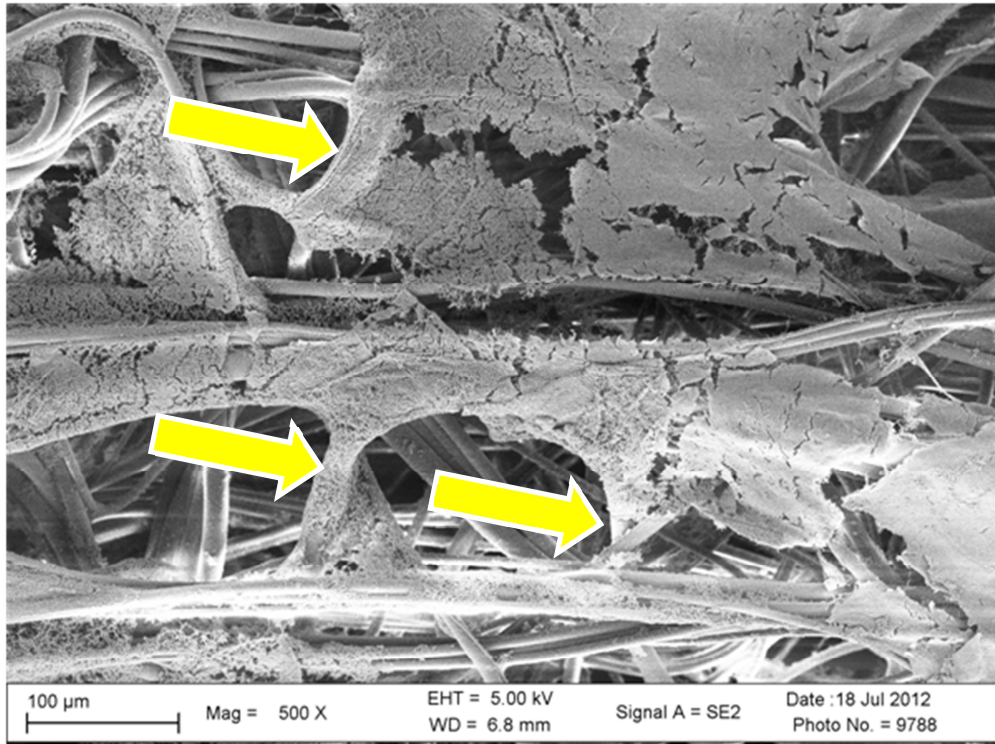


Figure 68. Cell spreading using crossing fibers as bridges. Yellow and white arrows point out the areas of interest

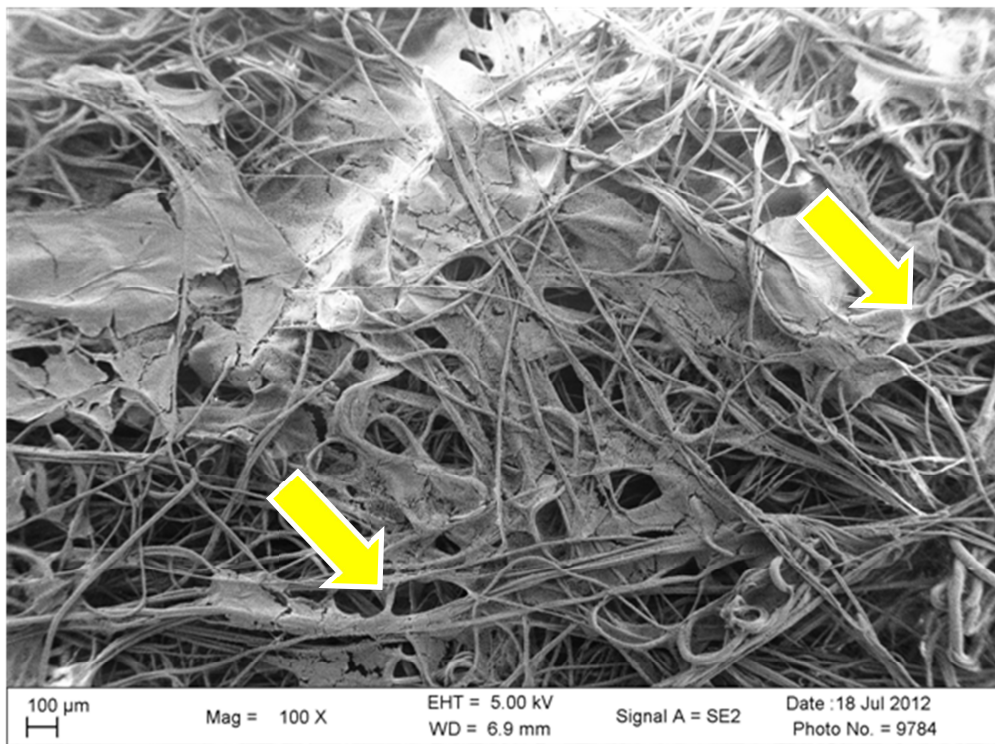


Figure 69. Cell migration frontal boundary

Figure 56 contain two images of a bundle of fibers that were engulfed by migrating cells at two different magnifications. In the image on the left it can be seen that the cells migrated along the long axes of the micron-sized fibers as well as around the circumference of the fiber bundle.

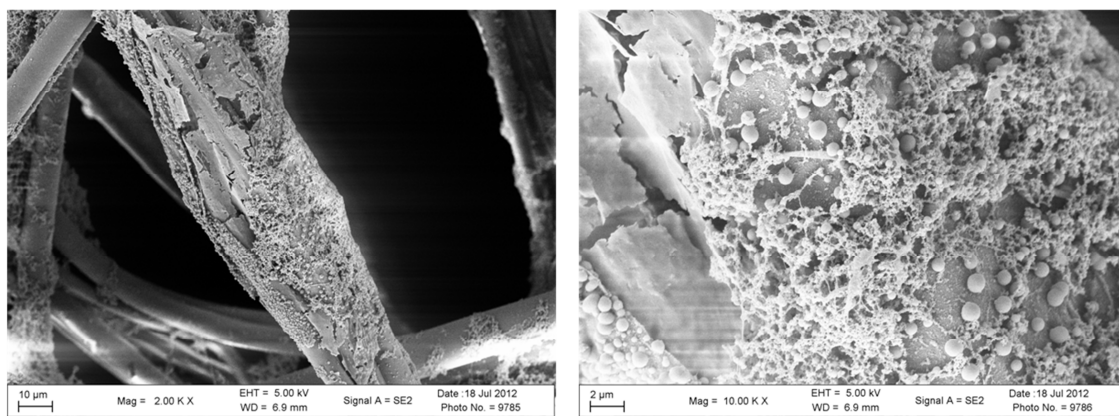


Figure 70. Cells engulfing a cluster of micron-sized fibers in a PLA web MB using a primary air flow rate of 1.5cm, collected at 50cm from the die exit

This provides more evidence that the cells will not spread beyond the boundaries on the fiber bundle diameter when no adjacent fibers or bridging fibers are present. The image in the right of figure 56 was taken at 10kX. The image depicts the morphology associated with the malignant melanoma cells seeded on the scaffold.

3.2.2.3 Nano-Fiber Size Webs Produced using a Air Flow of 0.8cm Collected at 30cm

Figures 57-59 contain images of cell colonization on PLA webs MB with a primary air flow rate of 0.8cm collected at 30cm from the die exit. Figure 57 shows that the colonization of the nano-sized web was much more thorough than that displayed on the micron-sized webs. Evidence of this can be seen by the lack of visible open pores. The image also shows that there is no preferred direction for cell migration. The cells appear to spread evenly across the scaffold and do not use particular fibers as their

directional guides. It should also be noted that the nucleus of many more cells are visible as spherical masses on the webs.

Figure 58 displays the spreading of the cells across the nano-sized fibers. The cells appear to spread across the gaps between the fibers without the aid of bridging fibers. The spread cells also appear to be very flat or thin in nature compared to those spread across the micron-webs. Also, cell penetration into the depth of the scaffold seems to be deeper than that exhibited on the micron-webs. Figure 59 displays the frontal growth boundary of the propagating cell colonies. It can be seen that the cells fill out the nano-sized scaffold more uniformly in the left to right direction before making forward progress, as the boundary appears to be one solid moving mass. This again signifies that the cell propagation is not as strictly regulated by the macro-orientation of the fibers.

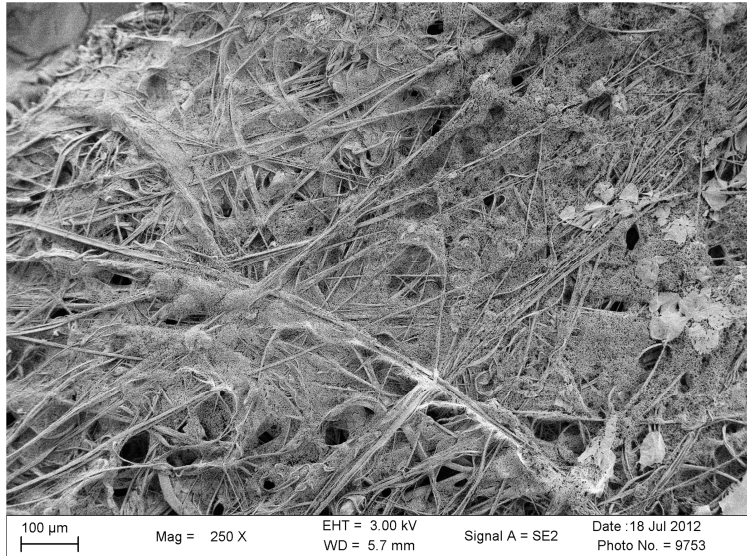


Figure 71. Cells seeded on a nano-fiber melt blown scaffold created using primary air flow of 0.8cmm collected at 30cm

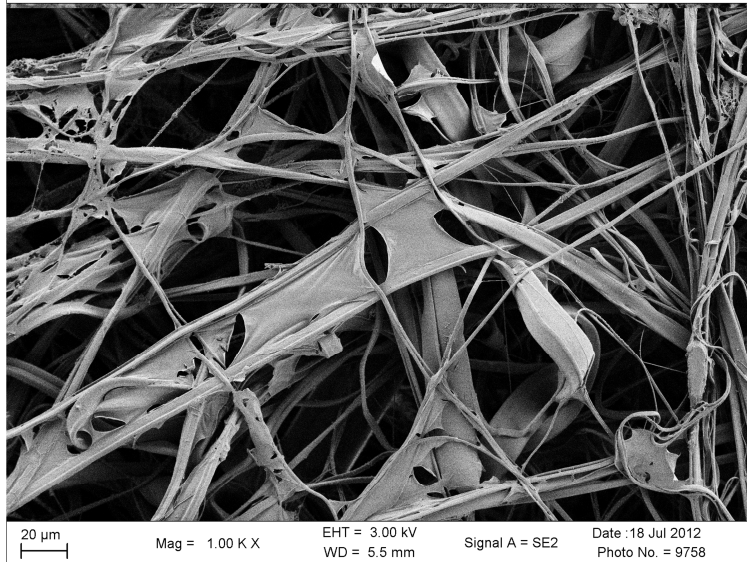


Figure 72. Cell spreading across nano-sized fibers for a MB scaffold using a primary air flow of 0.8cmm collected at 30cm



Figure 73. Frontal boundary of cell growth for cells seeded on a nano-sized scaffold MB at 0.8cmm collected at 30cm

3.2.2.4 Nano-Fiber Size Webs Produced using a Primary Air Flow of 0.8cmm Collected at 50cm

Figure 60 displays the dense colonization of nano-fiber sized MB PLA webs produced using a primary air flow rate of 0.8cmm, collected at 50cm. It can be seen that the spreading of the cells does not appear to occur in any one primary direction. As was the case with the micron-sized webs collected at 30 and 50cm, the nano-sized web collected at 50cm has more random and more dense fiber lay down than the web collected at a DCD of 30. Evidence of this can be seen by the lack of large pores between fibers and lack of fibers preferentially oriented in one direction. Figure 61 shows the boundary line of the migrating cell colonies as they approach a large pore. It can be seen that the cells migrated around the large pore and were unable to bridge the gap it produced. Once again, the cell colonies appear to migrate uniformly across the scaffold. Figure 61 also shows evidence of cell damage caused by the sample preparation process.

