**University of Victoria**
**Faculty of Engineering**
**Work Term Report**
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# The Effect of Scaffold Architecture on Directing Neuronal Differentiation of Human Induced Pluripotent Stem Cells

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**Work Term 2**
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**September 6th, 2013**

**In partial fulfillment of the requirements of the**
**B.Eng. Degree**

**Supervisor's Approval: To be completed by Co-op Employer**

I approve the release of this report to the University of Victoria for evaluation purposes only.
The report is to be considered (**select one**): ☐ NOT CONFIDENTIAL ☐ CONFIDENTIAL

Signature: _____ Position: _____ Date: _____

Name (print): _____ E-Mail: _____ Fax #: _____

Mr. Calvin Tripp
Co-op Coordinator
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September 6th, 2013

Dear Mr. Calvin Tripp,

Please accept the attached Work Term Report entitled “The Effect of Scaffold Architecture on Neuronal Differentiation of Human Induced Pluripotent Stem Cells.”

I am a 2B Biomedical Engineering student at the University of Victoria and I have just completed my second work term. My employer was the University of Victoria and I worked in the Willerth Lab. The focus of the Willerth Lab is in Tissue Engineering and Regenerative Medicine. My main research project was to investigate the architectural effects of scaffolds on neural differentiation of human induced pluripotent stem cells (hiPSCs). The experiment involved seeding neural progenitor cells derived from hiPSCs onto electrospun, microfiber scaffolds for a time period of 12 days. The cell viability and differentiation was examined using multiple staining assays. I would like to acknowledge my supervisor Dr. Willerth, and Graduate Students Nima Khadem Mohtaram, Amy Montgomery, Jose Carlos Gomez, Lin Sun, and Junghyuk Ko for their guidance, mentorship, and patience with me during this work term. I would also like to acknowledge the members of the Advanced Microscopy Facility for using their laboratory equipment. The experience and core competencies I gained from this work term will certainly be very valuable for my future endeavors.

Sincerely,

Craig King

# Summary

The central nervous system is incapable of regeneration, and the peripheral nervous system's self-repair capabilities are limited. The Willerth Lab is researching the physical effects of three dimensional (3D) scaffolds on stem cell differentiation into neurons in hopes to engineer tissue therapies that regenerate damaged nervous tissue. This report specifically focuses on the physical effects of electrospun, microfiber scaffolds on the viability and neuronal differentiation of human induced pluripotent stem cells (hiPSC).

Neural progenitor cells (derived from hiPSCs) were seeded on two different scaffold topographies, loop mesh and biaxial mesh, for a duration of 12 days. Both scaffolds were fabricated using melt electrospinning techniques. The average fiber diameter for the loop mesh and biaxial mesh scaffolds was 43.7 μm ± 3.90 μm and 42.3 μm ± 2.78 μm, respectively. The average separation distance for the loop mesh and biaxial mesh scaffolds was 177.9 μm ± 106.4 μm and 161.1 μm ± 99.2 μm, respectively. Bright field images showed that both scaffold topographies encouraged cell adhesion and cell migration since cells migrated along the fiber morphologies and filled porous structures. The Live/Dead images proved that both microfiber scaffolds were viable substrates to neural progenitor cells due to the high amount live cells compared to dead cells. Lastly, both microfiber scaffolds fostered neuronal differentiation as a majority of the seeded cells expressed the neuron specific protein β-III-tubulin. These results give insight into the physical effects that electrospun, microfiber scaffolds have on the mechanism responsible for hiPSC neuronal differentiation; however they are exclusively qualitative. The next experimental steps are to generate quantitative results.

# Table of Contents

# Glossary

**Antibody** – A protein produced by B-cells of the immune system that bind to a specific substance

**Antigen** – The substance that binds to a specific antibody

**Blastocyst** – A structure formed in early development of a fetus; consisting of 70-100 cells

**Confluency** – The area covered by cells in a cell culture dish or flask

**Differentiate** – A process where less specialized cell become a specialized cell type

**Esterase** – An enzyme that splits esters into an acid and an alcohol

**CNC Feed Rate** – The velocity at which a CNC machine tool moves

**Glial Cell** – Non-neuronal cells that provide support and protection for neurons

**Multipoten**t – A property of cells that have the potential to differentiate into multiple, but limited cell types

**Nucleic Acids** – Large biological molecules which include deoxyribonucleic acid (DNA) and ribonucleic acid (RNA)

**Passage** – The transferring of a small amount of cells into a new vessel

**Pluripotent** – A property of cells that have the potential to differentiate into any cell type in the body

**Proliferate** – The process where cells multiply

**Scanning Electron Microscopy/Microscope (SEM)** – a type of microscope that produces an image by scanning the sample with a focused beam of electrons

**Vector Virus** – A virus used as a tool used to deliver genetic material into a cell

# Introduction

The research focus of the Willerth Lab is in the area of tissue engineering and regenerative medicine. The lab studies the behavioural response of stem cells seeded on three dimensional (3D) scaffolds. Various physical and chemical factors, such as scaffold morphology and encapsulated growth factors, can influence stem cell differentiation into specialized, mature cell types [1]. The main goal of the Willerth Lab is to understand the mechanisms that direct stem cell differentiation, specifically into neurons. The clinical goal of this research is to engineer tissue therapies, which can regenerate nervous system injuries. This report investigates the physical effects of electrospun, microfiber scaffolds on the viability and neuronal differentiation of human induced pluripotent stem cells (hiPSC).

## 1.1 Stem Cells

All tissues are composed of cells, which are derived from stem cells [2]. They are responsible for the fetal development, and for the daily maintenance and repair of injured tissue and cells [2]. A stem cell is defined by two properties: self-renewal and potency. Self-renewal is the ability to replicate and remain in an undifferentiated state and potency is the ability to differentiate into specialized cell types.

Figure 1 shows the process of stem cell proliferation (cell division) and differentiation (specialization). Cell “A” is a stem cell that can proliferate (process 1) and differentiate (process 2). Cell “B” is a progenitor cell; it is limited to proliferate (process 3) or differentiate (process 4). Cell “C” is a specialized adult cell.

