Integrating Electrospun Biocomposites Fibers with Microfluidics to Regulate Cell Behavior

Douglas Lafleur,[a] Garrett Hunter,[a] Tran Truong,[b] Dr. Linqing Li [b]
[a]Department of Mechanical Engineering, University of New Hampshire
[b]Department of Chemical Engineering, University of New Hampshire

Background

The Goal of the Research

Electrospun nanofiber materials are used to create scaffolding that cells can migrate across, facilitating tissue growth. This mimics the **extracellular matrix (ECM)**, the fiber networks which cells bind to and grow on, allowing the creation of 3D tissue networks for medical applications in the field of **regenerative medicine**. ECM fiber widths average **0.06-0.12 µm.**

The Process Itself

Mixtures of varying concentrations of **dextran** (a branched polymer of dextrose) and **silk** from the domestic silk moth (Bombyx Mori) formed scaffolding due to **electrospinning,** resulting in nanofibers of differing cross-sectional widths, patterns, and concentrations. By crosslinking these fibers, structural integrity for **dermal fibroblast cell cultures** was ensured, allowing the team to identify patters of growth on/within the scaffolding.

Applications

Regenerative Medicine

Electrospun nanofibers bear myriad uses. Most commonly, **scaffolding** for cells. To manipulate the viability of the fibers, thickness, density, strength, and even the presence of chemical signals is altered. This is critical for ensuring the growth of **desired lineages of soft tissue**. Once the tissue is grown, the **scaffolding can be absorbed**, leaving free-standing replacement tissue. With these methods, there arises the possibility of allowing the body to grow back what it lost, **instead** of utilizing **invasive methods** such as skin grafts. In addition, the tissues produced with this technique have the potential to be **applied to the heart** as a pericardial patch.

Drug Delivery

The created structure both has a **large surface area** and is **very porous**. Therefore, the fiber's unique characteristics could make it ideal for **prolonged drug delivery**. By utilizing its porous nature, large doses of medicine may be secreted slowly. This may allow even antibiotics to be built-in, **lowering overuse and preventing infection**.

Cell Research

What sets this apart is the porous and **3D nature** of the structure. In culture, cells are typically grown in 2D, **altering their behavior**. 3D space allows researchers **a better understanding** of cell function. Applied to cancer research, understanding their true function in the body **is paramount**.

Research Objectives

Investigating electrospinning conditions and material properties to optimize dextran-based and silk nanofibers for dermal fibroblast cell-culture.

- **Synthesize** solutions
- **Electrospin** Scaffolding
- **Characterize** Nanofibers
- **Crosslink** to Form Hydrogels
- **Culture** Dermal Fibroblast Cells

Fig. 1: Electrospinning Setup

Materials & Methods

Solution Preparation For Spinning

Materials for Solution Synthesis

- Dextran (86 kDa, 150kDa)
- Deionized (DI) water
- Syringe with 23-gauge needle
- Electrospinning pump
- High voltage source
- Conductive collection plate

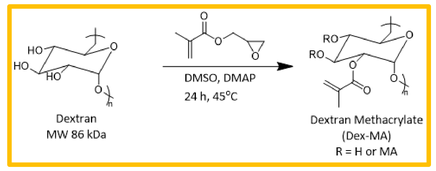

Fig. 2: Molecular Structure of Dextran and Dex-MA [4]

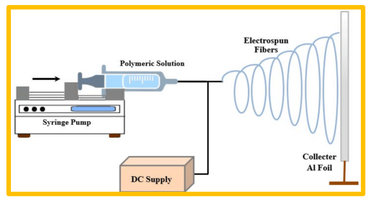

Fig. 3: Electrospinning Schematic and Taylor Cone [6]

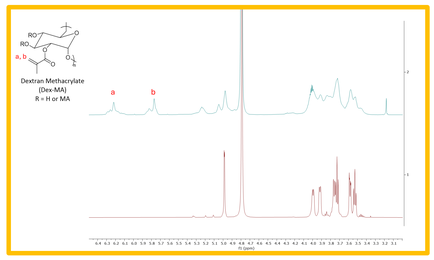

Fig. 4: ^{1}H NMR Confirmed Structure of Dex-MA

Cell Culture

Materials for Cell-Culture

- Human Dermal Fibroblasts **(HDF)**
- 50% dextran methacrylate **(Dex-MA)**
- Phosphate buffered saline **(PBS)**
- Fibronectin coating
- Irgacure 2959 **(photoinitiator)**
- Trypan Blue
- Phalloidin 488 (staining)
- DAPI (staining)

Hydrogel Formulation

Due to the **high solubility** of dextran, the scaffold was dissolved in a solution of Dex-MA **itself** dissolved in 150 µL (1X) **PBS** with 3 µL **Irgacure** (I2959) added. Then, by transferring it to a **MatTek dish** to **photo-crosslink** the solution under UV light for 60 seconds to form a **gel.**

The hydrogels were then coated with 0.1 mg/mL **Fibronectin** for 1 hour [1]. Once the hydrogels were coated, they were washed once with (1X) **PBS**. 50,000 cells were seeded for each setting.