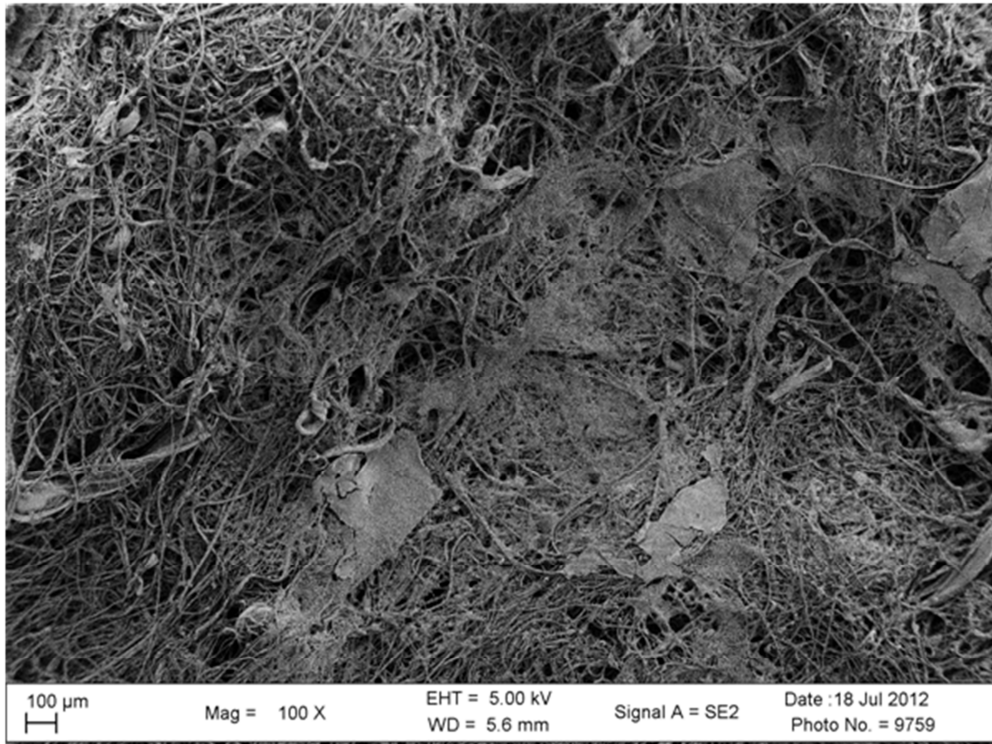


Figure 74. Cell colonies seeded on a micro-fiber sized MB scaffold processed using a primary air flow rate of 0.8cm/s, collected at 50cm.

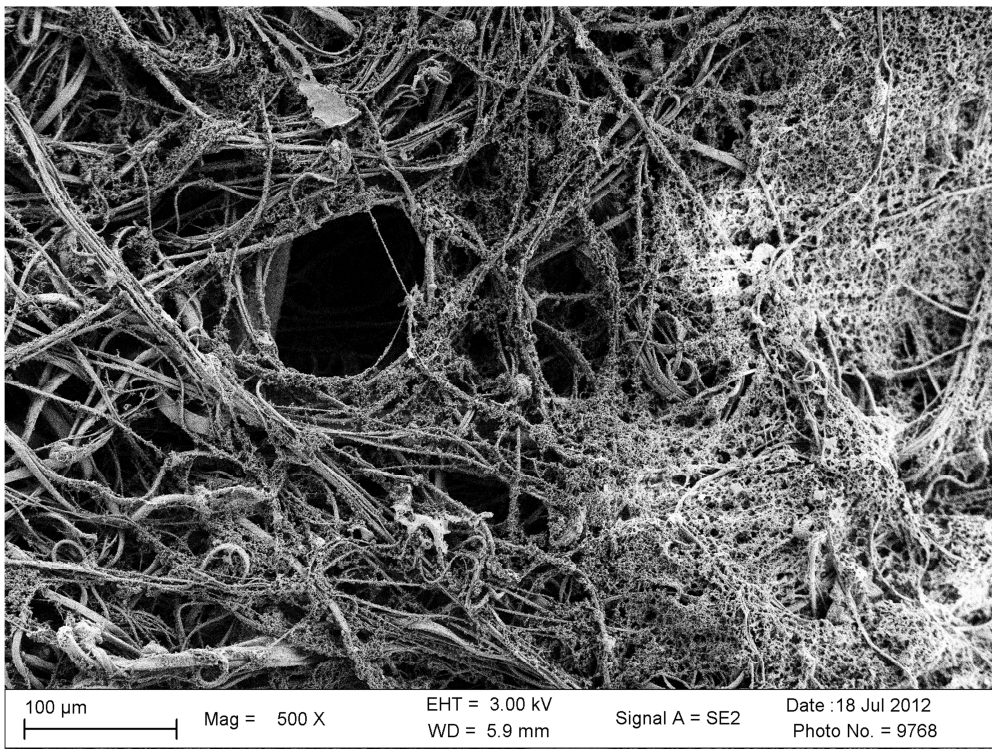


Figure 75. Frontal boundary of the migrating cell colonies

Low and high magnifications of the cellular damage due to the SEM preparation process are presented in figures 62 and 63. Figure 63 contains an imaging taken at 5kX of the damage imparted to the cells caused by sample preparation. The figure clearly displays the pock mark like structure seen at the boundary of the migrating cell colonies.

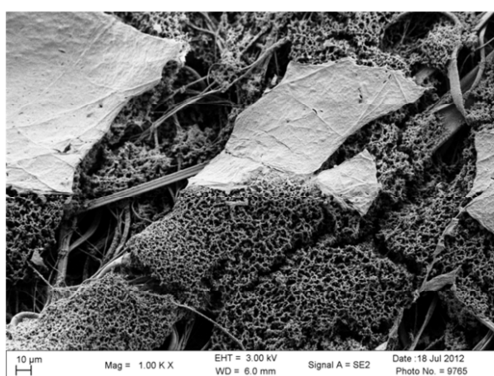


Figure 77. Cell propagation in 3-dimensions

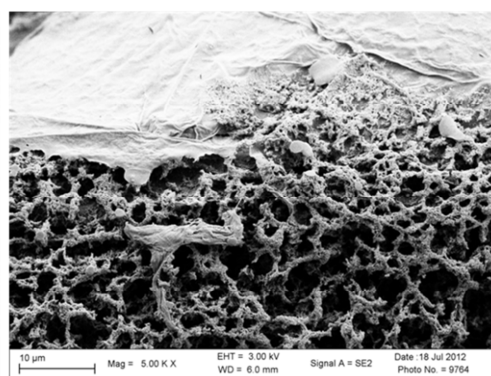


Figure 76. High magnification image of the damaged imparted to the cells caused by sample preparation

3.3 Confocal Microscopy of Seeded Scaffolds

Z-mapping performed with a confocal microscope was used to examine the amount of cell penetration into the depth of each MB scaffold chosen for cell seeding. The Z-mapping of MB scaffolds produced using a primary air flow rate of 1.5cm/s, collected at 30cm are presented in figure 64. The top set of images in figure 64 represent z-mapping from the top surface into the bulk of the scaffold, while the bottom set of images display mapping performed from the bottom of the scaffold into the bulk. Each square represents an image taken 5μm further into the depth of the scaffold than the image preceding it. Only the brightest images per series are displayed in the figure. In the images taken from the top surface of the scaffold into the bulk, fluorescence was detected down to depth of 145μm. Although cells

are visible on, or near, the surface of the scaffold, the bulk of the cells appear to be located between depths of 45-140 μ m. Z-mapping from the bottom surface of the scaffold into the bulk showed that cells were able to penetrate 160 μ m into the scaffold. Present in both sets of images are the local alignment of cells along the long axis of fibers. The local alignment seen in the cells is presented in figure 65 represented by two images taken at the same location. The image on the left was acquired using fluorescence, while the image on the right was captured using transmitted light. The circled regions represent areas of overlap. The Z-mapping of MB scaffolds produced using a primary air flow rate of 1.5cm/min, collected at 50cm are presented in figure 66. Each square represents an image taken 5 μ m further into the depth of the scaffold than the image preceding it. Only the brightest images per series are displayed in the figure. The series of images show that cells were able to penetrate about 190 μ m into the bulk of the scaffold. Although cells can be seen near the surface, the majority of the cells are located between 35-180 μ m. The presence of local cell alignment is clearly visible on the seeded scaffold. The Z-mapping of the same scaffold from the bottom surface into the bulk is presented in figure 67. The maximum cell penetration depth from the bottom of the scaffold into the bulk was location dependent, and varied from 155-190 μ m. The series of images in figure 67 represent the penetration depth of 155 μ m. This series of images displays the heavy influence of fiber alignment on cell migration. An enlarged image of the preferential fiber alignment is presented in figure 68.