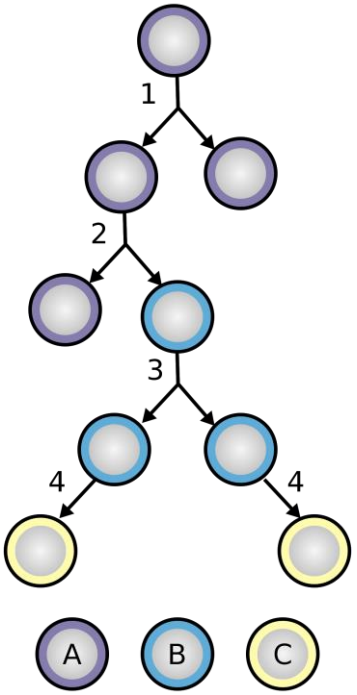


**Figure 1: The Process of Stem Cell (A) Division and Differentiation into Progenitor Cells (B) and Specialized Adult Cells (C)**

The main types of stem cells are embryonic stem (ES) cells, adult stem cells, and induced pluripotent stem (iPS) cells. ES cells are derived from a blastocyst, which is an early stage embryo. They are pluripotent meaning they can differentiate into any cell from the body [2]. Adult stem cells are semi specialized cells found in various tissues in the body. However, they are multipotent, meaning they can differentiate into multiple cell types specific only to the tissue in which they reside [2]. iPS cells are specialized adult cells that have been engineered to become pluripotent and behave like ES cells. Generally, vector viruses are used to genetically reprogram adult cells to become pluripotent by inserting ES cell genes [2].

Since ES cells are derived from embryos they are shrouded with ethical controversy; however, adult stem cells and iPS cells do not have this issue. Disadvantages of adult stem cells include their limited access (locked in deep tissue) and potency. iPS cells appear to be the best option since they are pluripotent and can provide patient specific cell lines. The Willerth Lab studies the behaviour of both ES cells and iPS cells. Specifically, this report focuses on the behaviour of hiPSCs.

When the factors that maintain the pluripotent state are removed, pluripotent stem cells form 3D aggregates called embryoid bodies (EB). It is believed the cells are mimicking the early stages of embryonic development as they begin to differentiate [3]. EBs will differentiate non-specifically to form all cell types of the human body in the absence of additional physical or chemical cues. Differentiation can be directed into specific lineages using optimized media formulations, and stimulatory or inhibitory factors [3]. The differentiation of the hiPSCs discussed in this report are directed into neural progenitor cells before being seeded onto 3D scaffolds.

## 1.2 Electrospinning

Electrospinning is a simple, fast, and cost-effective fiber fabrication technique that uses electric forces to produce polymer fibers on the nano to microscale [4]. This process is widely used in Tissue Engineering research because of its ability to produce high surface area, porous scaffolds which fosters cell adhesion, cell invasion, and diffusion of nutrients and waste [5]. The two major methods of electrospinning include melt and solution electrospinning. Both solution and melt electrospun scaffolds are proven to be stem cell compatible; however, solution electrospinning is the most commonly used in Tissue Engineering applications [6] [7]. Both solution and melt electrospinning require a high voltage to be applied between a liquid polymer and a collector plate. The electrostatic force and gravity will counteract the surface tension of the polymer liquid and draw a fiber towards the charged collector plate.

Solution electrospinning requires the polymer to be dissolved into solution using harsh, volatile organic solvents such as dichloromethane. Therefore, solution electrospinning can take place at room temperature. During the electrospinning process the majority of the solvent evaporates, however, residues may remain that possibly have cytotoxic effects. Melt electrospinning avoids these harsh chemicals by melting the polymer into a liquid. Consequently, the working temperature of melt electrospinning must be equal or greater than the polymer melting point. Bioactive molecules such as growth factors and proteins can be encapsulated in electrospun fibers, which can diffuse out from the fibers for controlled drug delivery purposes. The lower working temperature of solution spinning is better suited for encapsulating drugs because the higher working temperature of melt electrospinning can denature the bioactive molecules.

In solution electrospinning, the solution is more conductive and less viscous then the polymer melt in melt electrospinning. This causes the attractive and repulsive electrostatic forces to become more significant causing the fiber to whip and lash randomly. This random whipping and lashing causes the fiber to be drawn out on the nanoscale; however it limits the control over scaffold topography and porosity. The polymer melt has a lower conductivity and higher viscosity resulting in larger fibers and more control over scaffold morphology. Fiber morphologies such as diameter, separation and orientation can be altered by changing the electrospinning operating parameters found in Table 1. A variety of scaffold topographies including the ones discussed in this report can be fabricated by tailoring these operating parameters. A list of the advantages and disadvantages of solution and melt electrospinning can be found in Table 2 and 3.

**Table 1: Electrospinning Operating Parameters**

| |
|---|
| Applied voltage |
| Collecting distance |
| Working Temperature |
| Motion of collector/nozzle |
| Nozzle diameter |
| Net time electrospinning |
| Viscosity of solution/melt |
| Flow rate of solution/melt |
| Conductivity of solution/melt |

**Table 2: Advantages and Disadvantages of Solution Electrospinning**

| **Advantages** | **Disadvantages** |
|---|---|
| Fiber diameter on nano and microscale | Requires toxic solvents |
| Low working temperature | Limited control over topography of scaffolds |
| | Limited reproducibility of scaffolds |
| | Limited control over porosity of scaffolds |

**Table 3: Advantages and Disadvantages of Melt Electrospinning**

| **Advantages** | **Disadvantages** |
|---|---|
| Precise control over scaffold topography | High working temperature |
| Higher reproducibility of scaffolds | Fiber diameter limited to microscale |
| Does not require toxic solvents | |
| Precise control over scaffold porosity | |

# 2 Methods and Materials

The physical effects of electrospun, microfiber scaffolds on the viability and neuronal differentiation of hiPSCs were investigated using the following methods and materials. The 3D scaffolds were fabricated in two different topographies using a melt electrospinning setup. The microstructure of these scaffolds were characterized using scanning electron microscopy (SEM). The details of the hiPSC culture, neural aggregate formation, and progenitor cell

seeding are explained. Lastly, the staining methods used to qualitative analyze cell viability and cell differentiation are described.

## 2.1 Melt Electrospinning

The 3D scaffolds are fabricated using a custom made melt electrospinning apparatus seen in Figure 2. The major parts of the melt electrospinning apparatus are the CNC machine from K2CNC, the syringe pump from New Era Pump Systems, the heating band from Orion Telescopes, and the high applied voltage supply from Gamma High Voltage Research. The custom made parts include the melting chamber, chamber press, and the interchangeable nozzles.