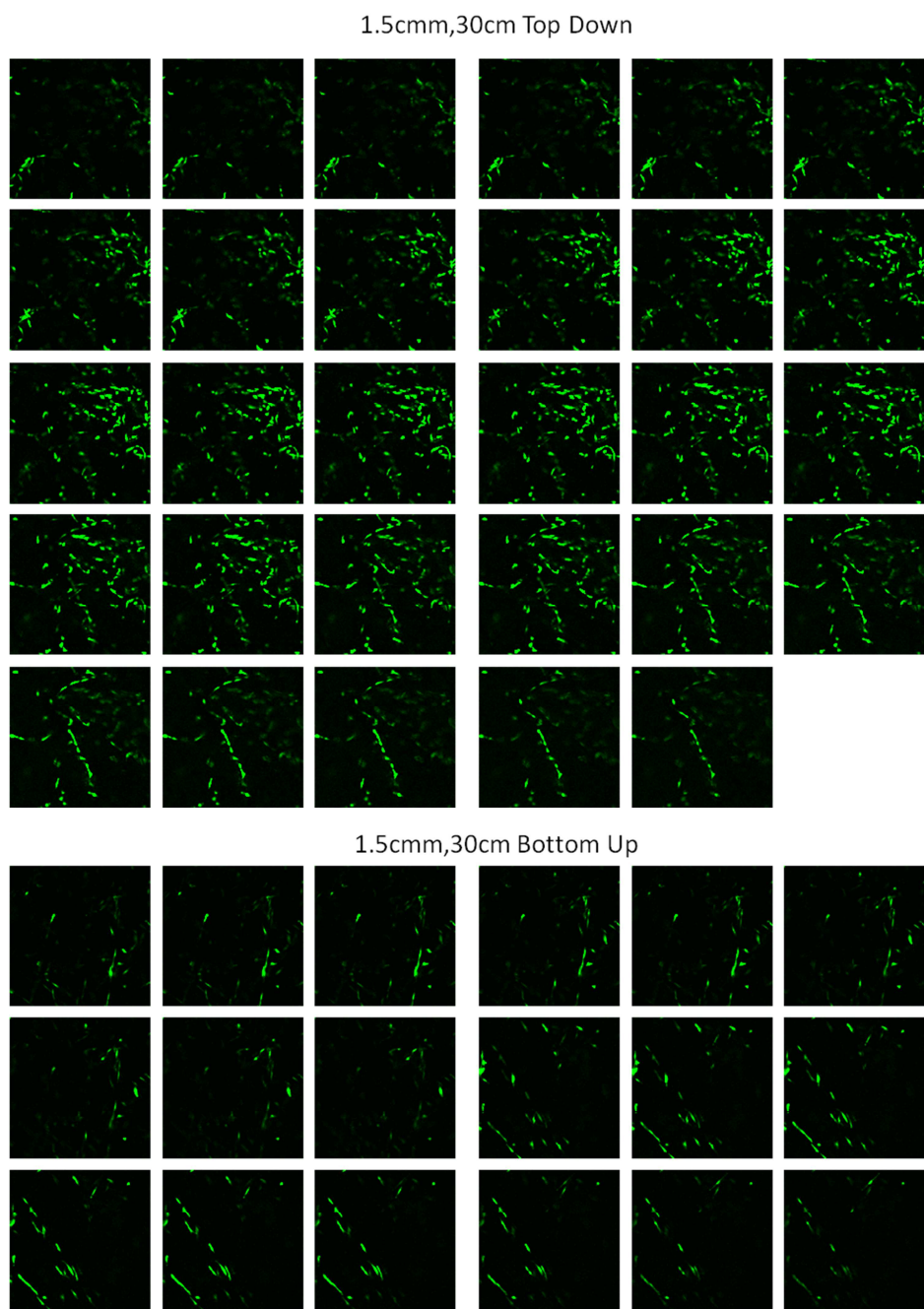


Figure 78. Confocal Z-mapping from the top and bottom surfaces into the bulk of a cell seeded micron-sized web MB using a primary air flow rate of 1.5cmm, collected at 30cm

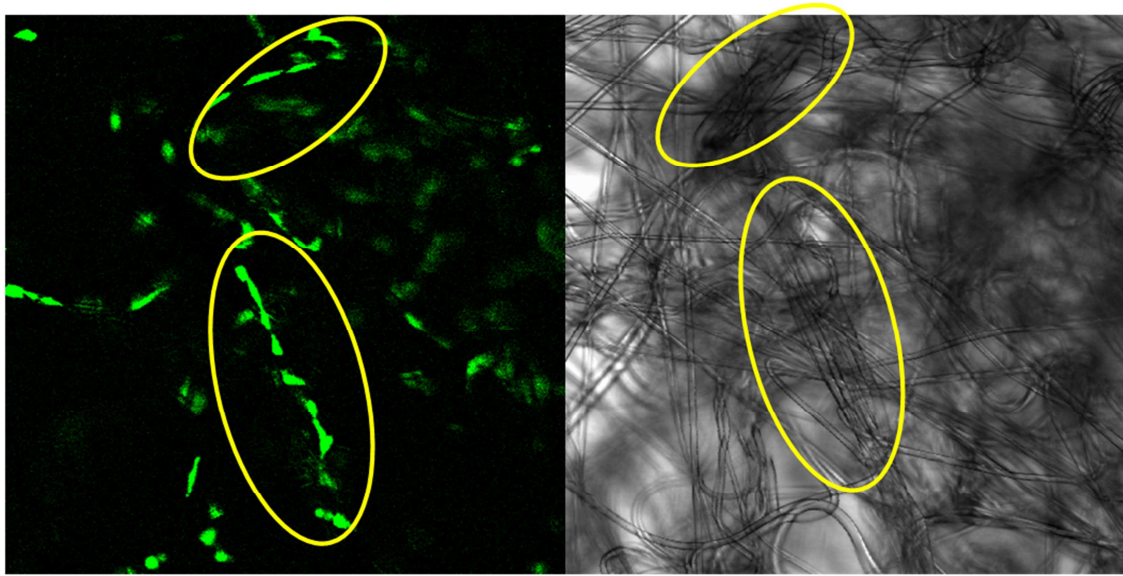


Figure 79. Local alignment on seeded cells on the long axis of MB micron-fibers

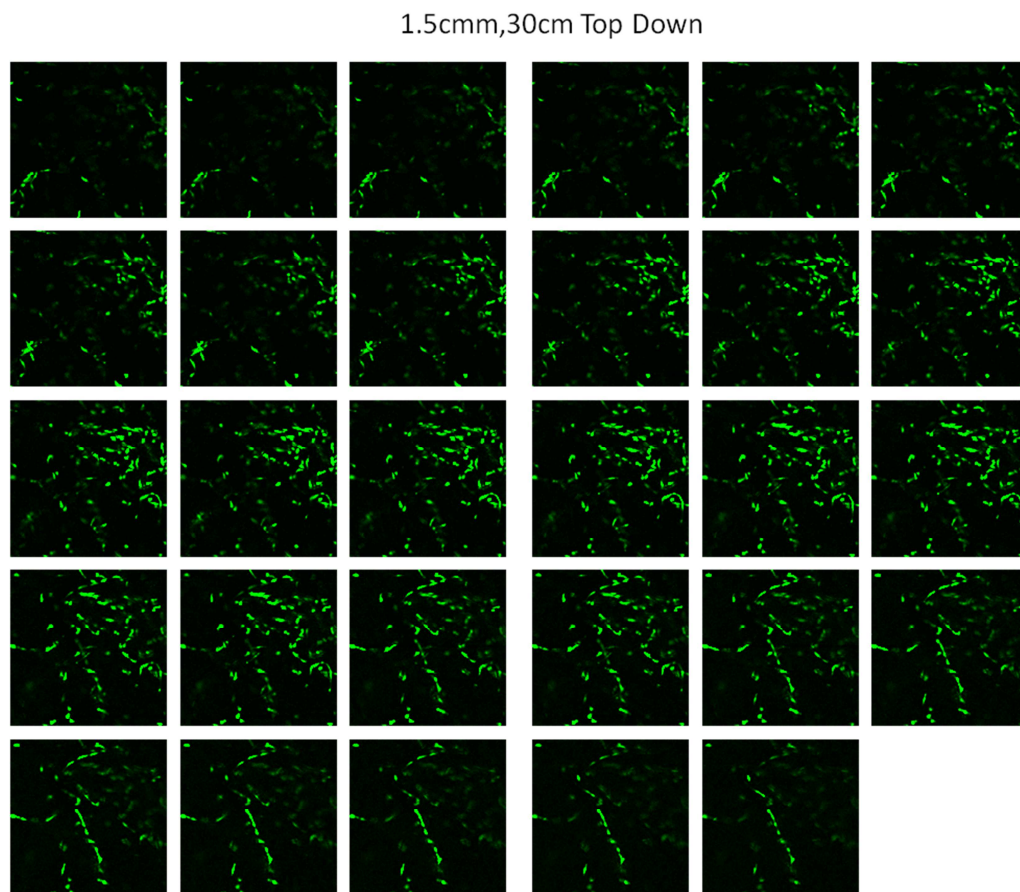


Figure 80. Confocal Z-mapping from the top surface into the bulk of a cell seeded micron-sized web MB using a primary air flow rate of 1.5cmm, collected at 50cm

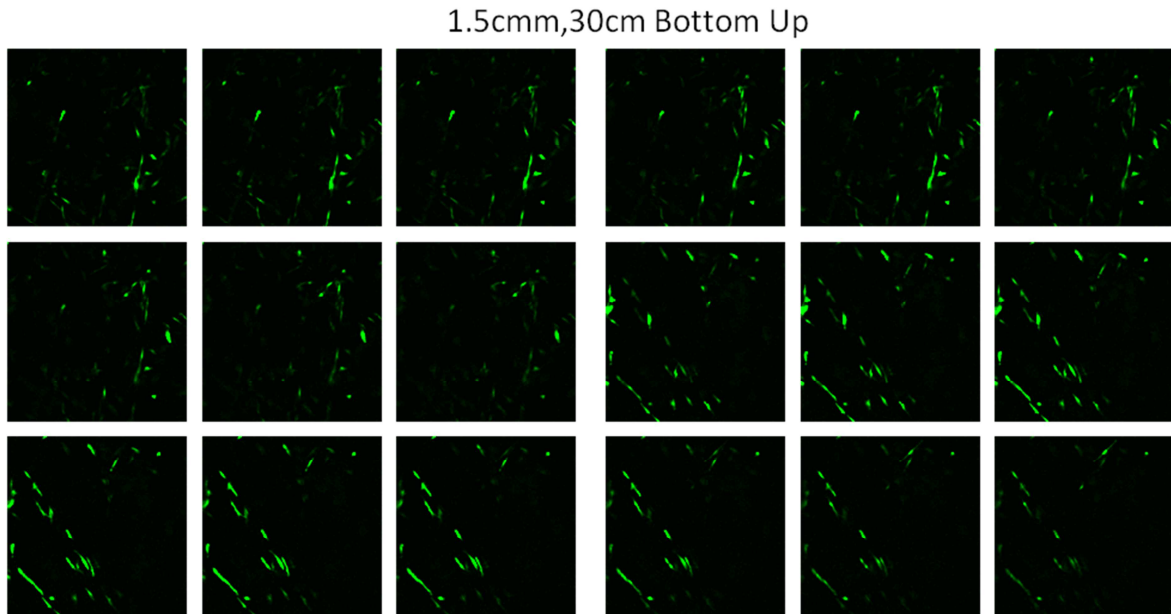


Figure 81. Confocal Z-mapping from the bottom surface into the bulk of a cell seeded micron-sized web MB using a primary air flow rate of 1.5cmm, collected at 50cm

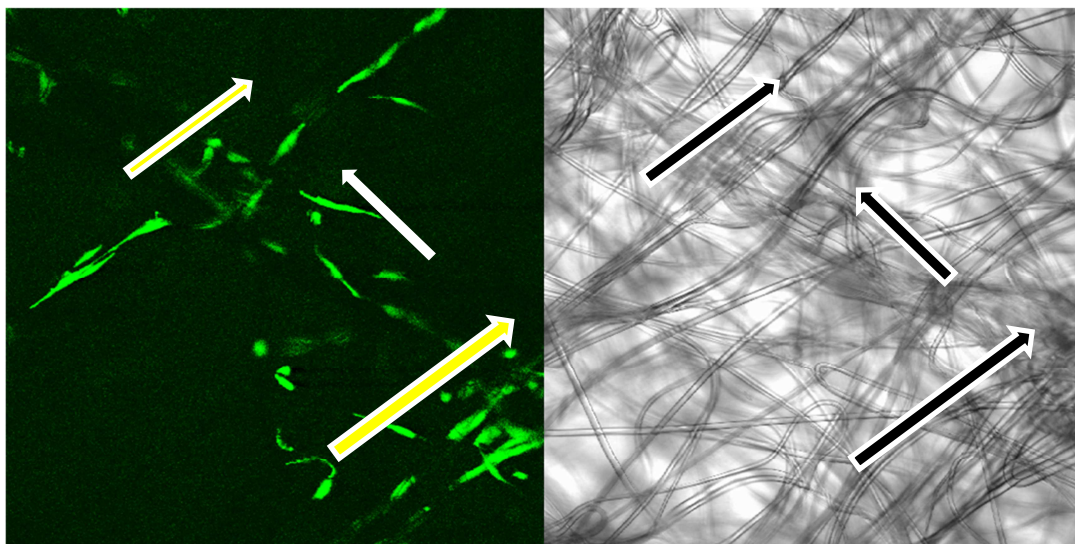


Figure 82. Confocal image of preferential cell alignment on bundles of micron-sized fibers. Cell alignment in the fiber direction is denoted by the yellow and black arrows.

The Z-mapping of nano-sized MB scaffolds produced using a primary air flow rate of 0.8cmm, collected at 30cm is presented in figure 69. Each square represents an image taken 5 μ m further into the depth of

the scaffold than the image preceding it. Only the brightest images per series are displayed in the figure. Examination of the series of images shows that the cells were able to migrate into the bulk of the scaffold to a depth of 180 μ m. Although cells fluoresce on the surface of the scaffold, the majority of the cells are located between 35-100 μ m in depth. A noticeable difference between the confocal images of the nano-sized webs and the micron-sized webs is that the alignment of the cells on the nano-webs appears to be random. In other words, no preferential alignment of the cells can be seen.

The Z-mapping of nano-sized MB scaffolds produced using a primary air flow rate of 0.8cm/min, collected at 50cm are presented in figure 70. Each square represents an image taken 5 μ m further into the depth of the scaffold than the image preceding it. Only the brightest images per series are displayed in the figure. The Z-mapping performed from the top surface into the bulk of the scaffold shows that the cells were able to migrate about 80 μ m into the PLA web. Z-mapping performed from the bottom surface into the bulk show that the cells were able to penetrate about 140 μ m into the bulk of the scaffold. Although cells can be seen on or near the surface, the bulk of the cells appear to reside between 30-90 μ m into the scaffold. Evident in both series of images is that the cells do not display any preferential degree alignment and appear to be seeded randomly.

.8cmm,30cm

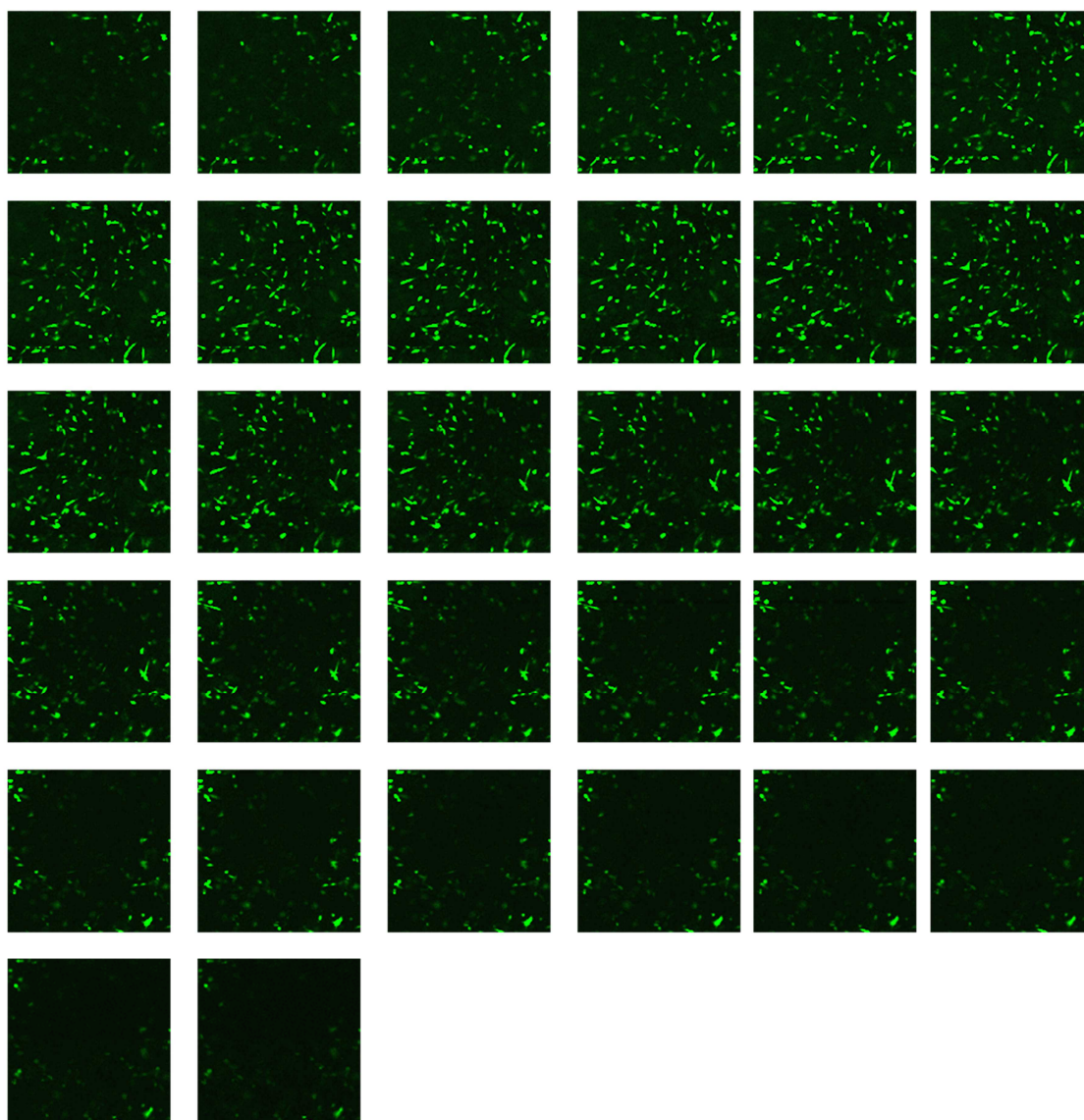


Figure 83. Confocal Z-mapping from the top surface into the bulk of a cell seeded nano-sized web MB using a primary air flow rate of 0.8cmm, collected at 30cm

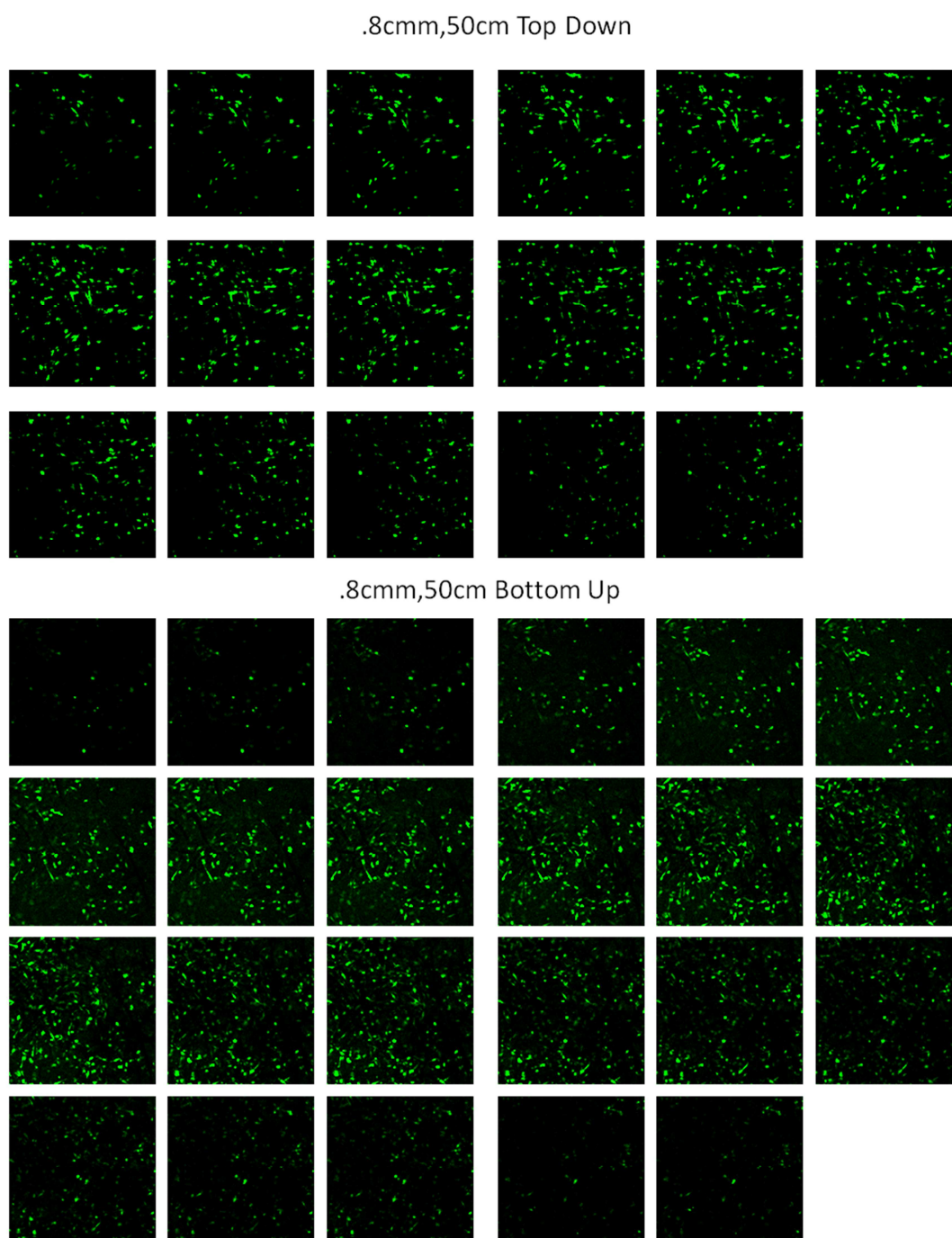


Figure 84. Confocal Z-mapping from the top and bottom surfaces into the bulk of a cell seeded nano-sized web MB using a primary air flow rate of 0.8cmm, collected at 50cm

4.0 Discussion

4.0.1 Intrinsic Viscosity and TGA

Polymer chains in solution can take on many different coiled configurations depending on the type of solvent used, poor, theta or good, and the associated interactions between them. Due to the coiled configurations of the polymer chains, the viscosity of a dilute solution increases with respect to that of the solvent as the polymer concentration increases. The contribution that the polymer coils provide to the solution viscosity, η , can be modeled by;

$$\eta_{sp} = \frac{\eta - \eta_s}{\eta_s} \quad (8)$$

where η_s is the solvent viscosity and η_{sp} is known as the specific viscosity. The numerator in equation (8) is the polymer contribution to the solution viscosity, which makes the specific viscosity a dimensionless measure of the polymer contribution to the solution viscosity. The intrinsic viscosity of the polymer is found by creating a plot of η_{sp} vs. concentration of polymer in solution and then extrapolating the concentration back to 0.0g/dL until the intercept with the y-axis is found. Figure 12 graphically displays the use of this method. The viscosity average molecular weight can then be deduced using equation (7), the MHS equation.