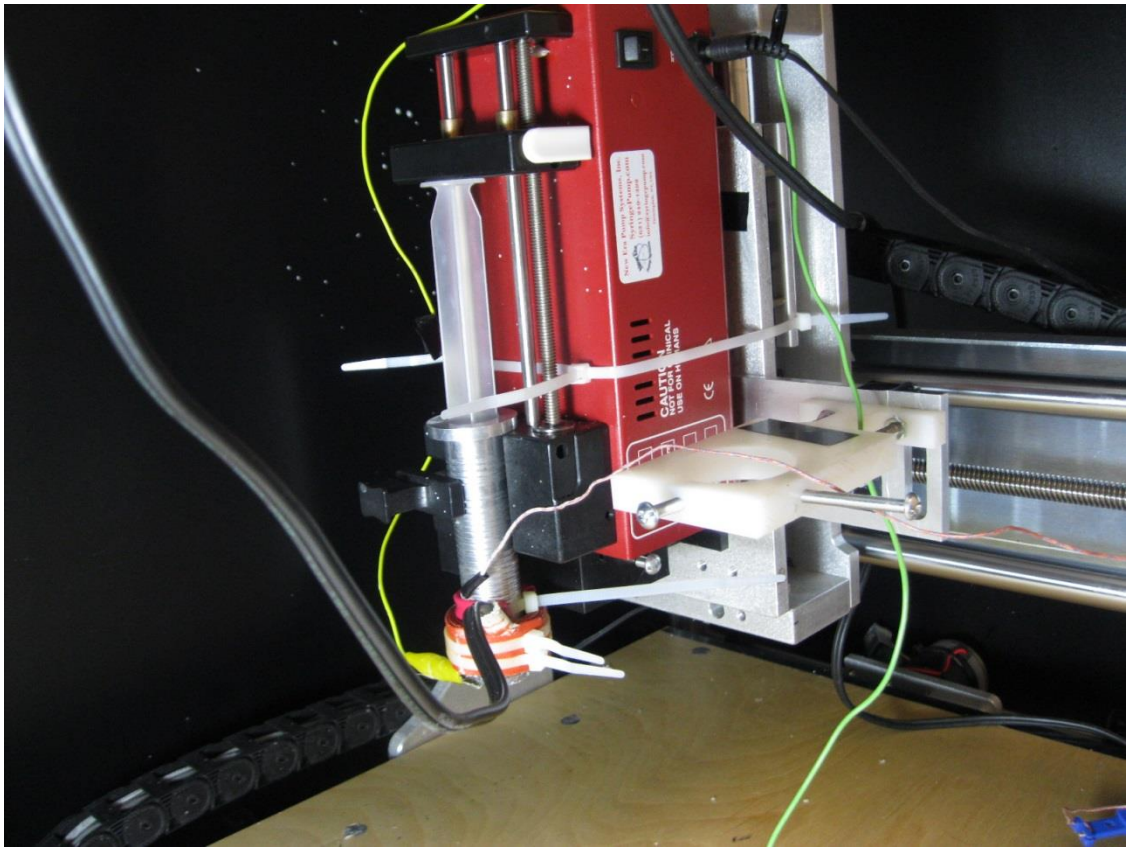

**Figure 2: Electrospinning Apparatus**

The polymer used in the melt electrospinning process was polycaprolactone (PCL), a biodegradable polyester. The PCL granules used, purchased from Sigma Aldrich, had a molecular weight of 45,000 Da and a melting point of 60 ºC.

The PCL granules were heated in the melt chamber by the heating band, and the syringe pump coupled with the chamber press exerted a continuous flow rate of the molten PCL. A high voltage was applied to the molten PCL and the collecting surface, which was a wooden plate wrapped in conductive aluminum foil. The spinneret was coupled to the CNC machine giving control of the fiber dispensing in the X, Y, and Z direction; however the Z (collecting) distance was constant. The melt electrospinning operating parameters for each scaffold topography, loop mesh and biaxial mesh, were adjusted accordingly.

### 2.1.1 Loop Mesh

The loop mesh scaffolds consisted of two layers of looped fibers. The CNC machine was programmed to lay a layer of horizontal fibers on top of a layer of vertical fibers. A low CNC feed rate was used causing the fibers to bunch up in loops. A loop mesh scaffold can be seen in Figure 3.

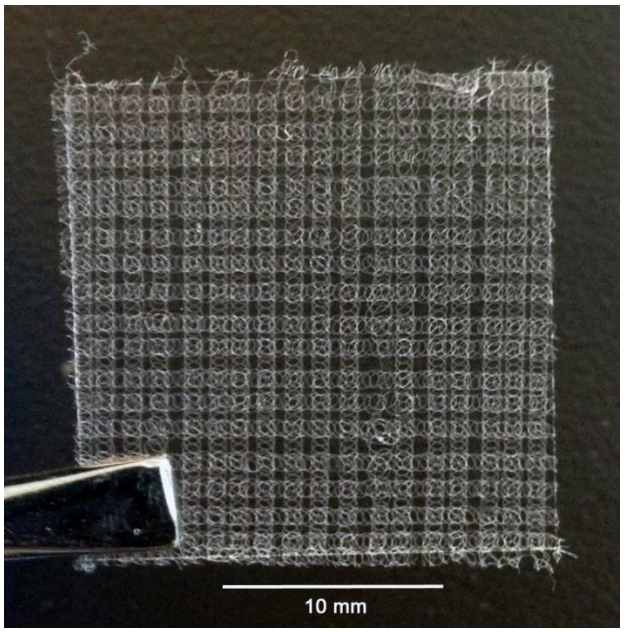


**Figure 3: Loop Mesh Scaffold**

The following were the melt electrospinning operating parameters for the loop mesh scaffolds:

- Applied Voltage: 20 kV
- Collecting distance: 5 cm
- Working temperature: 80 ºC
- CNC feed: 200 mm/s
- Nozzle diameter: 200 µm
- Layers: 2
- Viscosity of melt: 295.1 Pa·s
- Flow rate of melt: 2 mL/hr

### 2.1.2 Biaxial Mesh

The biaxial mesh scaffolds consisted of 20 layers of straight fibers. The CNC machine was programmed to lay a layer of horizontal fibers on top of a layer of vertical fibers; this was repeated ten times. A high CNC rate was used drawing the fibers straight. A biaxial mesh scaffold can be seen in Figure 4.