The intrinsic viscosity of NatureWorks 6252D PLA was 0.9734. The M_η calculated from the measurement of intrinsic viscosity was approximately 27000g/mol. Figure 12 shows that the points used to determine the linear fit are not co-linear. Measurements of flow time for each solution concentration of PLA through the Ubbelohde viscometer were taken in duplicate and did not vary from one another for one another by more than a few seconds. As the repeat measurements were similar, the movement away from co-linearity could have been caused by thermal fluctuations of the water during the determination of flow times. Chloroform is only a theta solvent for PLA at 25°C and any variance in temperature away

from 25°C may change the unperturbed coil radius of the PLA molecules. Also, the viscometer and solutions used may not have needed more than 5 minutes to reach equilibrium temperature. Nevertheless, the calculated M_n agrees with values obtained by other researchers performing similar analysis on PLA¹⁸.

The non-isothermal mass loss in figure 13 for PLA under N₂ atmosphere begins around 300°C and mass loss appears to be completed at approximately 375°C. This data agrees with work conducted by other researches on PLA^{20, 21}.

4.1 Effects of Processing Conditions on the Mechanical Properties of MB Webs

4.1.1 Fiber Size Distributions

Figure 14 contains the mean fiber diameter and standard error of the mean for micron-webs. The figure shows that an increase in the primary air flow rate used to MB the webs results in a decrease in the mean fiber diameter. The linear fit to the plot states that an increase in primary airflow rate of 1cm/min results in a reduction in fiber diameter by about 1µm. The observed reduction in fiber diameter with an increase in primary air flow rate was expected, as equation (1) shows that the largest contributing factor to the parallel force acting on the fibers is the effective air velocity. This trend agrees with those produced by other researchers producing MB micron-webs^{14, 16}. Figure 14 also shows that the fiber diameter is not affected by the DCD in a statistically significant manner, as the mean values of all fiber diameters produced at the same primary air flow rate fall within the standard error of the mean. It would be expected that at larger DCDs the fiber diameters would continue to decrease due to fiber-fiber interactions, and that the number of large fibers would increase due to coalescing of the molten polymer filaments as they approach the collector. Tables 4-7 show that this is not the case for any of the primary air flow rates used. The findings presented in this report strongly disagree with those reported by Bresee et al in 2006, on the MB of micron-sized fibers of polypropylene, which claim that an increase

in DCD will result in a decrease in fiber diameter. To explain the discrepancy between the two sets of results the solidification of the fibers will be examined.

Figure 5 shows that the maximum amount of stress produced in the fibers occurs within 2cm of the die exit and the stopping point is reached by 8cm away from the exit. Also within this distance, the temperature of the primary air flow rate reaches its local minimum as is presented in figure 71.

Therefore, it is safe to assume that at this point the fibers have reached their maximum level of attenuation due to an increase in velocity and that both steady state velocity and ambient temperature have been reached. We can now model the transient conduction of heat out of the fibers neglecting spatial variation in temperature. Spatial variation in temperature is the temperature drop across the radius of the fibers from the core temperature to the ambient air temperature. The spatial variation in temperature can only be neglected if the Biot number is less than 0.1³⁵;

$$Bi = \frac{h\delta}{k} \quad (8)$$

where h is the heat transfer coefficient of the fluid, k is the thermal conductivity of the material, and δ is the length equal to the distance over which the temperature drop would occur. As the thermal

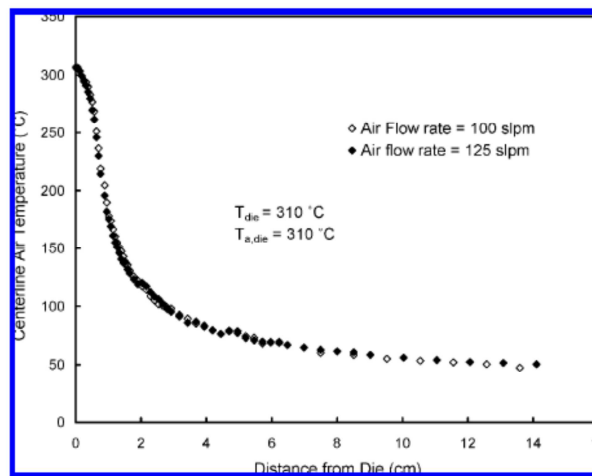


Figure 85. Decrease in air temperature versus distance from the melt blowing die (Marla et al 2009)

conductivity of polymers ranges between 0.1-0.5, it can be seen that the dominating factor in the Biot number is δ ³⁶. As the largest diameter of any of the MB fibers produced for this study is about 10 μ m, it is evident that the Biot number is less than 0.1.

Neglecting spatial variation and taking into account that the fibers are not generating any additional heat input, equation 9 may be used to calculate the cooling time of the fibers by convection³⁵;

$$\frac{T-T_{\infty}}{T_i-T_{\infty}} = e^{\frac{hA_s t}{\rho C_p V}} \quad (9)$$

where;

A_s = surface area, m²

C_p = specific heat capacity of the cooling material, J/kg-°C

h = heat transfer coefficient of the fluid, W/m²-°C

V = volume of the cooling material, m³

T = target temperature, °C

T_{∞} = ambient temperature of the fluid, °C

T_i = initial temperature of the cooling material, °C

ρ = density of the material, kg/m³

t = time, s

For our system, the time necessary to cool below 170°C, the T_m of the PLA used, is what was investigated. Fiber temperature leaving the die is 220°C, the ambient temperature is 50°C (air temperature at the stopping point), the C_p of molten PLA is 6.3J/kg-°C³⁷, and the diameter of the fibers used to calculate the heat transfer are found in table 19. As the air and the fibers are traveling at the same velocity, the heat transfer coefficient for natural convection in air can be taken as 5-25W/m²K. Five W/m²K was used to represent the worst possible scenario for heat transfer. A length of 0.01m was used

to calculate the surface areas and volumes. The stopping point velocity was determined by direct measurement using an ExTech Anometer with an attached vane probe.

The data from the calculations are presented in table 20. The calculated cooling times to the melt temperature clearly show that all of the fibers produced, except those at the highest primary air rate and smallest DCD, solidified before they reached the collector.

Table 6. Calculated cooling times for MB fibers and travel time to the three DCDs used to collect the webs. The parameters for fibers that would not cool below the melting temperature before reaching the collector are highlighted in yellow

Fiber Diameter (μm)	Cooling Time to T_m (s)	Velocity at Stopping Point (m/s)	Time to .1m (s)	Time to .3m (s)	Time to .5m (s)
10	0.001184939	13	0.00769	0.02308	0.03846
7	0.000829458	26.5	0.00377	0.01132	0.01887
3.5	0.000414729	204	0.00049	0.00147	0.00245
1.8	0.000213289	706	0.00014	0.00042	0.00071
(nm)					
609	7.22813E-05	13	0.00769	0.02308	0.03846
580	6.87265E-05	42	0.00238	0.00714	0.01190
570	5.56922E-05	106	0.00094	0.00283	0.00472

This helps to explain why no decrease in fiber diameter was observed as the DCD was increased for each primary air flow rate used. It appears that the quickly solidifying fibers negated the effects of any fiber-fiber interaction that may have occurred away from the die exit. Also, tables 4-7 show that the number of large fiber bundles did not increase with an increase in DCD as was expected. This evidence further suggests that the fibers solidified close to the die exit, and that any coalescing that may have occurred could only have done so between small diameter fibers.

The mean diameters of the nano-sized webs are presented in figure 17. Clearly there is no correlation between primary air flow rate and fiber size diameter. Table 12 shows that the fibers are cooling below T_m in a very short amount of time, thus they solidify well before the stopping point is even reached. Due to their size, it is likely the fibers remain molten as a function of air temperature and cool below T_m

when the air temperature does. Figure 71 shows that a starting air temperature greater than the 260°C used in our MB trials will cool below the T_m of PLA before the maximum amount of attenuation stress will be imposed upon the fiber. Therefore, the increase in force accompanying an increase in primary air flow rate will not have enough time to be transferred to the MB fibers. Consequently, all of the fibers produced using the AGR die have a similar diameter.

4.1.2 Pore Size Distributions and Air Permeability

The mean pore sizes of micro-sized webs are presented in figure 20. The mean pore size decreases with an increase in primary air flow rate. This trend was expected as an increase in the primary airflow rate caused a decrease in fiber diameter of the webs. A decrease in the fiber diameter results in lengthened collection times in order to obtain the same basis weight as webs collected with larger fiber diameters. The increase in collection time means that more fibers will be laid down in the same unit area, thus more random fiber overlapping will occur and the mean pore size will decrease.

Figure 20 also shows that as DCD is increased the mean pore size increases for all of the primary air flow rates used. As the fibers possess velocity in three dimensional space, an increase in DCD will cause an increase in the area over which the fibers are laid down. Consequently, fiber overlap is reduced and an increase in both the mean pore size and pore size distribution is seen¹⁴.

The mean pore size data for nano-webs is contained in figure 22. Once again an increase in primary air flow rate caused a decrease in the mean pore size, while an increase in DCD resulted in larger pores and pore size distributions. The difference in mean pore size and PSD is much less exaggerated than the changes caused by processing conditions in micron-webs. As the fiber size diameter for the nano-webs did not vary with primary air flow rate, the decrease in pore size cannot be associated with fiber diameter. Here the decrease in pore size with primary air flow rate was caused by the slow collection speed necessary to reach a BW of 20g/m². The collection speed was much slower due to the small size

of the fibers compared to the micro-webs (less mass per fiber collected). An increase in the primary air flow rate lays the fibers on the collector with more force causing the web thickness to decrease. The slower collection speed provided more time for the web thickness to decrease, causing a collapse in the web architecture and thus a decrease in pore size. Figure 72 shows the change in fiber web morphology caused by an increase in the primary air flow rate for nano-webs.