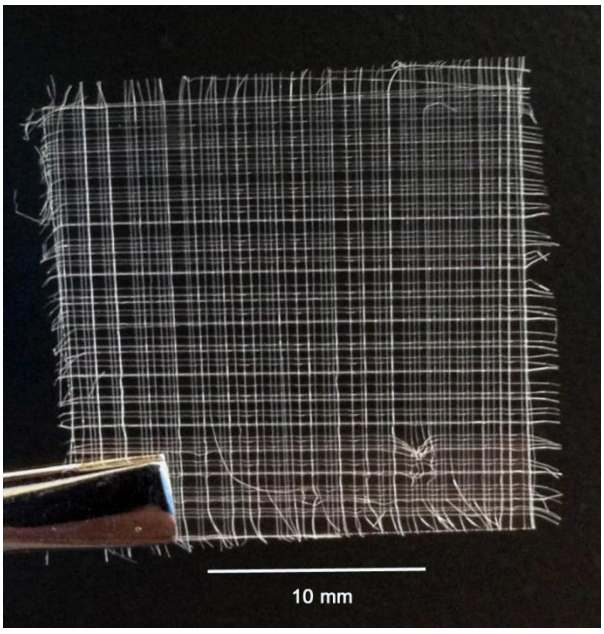


**Figure 4: Biaxial Mesh Scaffold**

The following were the melt electrospinning operating parameters for the biaxial mesh scaffolds:

- Applied Voltage: 20 kV
- Collecting distance: 5 cm
- Working temperature: 80 ºC
- CNC feed: 1700 mm/s
- Nozzle diameter: 200 μm
- Layers: 20
- Viscosity of melt: 295.1 Pa·s
- Flow rate of melt: 2 mL/hr

## 2.2 Microstructure Characterization

The microstructure of the loop mesh and biaxial mesh scaffolds were characterized using SEM and image processing software. Loop mesh and biaxial mesh scaffold samples were transferred to loading stubs and carbon coated for SEM preparation. A 3 nm thick carbon layer was coated on the polymer microfiber surface with a Cressington 208 Carbon Coater; this increased the image quality by reducing the electron charge up on the non-conductive polymer fibers. After preparation the samples were loaded and imaged in the Hitachi S-4800 field emission SEM. Low magnification images were taken at a 1.0 kV and 10 μA beam intensity and a working distance of approximately 8 mm [6]. A large sample size of fiber diameters and separation distances for the loop mesh and biaxial mesh scaffolds were measured with Quartz PCI Digital Imaging and Processing software. From these measurements the average fiber diameter and separation distance for each scaffold were calculated.

## 2.3 Human Induced Pluripotent Stem Cell Culture

hiPSCs were cultured on a Vitronectin XF™ matrix and in TeSR™-E8™ media, both from STEMCELL Technologies [8]. Vitronectin is a protein from the extracellular matrix that promotes cell adhesion and cell invasion [8]. TeSR™ is a feeder free media that maintains undifferentiated human ES and iPS cells. The hiPSCs were incubated at a 5% carbon dioxide ($CO_2$) level and a temperature of 37 ºC to mimic body conditions. When cell confluency reached approximately 90% the hiPSCs were passaged. A colony of hiPSCs at approximately 70% confluency can be seen in Figure 5.

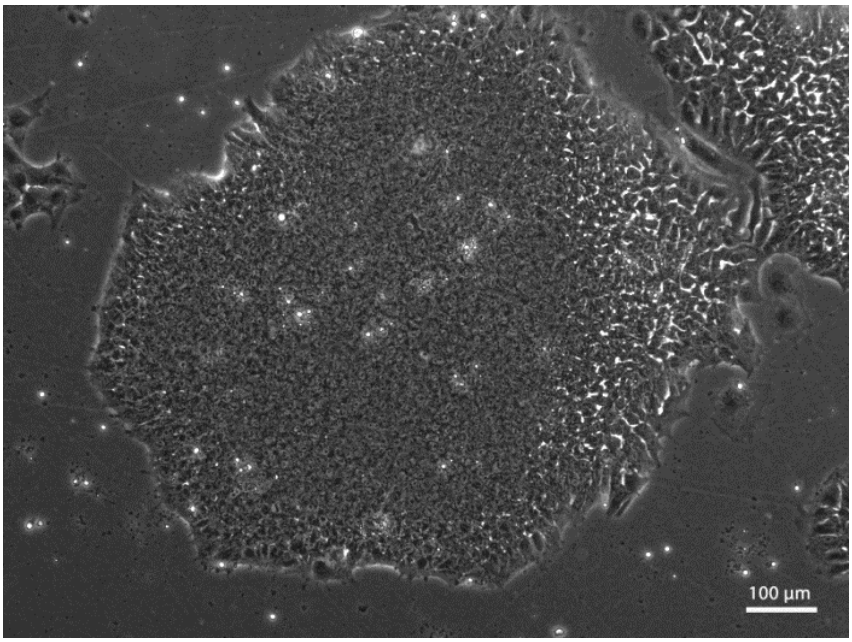


**Figure 5: Human Induced Pluripotent Stem Cell Colony**

## 2.4 Human Neural Aggregate Formation

Human neural aggregates or EBs were formed by dissociating 90% confluent hiPSC colonies into a single cell solution using STEMCELL Technologies' Gentle Cell Dissociation Reagent. The single cell solution was distributed into STEMCELL Technologies' 8 well Aggrewell™ 800 plate [9]. The Aggrewell 800 plate has small pyramidal depressions that use gravity to form consistent, spherical EBs [9]. At 90% confluency the colonies dissociate into

approximately 6 million cells yielding approximately 20,000 cells per EB [3]. The EBs were incubated in STEMCELL Technologies' STEMdiff™ Neural Induction Medium (NIM) for 5 days at a 5% $CO_2$ level and 37 ºC. 2 mL of NIM was added to each well and 1.5 mL was changed daily. NIM directs the differentiation of the EBs into neural progenitor cells. An EB after 1 day of formation can be seen in Figure 6.