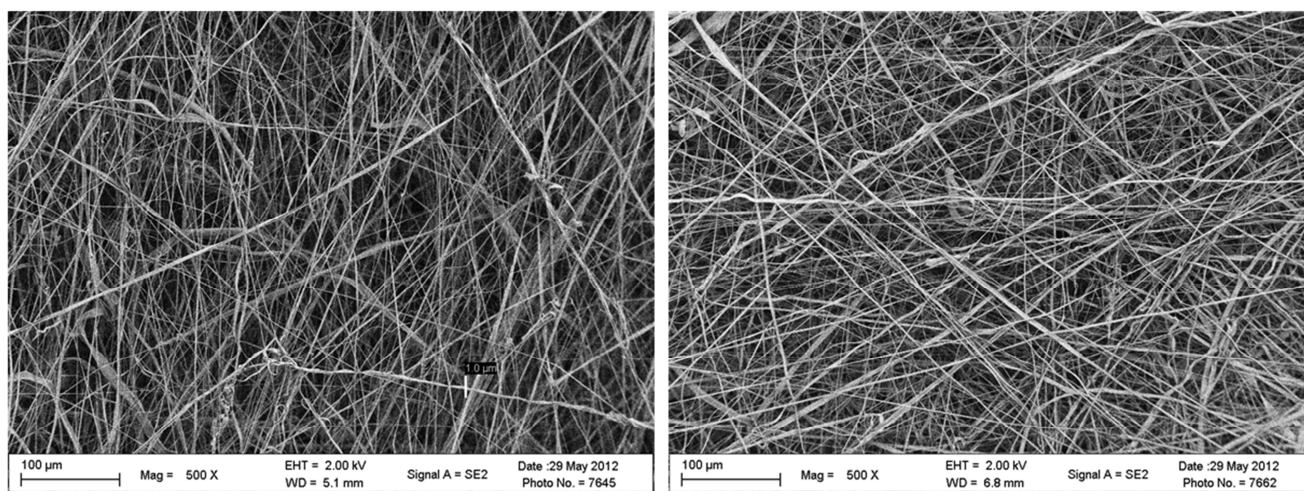


Figure 86. (Left) SEM micrograph of the MB nano-web using a primary air flow rate of 2.3cm/s, collected at 10cm. (Right) SEM micrograph of the nano-web produced using a primary air flow rate of 5.5cm/s, collected at 10cm

Air permeability through the MB webs was investigated as a safe check to ensure that the trends seen in the porosity data were in fact correct. As mean pore size increases, so should air permeability and vice versa. It is encouraging to see that the trends for air permeability presented in figures 26 and 27 closely match those for porosity seen in figures 20 and 22. Air permeability also allows us to determine whether or not the pores seen in the webs are through pores or closed pores. Through pores are necessary for the successful delivery of nutrients and removal of waste to and from cells in a scaffold. A structure may be highly porous, but if it displays poor air permeability it most likely has closed pores and would not be a good candidate for successful cell culturing.

4.1.3 Non-Woven Web Thickness

The web thickness observed for the MB micro and nano-webs fell between 0.2-0.5mm. In general, matt thickness decreased inversely to primary air flow rate. This trend was expected in micro-webs as an increase in air flow caused a decrease in the mean fiber size. A decrease in the mean fiber size results in longer collection times in order to achieve the same basis weight. The longer collection times cause more layering, which coupled with high air flow rates result in the layers being compacted on top of one another. Therefore we see a decrease in thickness and pore size with increased layering. A similar trend was seen in the nano-webs, and as with pore size, the decrease was not caused by a change in fiber diameter. Here, the decrease was caused solely by the increase in force associated with an increase in primary air flow rate. Another noticeable trend seen when examining web thickness is that as DCD is increased the web thickness also increases. As the DCD increases the fibers are laid down with less impact force and the effect of primary air flow rate are reduced. Also, air turbulence is high at the collector and longer DCDs allow the fibers to be laid down over a wider area and in 3-dimensions. A frequent occurrence is MB web collected at large DCDs is the appearance of fibers being laid down perpendicularly to the collector's surface, rather than laying flat on the collector.

4.1.4 Tensile Modulus

The tensile modulus data for micro and nano-webs are presented in figures 28 and 29, respectively. The data shows that webs MB using DCDs of 10cm do not behave isotropically when subjected to tensile testing. It is evident that at close DCD the fibers orient themselves in the machine direction, and the effect is enhanced by increasing the primary air flow rate. The micro-web morphology of four webs collected at a DCD of 10cm with increasing primary air flow rate is presented in figure 73.

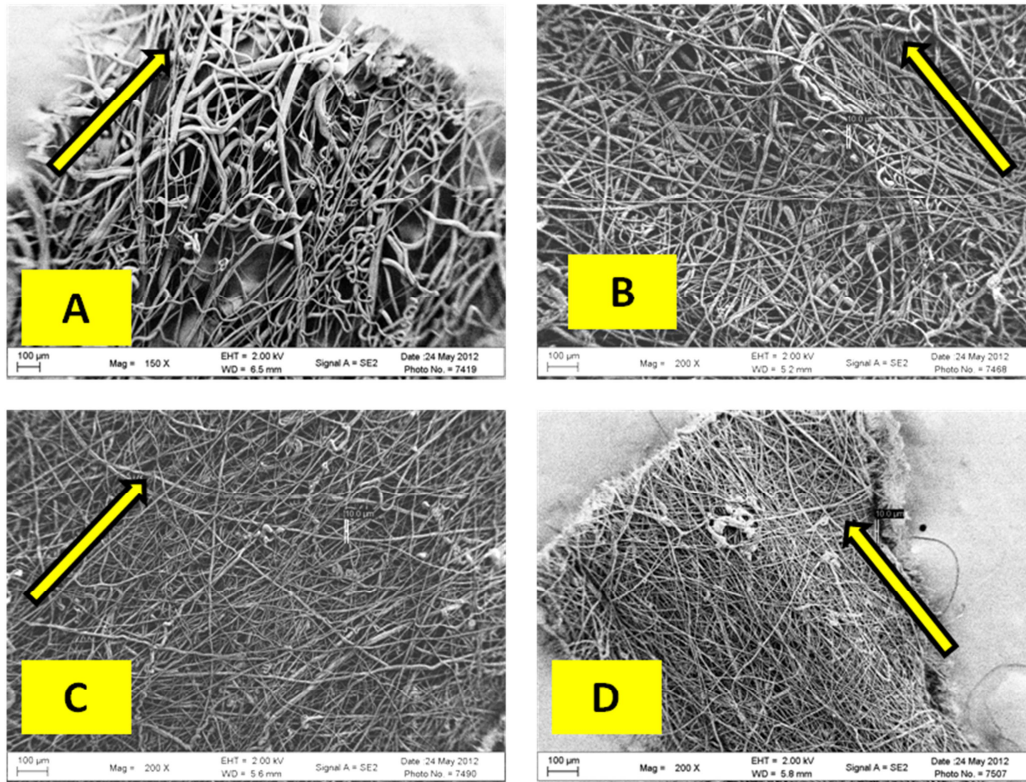


Figure 87. (A) Micro-web produced at 0.8cm, collected at 10cm. (B) Micro-web produced at 1.5cm, collected at 10cm. (C) Micro-web produced at 10cm, collected at 10cm. (D) Micro-web produced at 35cm, collected at 10cm

Coinciding with this, at higher primary air flow rates, the fibers are kept in closer proximity to one another as they have less time to spread before they reach the collector. This produces webs with more fibers per unit area oriented in the same direction. Examination of figure 73 from A to D displays the increase in both density of fibers and orientation of fibers in one direction denoted by the yellow and black arrows in the figure.

The increase in tensile modulus for micro-webs with primary air flow for all DCDS may have been caused by the increase in crystallinity associated with the increase in DCD. Crystalline regions of a polymer are stiffer than amorphous regions and an increase in tensile modulus is seen with increasing crystallinity of a polymeric material. As the DCD is increased past 10cm, both the micro and nano-webs behave isotropically under tensile load. Air turbulence is high at the collector and larger DCDs allow the

fibers more time to move in 3-dimensions. Consequently the fibers are globally laid down in a random fashion.

4.1.5 Crystallinity

The crystallinity data for the PLA micro and nano-webs is located in table 18. Table 18 shows that there is a slight increase in crystallinity with primary air flow rate in the micro-webs, while the nano-webs all exhibit relatively the same amount of crystalline region. One reason for this may be the high primary air flow rates used to MB the micro-webs caused orientation in the fibers at the molecular level. This is a plausible explanation, as PLA has an entanglement molecular weight of 16000g/mol, while the PLA used in this study has an M_n less than double that. Although not much research has focused attention on induced crystallization via melt blowing, researchers have confirmed that the crystallinity in PLA can be increased at high draw ratios and temperatures through orientation induced crystallization^{38, 39, 39}. This trend is most likely not seen in the nano-webs because the primary air flow rate used to produce them was not high enough to produce the shear force necessary to cause molecular alignment. FTIR would have to be conducted in order to substantiate this claim.

The exothermic peaks seen in the DSC heating curves of all the MB webs, but not in the DSC heating curve of natural PLA were caused by the rapid cooling of the small diameter fibers. PLA is a slow crystallizer, its half life for pure PLA crystallization has been reported to be between 17-45 minutes²⁷. MB PLA acts as a non-isothermal crystallization process and the samples are unable to reach maximum crystallinity before they cool below T_m . Therefore, the exothermic peaks seen near 100°C are cold crystallization exotherms²⁷. This is an interesting observation, because being able to control the amount of crystallinity in the webs could be used to augment the tensile modulus of the webs. For instance, webs blown at low primary air flow rates and large DCDs had large mean pore diameters and a low tensile modulus. Annealing the samples would be an excellent way to increase the modulus without

sacrificing pore size for scaffold creation. The effects of annealing the one PLA micro-web is presented in figure 37. The DSC heating and cooling curve in figure 37 no longer shows a cold crystallization peak as annealing for 12 hours allowed the material to reach a much higher level of crystallinity.

4.1.6 Degradation Rate

The degradation rate of the PLA samples in PBS found here agree with work performed by other researchers^{40, 28, 41, 42}. This is good news because it signifies that the processing conditions did not facilitate degradation during creation of the MB webs. Also, it is likely that formation of micro and nano-architecture will not negatively affect the long term mechanical properties of the material by facilitating early breakdown. Comparison of micro and nano-fibers produced via MB to electrospinning shows that the structure of the fibers made by MB do not contain surface abnormalities or fiber porosity as those created through electrospinning. Images depicting the differences in surface topography between electrospun and MB micro and nano-fibres are presented in figure 74 and 75. Figure 74 shows the large amount of porosity created in fibers during electrospinning, while figure 75 shows that MB fibers does not impart any structural abnormalities in the different sized fibers.