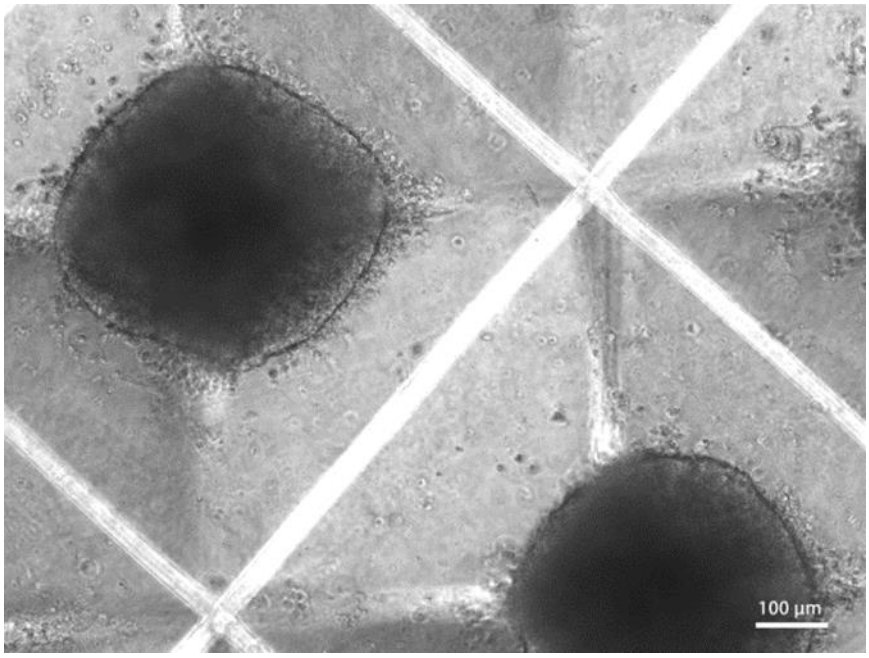


**Figure 6: Embryoid Body Formation After 1 Day in an Aggrewell Plate**

## 2.5 Neural Progenitor Cell Seeding

After 5 days of EB formation, EBs containing neural progenitors cells were seeded onto loop mesh and biaxial mesh scaffolds. Approximately four EBs were seeded into each well of the 6 well plates containing loop mesh or biaxial mesh scaffolds. The EBs were placed in NIM and incubated at 5% $CO_2$ and 37 ºC for 12 days.

## 2.6 Cell Viability

After 12 days the viability of the seeded cells was qualitatively analyzed with fluorescent microscopy and Invitrogen's Live/Dead® Viability/Cytotoxicity Kit *for mammalian cells*. The kit contains two fluorescent stains that measure cell viability: calcien AM and ethidium homodimer

[6]. Calcien AM freely diffuses into live cells where it is enzymatically cleaved into green fluorescent calcien by intracellular esterase [6]. Ethidium homodimer cannot diffuse through live cells, but can diffuse through the damaged membranes of dead cells, where it binds to nucleic acids and fluoresces red [6]. The cells were washed twice in Dulbecco's phosphate buffered saline (DPBS) from Invitrogen, then a 600 µL stain solution with a calcien AM concentration of 2 µM and ethidium homodimer concentration of 4 µM was added to each well. The seeded cells were incubated at room temperature for 45 minutes prior to viewing with an IncuCyte ZOOM Essen BioScience® fluorescent microscope. Images were captured at a wavelength of 515 nm for green fluorescence and 635 nm for red fluorescence then overlaid at 50% opacity.

## 2.7 Cell Differentiation

After 12 days the neuronal differentiation of the seeded cells was qualitatively analyzed with fluorescent microscopy and immunocytochemistry targeting β-III-tubulin, a microtubule protein found exclusively in neurons. The cells must be chemically fixed to maintain cellular structure in a state as near to live as possible. Then the cell membranes must be permeabilized (dissolved by detergents) giving access for the primary and secondary antibodies to the intracellular antigens. Primary antibodies target and bind to a molecule of interest, which in this case is the protein β-III-tubulin. Primary antibodies are typically unconjugated meaning they do not have a detectable probe; therefore a conjugated secondary antibody is needed that binds to the primary antibody that has a detectable fluorescent probe.

The seeded cells were fixed in a 10% formalin solution from Sigma for one hour at room temperature, and then permeabilized in a 0.1% Triton-X solution from Sigma for 45 minutes at 6 ºC. The permeabilization was then blocked with 5% normal goat serum from Millipore for 2 hours at 6 ºC. The primary antibody for β-III-tubulin from Millipore was then added to each well at a 1:500 dilution and incubated for 15 hours at 6 ºC. The cells were then washed three times with phosphate buffered saline (PBS) from Invitrogen then a 1:200 dilution of Millipore's Alexa

Fluor® 488-conjugated secondary antibody was added to each well. The cells were then protected from the light and incubated for four hours at room temperature. Again, the cells were washed three times with PBS then viewed with an IncuCyte ZOOM Essen BioScience® fluorescent microscope at four times magnification. For ten times magnification a LEICA 3000B inverted microscope was used with a X-cite series 120Q fluorescent light source from Lumen Dynamics coupled with a Retiga 2000R fast-cooled mono 12-bit camera from Q-imaging. Images were then captured at a wavelength of 515 nm for green fluorescence.

# 3 Results

The physical effects of electrospun, microfiber scaffolds on hiPSCs are discussed below. The loop mesh and biaxial mesh scaffolds were quantitatively characterized using SEM and image processing software. The resulting effect of these scaffolds on hiPSC viability and differentiation were qualitatively assessed using cell viability assays and immunocytochemistry.

## 3.1 Microscale Characterization

The loop mesh and biaxial mesh scaffolds were characterized using SEM and an image processing software. A large sample size of fiber diameters and separation distances were used to find the mean and standard deviation (SD) of each. The mean and SD for fiber diameter and separation distance of the loop mesh and biaxial mesh scaffolds can be found in the Table 4.

**Table 4: Microscale Characterization of Scaffolds**

| | **Mean Fiber Diameter** | **SD of Fiber Diameter** | **Mean Separation Distance** | **SD of Separation Distance** |
|---|---|---|---|---|
| **Loop Mesh** | 43.7 μm | 3.90 μm | 177.9 μm | 106.4 μm |
| **Biaxial Mesh** | 42.3 μm | 2.78 μm | 161.1 μm | 99.2 μm |

The fiber diameters and separation distances were measured with Quartz PCI for each scaffold. Six images were analyzed per scaffold topography; three for measuring fiber diameter and three for measuring separation distance. Figure 7 shows the technique used for measuring fiber diameter and separation distance for both loop mesh and biaxial mesh scaffolds, as well as an angled view of each topography.