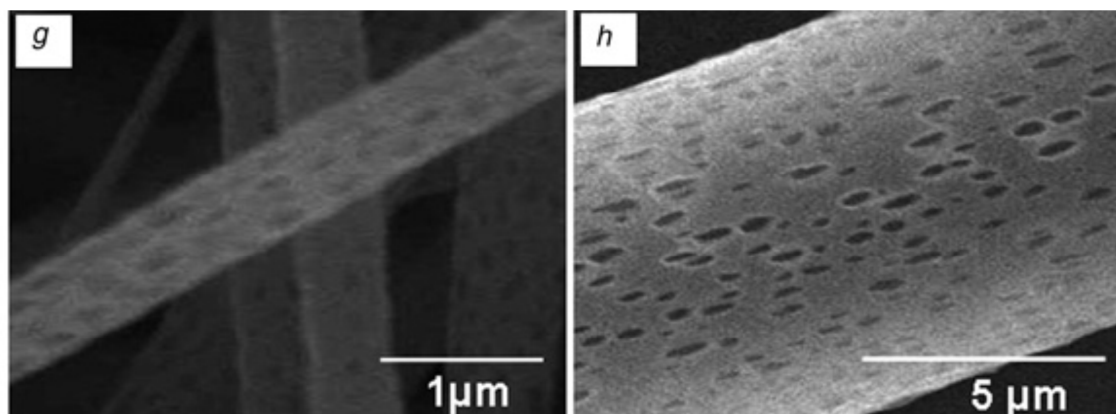


Figure 88. (Left) Surface topography of nano-fibers produced via electrospinning. (Right) surface topography of micro-fibers produced via electrospinning (Shalumon et al 2012)

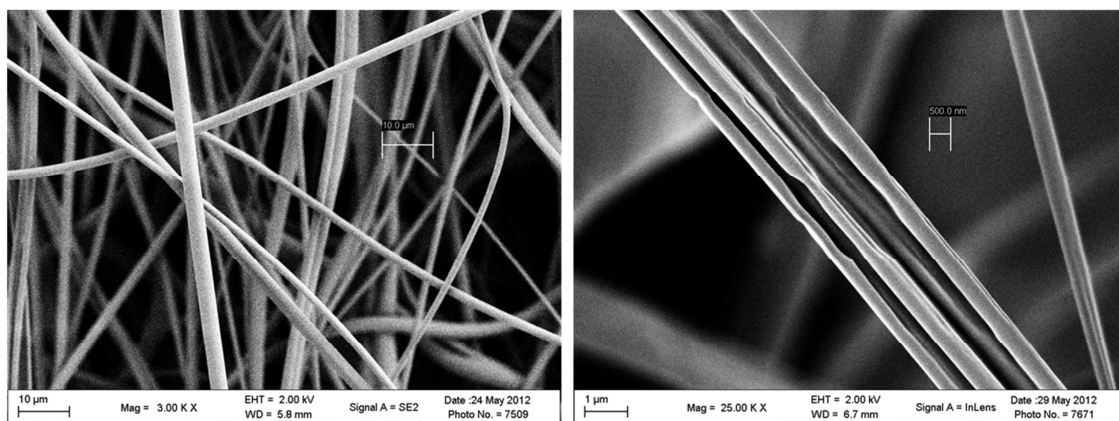


Figure 89. (Left) Surface topography of micro-fibers produced via MB. (Right) Surface topography of nano-fibers produced via MB

4.2 Scaffold Cell Seeding

4.2.1 The Effects of Fiber Morphology on Cell Morphology

Overall, the four scaffolds selected for cell culture performed as expected for cellular attachment, but performed better than expected for cell migration. Examination of similar studies did not show the same level of cell coverage of the scaffolds in 24hrs⁴². Comparison between cells seeded on micro and nano-webs show that there is a striking difference between the ways the cells both attach and spread across the scaffolds. When single cells attach to single micron sized fibers they tend to take on a globular morphology like those seen in figures 40 and 41. Also, the cells appear to spread to the width of the fiber before proceeding to track along the long axis of the fibers. On nano-webs it is obviously more difficult for the cells by chance to be seeded on a single fiber, and the morphology assumed by the cells is much more flat. The degree of lateral spreading is also greater by the single cells on the nano-webs than on the micro-webs as can be seen in figures 1, 44 and 47. If by chance a cell does find itself isolated on a single nano-fiber, figure 45 shows that the cell will ball up worse than if it was on a single micro-fiber. Similar morphologies have been seen by other researchers seeding cancerous cells on electrospun PLA scaffolds⁴².

Cell morphology was affected by the drying process used to ready the sample for SEM. Figures 62 and 63 highlight this damage that can be viewed as a crater like structure on the surface of the cell. The most likely cause of this damage was due to incomplete drying before vacuum sputter coating the scaffolds with gold for SEM. The cells spread so well on the scaffolds that gradient drying in ethanol followed by gradient drying in HMDS and then a soak in HMDS overnight could not leech all of the water out of the scaffolds. During the evacuation of the sputter coating chamber the rest of the moisture contained in the scaffolds was pulled off and appears to have torn through the cell walls. An image of a rupture cell is presented in figure 76.

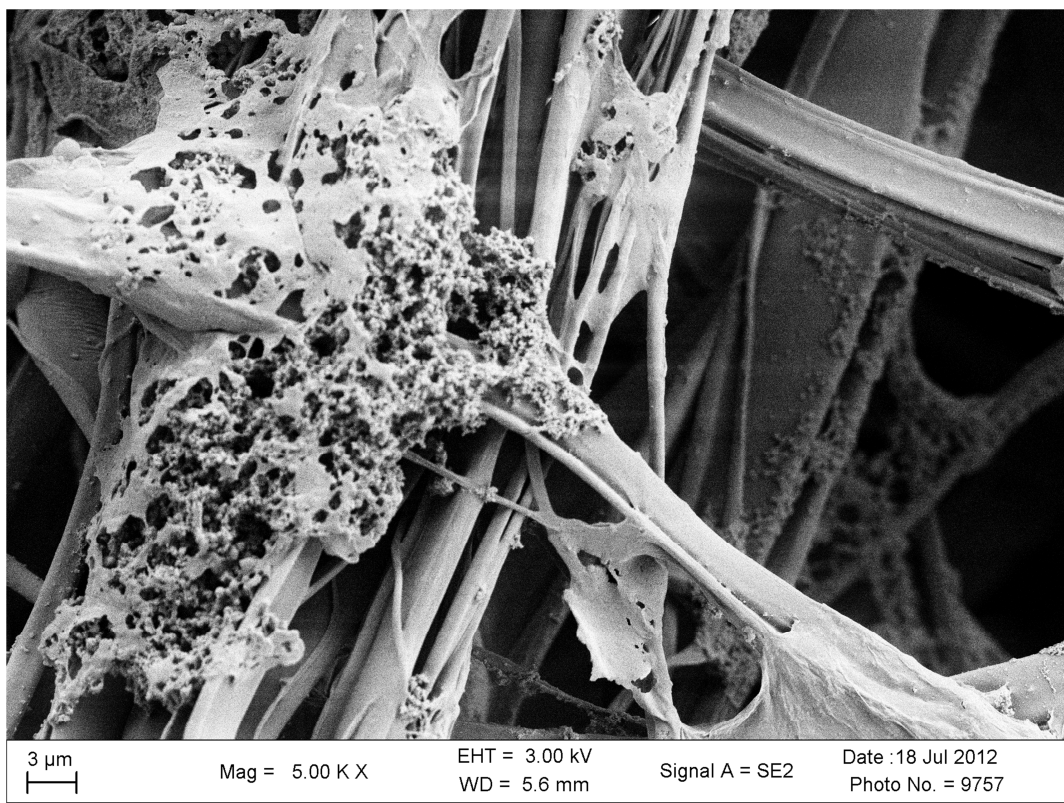


Figure 90. Image of a ruptured cell caused by vacuum drying

4.2.2 Web Morphology on Cell Migration

SEM and Confocal microscopy showed that cell migration was affected by web morphology. On micron-webs the migration of cells is heavily influenced by the long axis of the fibers and cells will not spread laterally without the aid of abutting parallel fibers or fiber junctions. The growth of cells along the long axis of fibers is presented in figures 77 and 78. These confocal images clearly show that the cells are being guided by the fibers and are unable to spread without the aid of crossing fibers. Maintaining the same basis weight between micro and nano-webs means that thousands more fibers will be laid down in same unit area. Figure 79 shows that with so many more fine fibers in the same weight web, the seeded cells no longer have a preferred direction of growth and can migrate freely. Here we see the effect of topographical feedback discussed in section 1.1.2.3. Altering the morphology to incorporate nano-scale features clearly aided in cell migration in the x and y-directions, not only because there were more points for cellular focal adhesion to occur, but also due to the reduction in the number of large pores. Figures 48 and 55 clearly show that the migrating cell went around pores greater than 100 μ m in diameter and were unable to cross them, disrupting migration.

The z-mapping performed with the confocal microscope is presented in figures 64, 66, 67, and 69-70. The imaging shows that there was little difference in overall cell migration into the bulk of the scaffold between the micro-web and nano-web scaffolds. The micro-webs had mean pore diameters about twice as wide as the nano-webs, but cell migration from neither the top nor bottom of the scaffold appeared to be different. The fact that cells were located on both faces on the scaffold, but were seeded from the top of the scaffold shows that the A375 cells were able to migrate through the entire thickness of both micro and nano-web scaffolds. The lack of fluorescence in the middle of the center of the scaffolds may have been caused too many layers of fibers blocking the transmission of focused light, or the scaffolds were compressed down to about 200 μ m when prepared for confocal microscopy. Regardless, it is

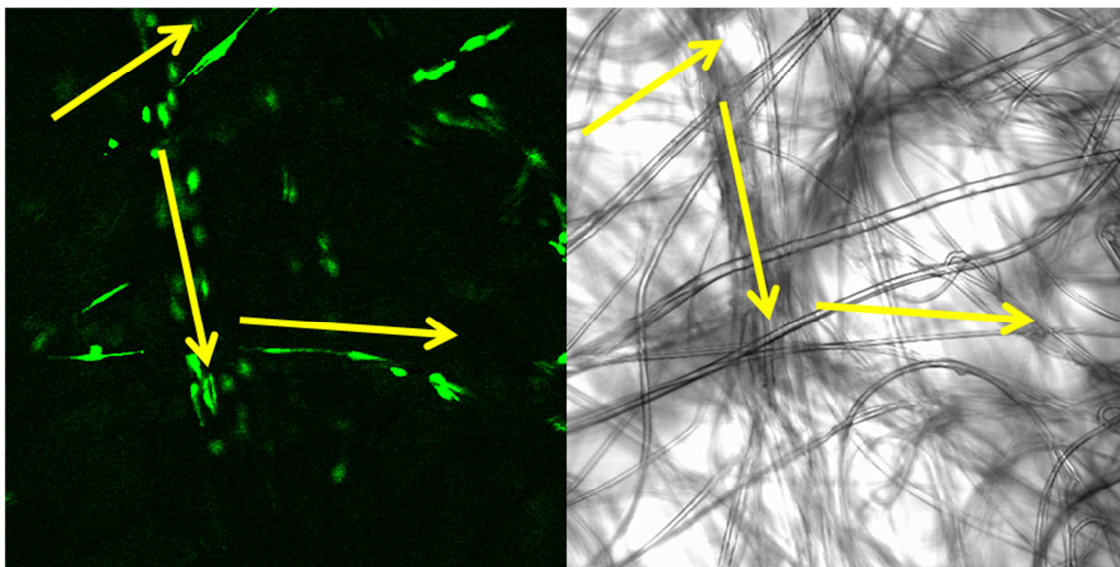


Figure 91. Confocal images of the cellular alignment on fibers in micro-webs; example 1