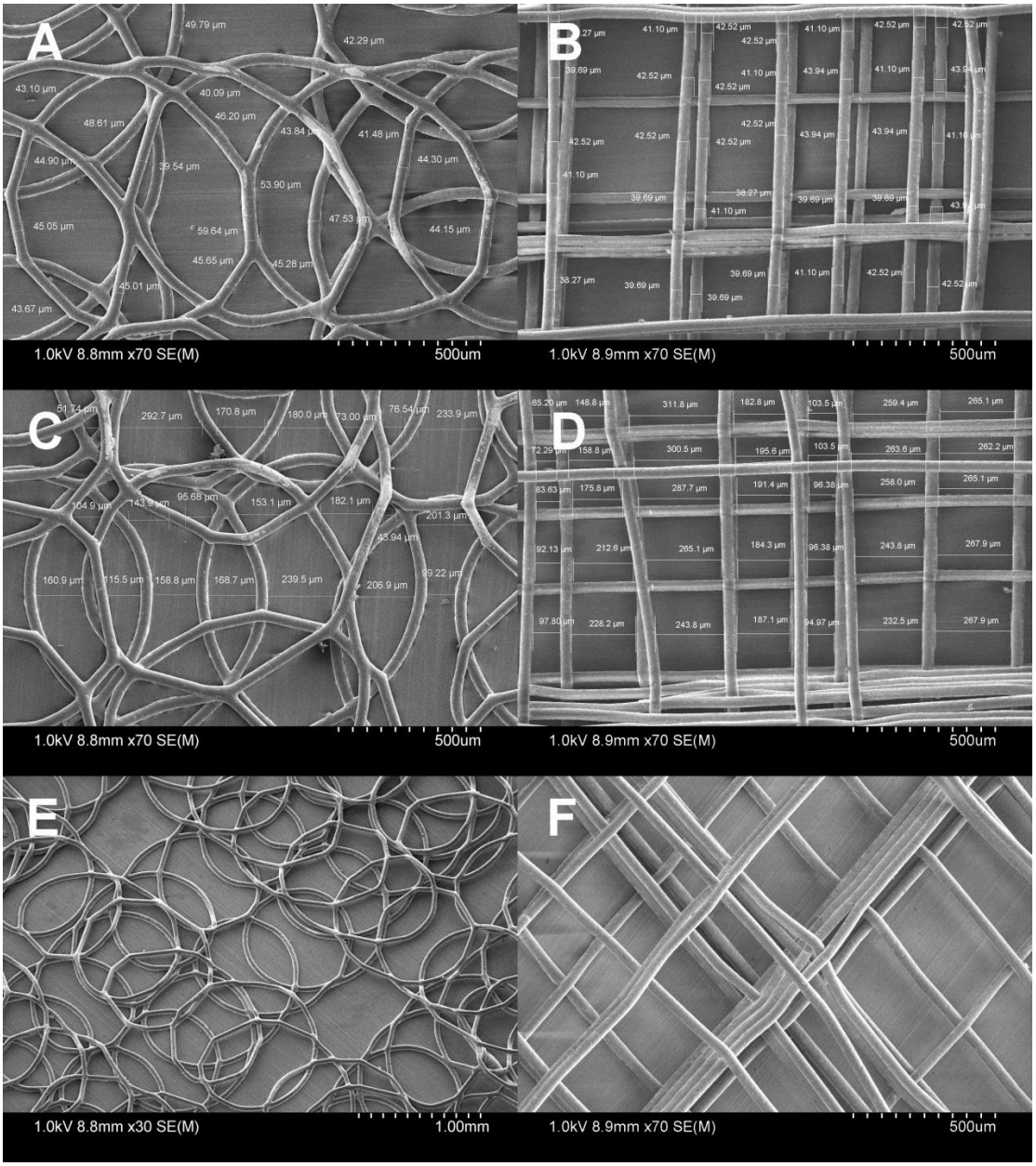


**Figure 7: Measuring technique for: loop mesh fiber diameter (A), loop mesh separation distance (B), biaxial mesh fiber diameter (C), and biaxial mesh separation distance (D). Angled view of loop mesh (E), and biaxial mesh scaffolds (F).**

## 3.2 Cell Viability and Differentiation

EBs containing neural progenitor cells derived from hiPSCs were seeded on loop mesh and biaxial mesh scaffolds for 12 days. The viability of the seeded cells was qualitatively analyzed with fluorescent microscopy and a Live/Dead viability kit. The kit contains stains that cause the cells to fluoresce green if they are alive and red if dead under fluorescent microscopy. This gave us a tool to quantitatively analyze cell viability. The differentiation of the seeded cells was qualitatively analyzed with fluorescent microscopy and immunocytochemistry. Immunocytochemistry uses primary antibodies and conjugated secondary antibodies to target β-III-tubulin, a neuron specific protein. The conjugated secondary antibodies bind to the primary antibodies, which in turn bind to β-III-tubulin. The conjugated secondary antibodies also fluoresce green under fluorescent microscopy, therefore, since β-III-tubulin is neuron specific only neurons should fluoresce. This gave us a tool to quantitatively analyze neuronal differentiation. High magnification bright field images of cells seeded on loop mesh and biaxial mesh scaffolds after 10 days can be seen in Figure 8. Bright field, Live/Dead, and immunocytochemistry images of cells seeded on loop mesh and biaxial mesh scaffolds after 12 days can be seen in Figures 9 and 10, respectively.

It can be seen in the bright field images that both scaffold topographies encourage cell adhesion and cell migration. The cells have started to migrate outward from the spherical EB along the straight or looped fibers, depending on the scaffold morphology. The cells have also started to migrate between the pores of the scaffold and fill the loop and rectangular structures. It appears that the smaller pores are easier for the cells to migrate and fill.

The Live/Dead images demonstrate that both scaffold topographies are viable substrates for hiPSCs because the majority of the seeded cells fluoresced green. Small amounts of cell death is unavoidable; however the dead (red fluorescing) cells present may be due to the media. The cells metabolize the nutrients in the media and release waste by-

products, which lowers the levels of nutrients and raises the level of toxins. This could be addressed by changing the media during the 12 day seeding period.