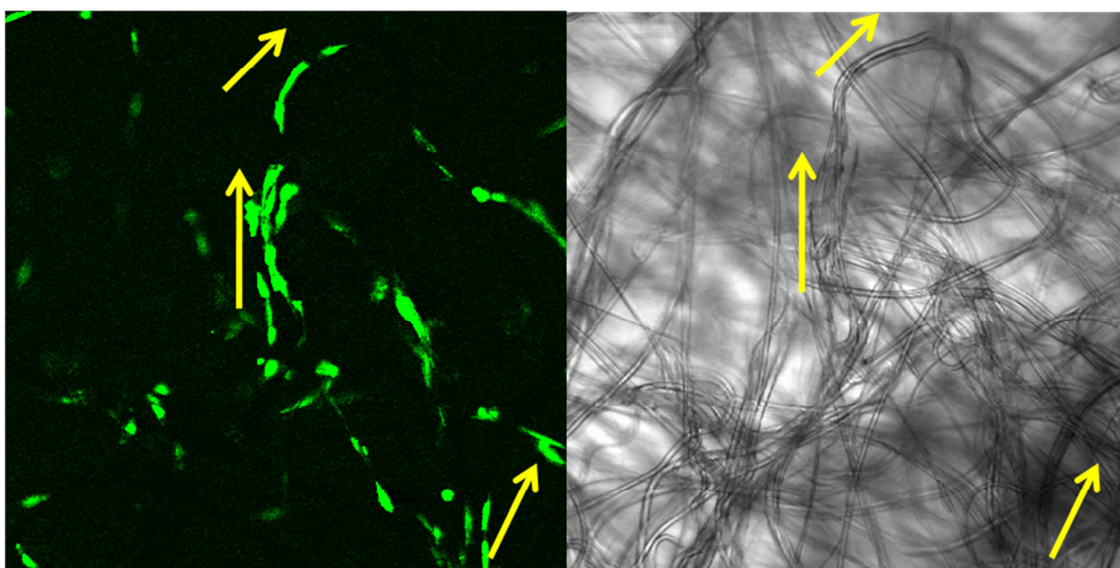


Figure 92. Confocal images of the cellular alignment on fibers in micro-webs; example 2

evident that a mean pore size greater than 20 microns is sufficient for cell migration into the bulk of the scaffold. This finding is important in that it shows 0.5mm PLA MB nano-scaffolds can successfully be seeded with cells that will migrate through the bulk of the material. Many researchers have found

difficulty in producing similar electrospun nano-scaffolds that can be successfully seeded with cells that will migrate into the bulk of the scaffold⁴. Generally, the problem encountered in electrospinning nano-scaffolds is the associated mean pore size this is too small for cell penetration. Many novel ideas have been proposed to overcome bulk migration difficulties including seeding multiple layers of thinner material (less fiber overlapping and a larger mean pore size) and then stacking them on top of one another, electrospraying thin layers of material on to cells, or even electrospinning scaffolds with cells encapsulated within the polymer-solvent solution⁴³. The data presented in this report shows that the melt blowing process is capable of producing a wide variety of scaffold architectures without the need to go to so much trouble for successful cell culture.

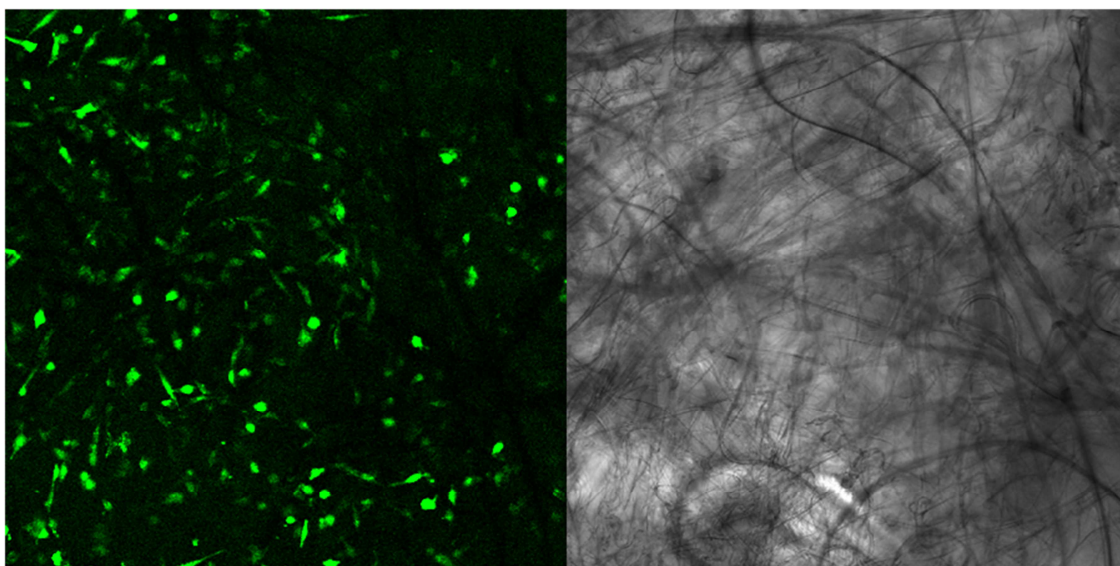


Figure 93. Confocal imaging showing the lack of cellular alignment on nano-webs

4.2.3 Tensile Modulus on Cell Migration

The four scaffolds used for seeding were specifically selected because they varied greatly from one another in tensile average tensile modulus. Ideally, the materials used to create the scaffold should be able to withstand the contractile forces imposed upon them by the cells, with a maximum force generated by the cell on the scaffold being 10-15% of the substrate's modulus. The migration of

epithelial cells has been studied both individually and as sheets of 10-20 cells^{5, 32}. Individual cells have the capability to produce $30.8 \pm 1 \text{ nN}/\mu\text{m}^2$ of tractional force, while sheets of epithelial cells can produced up to $12 \text{ nN}/\mu\text{m}^2$ at the proximal edge of the migrating cluster when placed on a substrate with a modulus of at least 150kPa. Therefore, four webs were chosen, two with a modulus near 150kPa and two with a modulus of about half that value to determine if there would be any discrepancies in migration.

The micro-web scaffolds had tensile moduli of 145kPa and 70, while the two nano-webs had moduli of 149 and 90kPa. Despite the stark differences in moduli between the two micro and two nano-webs, very little discrepancy was seen on cell migration across the surface or bulk of the scaffolds. For the micro-webs, the scaffold with the higher modulus exhibited better cell migration into the bulk of the scaffold, while in the nano-webs the opposite was seen. Clearly, the tensile modulus of the four scaffolds was large enough to provide the proper feedback to the A375 cells for good migration.

5.0 Conclusions

5.1 Melt Blowing

The most prominent conclusion from the fabrication and characterization of micron-sized and nano-sized webs made of PLA was demonstrating that PLA can be melt blown on a commercial line without the use of plasticizers. Published as of March 2012 in the Journal of Applied Polymer Science, the work conducted by Cui et al states that ;

“to blow PLA resins at extremely high rates, the melt index (MI) of PLA needed to be increased to proper values.”⁴⁴

It is obvious that there is a dearth of information available on MB PLA and this study will be able to add value to the field. A very interesting conclusion of this study is that an increase in DCD will not necessarily result in a decrease in fiber diameter for micro-webs as reported by other authors^{14, 16},. The findings reported here conclude that at DCDs greater than 10cm the micro fibers will have cooled below their melting temperatures, making it more difficult for fiber-fiber collisions to cause an increase or decrease in the diameters of the fibers. The calculations predict that nano-fibers, due to their size, cool with air temperature. As the air temperature drops below the T_m of PLA only 1-2cm from the die exit, no change in fiber diameter was seen with an increase in primary air flow rate. Essentially the fibers do not spend enough time in the molten state to fully undergo attenuation.

Data presented in the crystallinity section showed that it is possible to reduce the amount of crystallinity in PLA during web creation, and that full crystallinity can be restored upon annealing. This finding is very important because it means we have the capabilities to produce structures with morphological features associated with low tensile modulus, and then anneal the material to increase the strength of the webs.

5.2 Tissue Engineering

The results presented in table 19 show that MB web architecture including, fiber diameter, pore size and thickness can be precision controlled using the melt blown process. We have presented twenty-one

unique web architectures by varying the MB process parameters, but hundred if not thousands more can be produced by simply increasing the BW collected and varying the primary air flow rates used to blow the molten polymer. Also of importance is that all of the webs were produced without the use of solvents, signifying that MB is an effective alternative method to create non-woven webs for use as tissue engineering scaffolds. We have been successfully able to demonstrate that cells seeded on MB PLA scaffolds with both micro and nano-architectures will adhere to the fibers and migrate into the bulk of the scaffold. Cell spreading and migration was more successful on the nano-webs due to the lower occurrence of pores greater than 100 μ m in diameter. It is clear that epithelial cells cannot cross pore gaps of such distance without the aid of bridging fibers. Also, when seeded on nano-webs the cells simultaneously encounter multiple fibers rather than being seeded on a single fiber as in micro-webs. This provides the cells with more environmental feedback and consequently the cells flatten out and spread more quickly than cells seeded on a single micron-sized fiber. Finally, cell migration in micro-webs was heavily influenced by the long axis of the fibers they were seeded on. Confocal imaging and SEM microscopy were used to clearly identify the directional ailgment of cells seeded on micro-webs.

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Vita

William Gazzola grew up in the woods of southern New Hampshire. He attended high school at Coe-Brown Northwood Academy in Northwood, New Hampshire. After high school, William attended McGill University where he undertook the study of Materials Engineering. In 2008 he received his Bachelor of Engineering with a major in Materials from McGill University. He then proceeded to work in industry for two years before returning to obtain his Master's Degree at The University of Tennessee. William graduated with a Masters of Science Degree in Polymer Engineering in December 2012. He is now enrolled in medical school at The University of New England College of Osteopathic Medicine and plans to open his own primary care facility after he completes his residency.