The immunocytochemistry images show that both scaffold topographies foster neuronal differentiation because of the large number of green fluorescing cells. The green fluorescing cells have differentiated into neurons because they contain the neuron specific protein β-III-tubulin. After comparing the immunocytochemistry images to their corresponding bright field images it is apparent that not all the cells fluoresce. It appears that the neurons have grown along the scaffold fibers and dense cell areas which have the most support. The cells that are not fluorescing appear to be the outer cell growth in the porous areas between the scaffold fibers. It is suspected that these are glial (supportive) cells laying down a supportive structure that would promote neuronal differentiation given a longer seeding period.

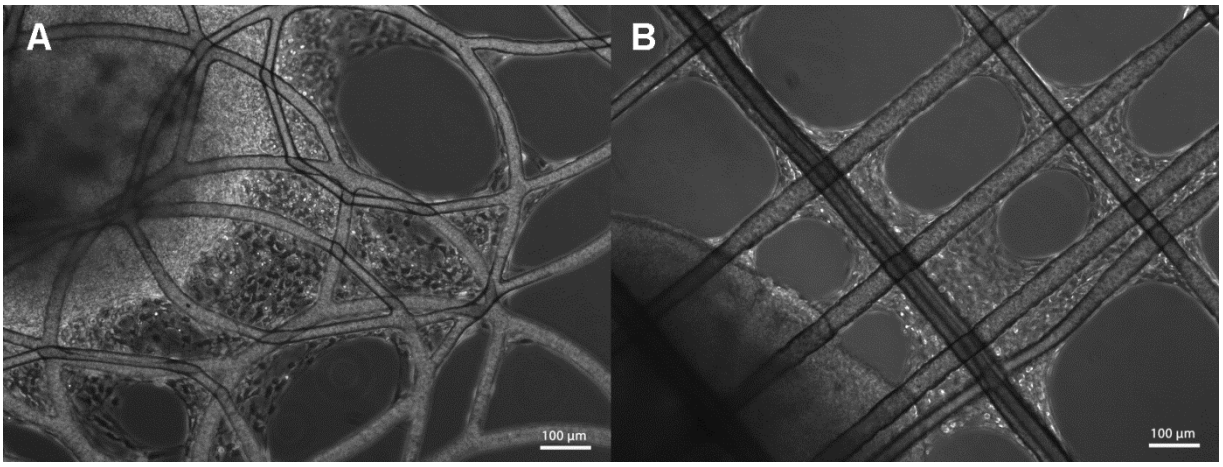


**Figure 8: Neural progenitor cells seeded on loop mesh (A), and biaxial mesh (B) scaffolds after 10 days**

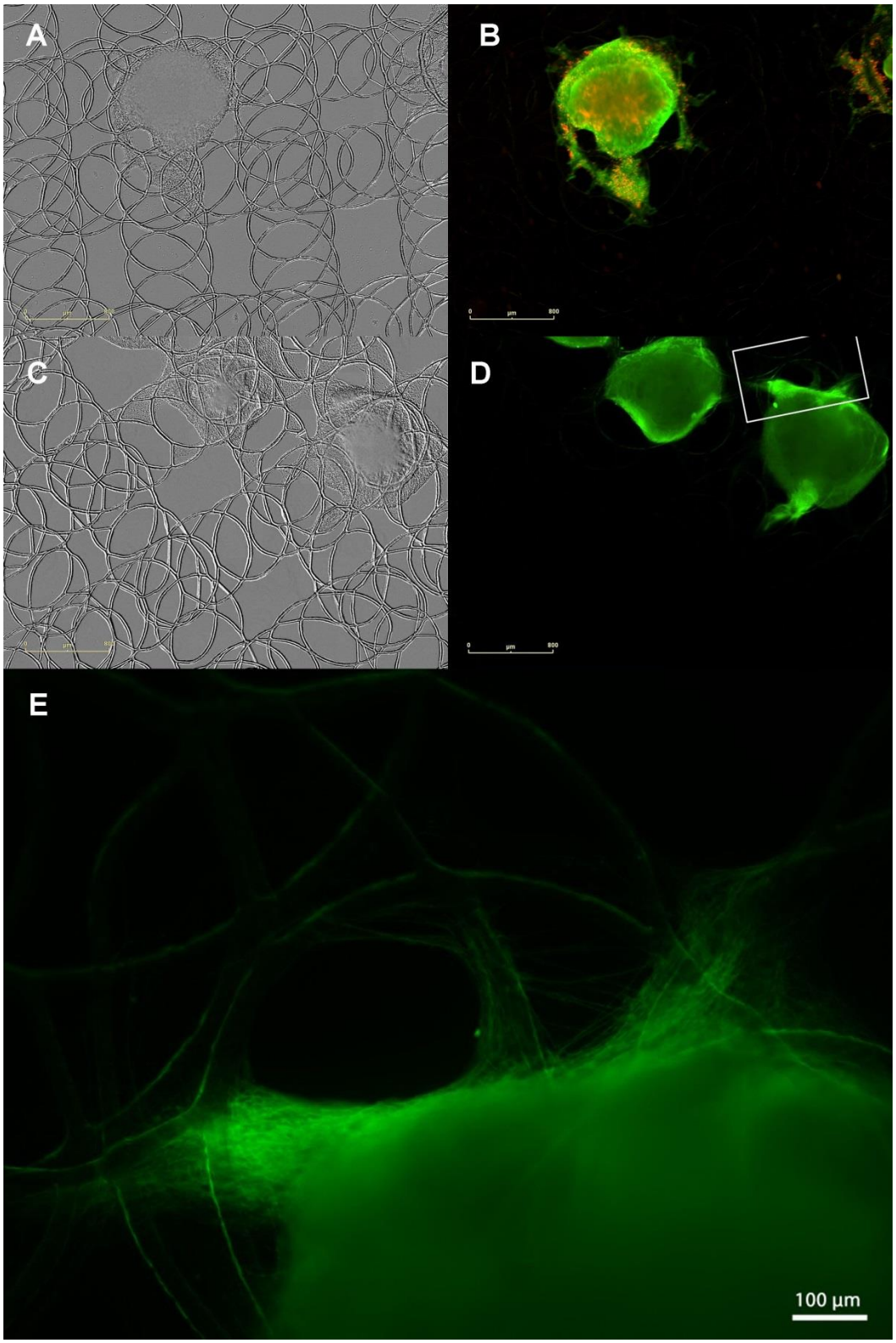


**Figure 9: Neural progenitor cells seeded on loop mesh scaffolds after 12 days. (A) is a bright field image corresponding to its Live/Dead staining counterpart (B). (C) is a bright field image corresponding to its immunocytochemistry staining counterpart (D). (E) is higher magnifiation image corresponding to the white rectangle in (D).**

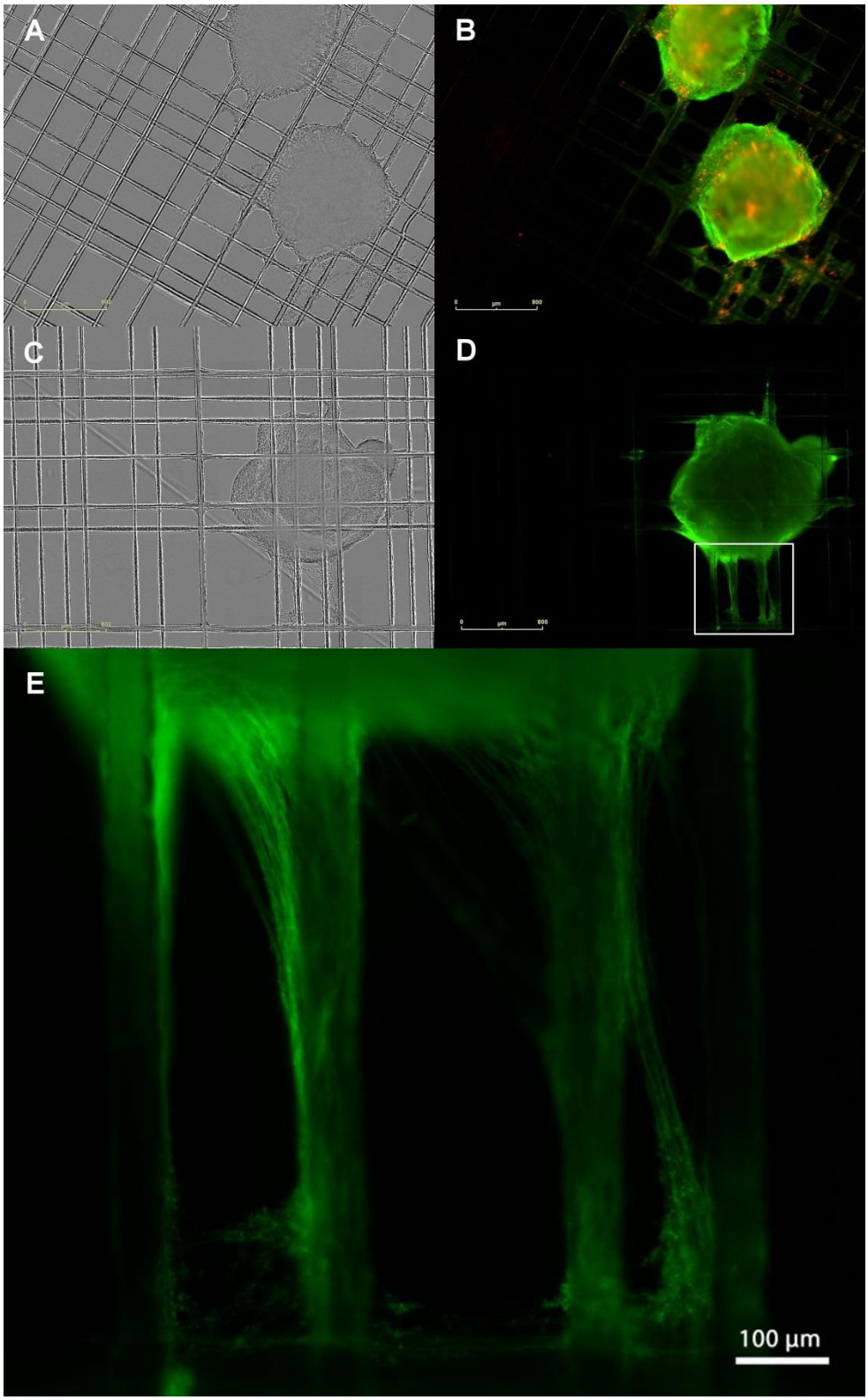


**Figure 10: Neural progenitor cells seeded on biaxial mesh scaffolds after 12 days. (A) is a bright field image corresponding to its Live/Dead staining counterpart (B). (C) is a bright field image corresponding to its immunocytochemistry staining counterpart (D). (E) is an higher magnification image corresponding to the white rectangle in (D).**

# 4 Conclusion

The physical effects of electrospun, microfiber scaffolds on the viability and neuronal differentiation of hiPSCs were investigated. Two different scaffold topographies, loop mesh and biaxial mesh, were fabricated using melt electrospinning techniques. The average fiber diameter for the loop mesh and biaxial mesh scaffolds was 43.7 μm ± 3.90 μm and 42.3 μm ± 2.78 μm, respectively. The average separation distance for the loop mesh and biaxial mesh scaffolds was 177.9 μm ± 106.4 μm and 161.1 μm ± 99.2 μm, respectively. After 12 days of seeding neural progenitor cells (derived from hiPSCs) on the loop mesh and biaxial mesh scaffolds the following conclusions were made. Both scaffold topographies encouraged cell adhesion and cell migration since bright field images showed that cells migrated along the fiber morphologies and filled porous structures. The Live/Dead images proved that both microfiber scaffolds were viable substrates to neural progenitor cells due to the high ratio of live cells over dead cells. Lastly, both microfiber scaffolds fostered neuronal differentiation because a majority of the seeded cells expressed the neuron specific protein β-III-tubulin. Although these results are exclusively qualitative they give insight into the physical effects that electrospun, microfiber scaffolds have on the mechanism responsible for hiPSC differentiation into neurons.

# 5 Recommendations

The next step in this research is to generate qualitative results. I recommend using an image analysis software like Matlab to analyze the large sample set of Live/Dead and immunocytochemistry images collected during this experiment. The software would be able to determine the percentage of live/dead cells and neurons in the seeded EB by counting the number of green and/or red pixels in a defined area (the seeded EB). Alternatively, flow cytometry could be used; however, the experiment protocols would have to be repeated. Flow cytometry is a laser based technique that counts and sorts specific cells from a single cell

solution including neural, live, dead, differentiated, and undifferentiated cells. I also recommend analyzing wells at time intervals throughout the 12 day seeding period. Progressive, quantitative data could yield a greater understanding of the mechanism behind hiPSC neuronal differentiation.

# 6 References


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