Cell Seeding and Culturing

Trypan blue was used to determine cell seeding density using the **Countess digital counting system**. After 24 hours, cells were fixed and stained with phalloidin and DAPI to reveal the actin **cytoskeleton** and nucleus of the cells and cell morphology.

Solution Synthesis

Scaffolds were produced by dissolving **dextran powder** in DI water and DMF in **centrifuge** tubes using a **vortex mixer** and **ultrasonic water bath**. The resulting solution is then electrospun using a 10 mL syringe with a 23-gauge needle. Dried samples were collected for **SEM analysis**.

Electrospinning Process

Screening Electrospinning Parameters

The physical features of fiber width and density in the support are **influenced** by limiting guidelines such as **distance, voltage, extrusion rate,** as well as the **concentration of silk**. The distance proved crucial during experimentation while maintaining a constant voltage of **15 kilovolts and a flow rate of 0.5 mL/hr**. The first analysis for all concentrations permitted the flow at 8 cm from the **needle tip to the collector plate**. Proceeding, other samples were spun at distances of 10 cm, 12 cm and 14 cm.

Concept of Electrospinning

Electrospinning involves the use of high voltage to create **nanofibers** from polymer solutions. As the **syringe plunger** is pressed down steadily, the solution is extruded from a needle tip. The use of high voltage **charges** the solution stream, resulting in the creation of a group of strands of a **conical** shape called a **Taylor cone**. These charged nanofibers are then collected on a **grounded conductive surface.** [2]

Nanofiber Characterization

Dextran Only

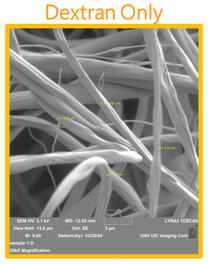

Fig. 5: SEM Image of Control Group

3:1 Dex : Dex-MA

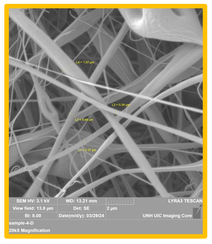

Fig. 6: SEM Image of Dextran and Methacrylate Sample

1:1 Dex : Dex-MA

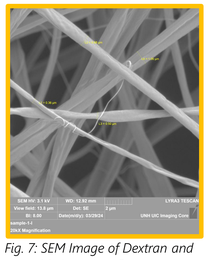

Fig. 7: SEM Image of Dextran and Methacrylate Sample

Dextran and Dextran-MA

Higher amounts of **Dextran Methacrylate (Dex-MA)** compared to regular dextran caused the amount of irregular **thin wires** to decrease. This is likely related to the strength of the threads.

Dextran and Silk

The sample with **higher concentrations** of silk appears to be **dense, torn, tangled**, and **messy**. However, with lower concentrations of silk, far **straighter and thicker bands** are achieved.

150 kDa 3:2 Dex:Silk

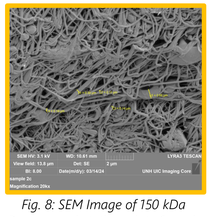

Fig. 8: SEM Image of 150 kDa Dextran and Methacrylate Sample

150 kDa 4:1 Dex:Silk

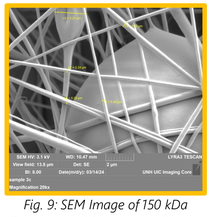

Fig. 9: SEM Image of 150 kDa Dextran and Methacrylate Sample

10 cm

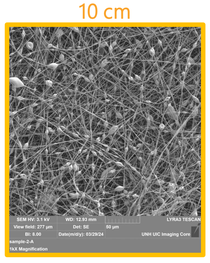

Fig. 10: SEM Image of 86 kDa Dex/DexMA (3:1) Sample

12 cm

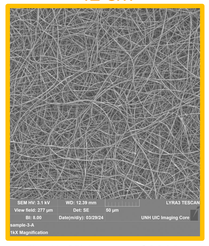

Fig. 11: SEM Image of 86 kDa Dex/DexMA (3:1) Sample

14 cm

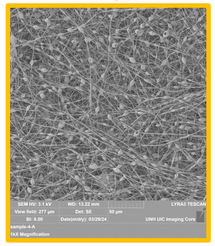

Fig. 12: SEM Image of 86 kDa Dex/DexMA (3:1) Sample

Electrospinning Distance

At 14 cm and 10 cm, the fibers would form irregularly-shaped beads of material. **At 12 cm,** however, **these abnormalities are all but gone.** This produces a more effective fiber for our purposes.

Molecular Weight of Dextran

These two samples bear **differences in molecular weight** of dextran. The more concentrated variety appears to **have less tangled** wires and far **larger bulbs** than the other.

86 kDa

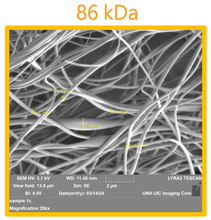

Fig. 13: SEM Image of 4 mL Dex to 1 mL Silk Sample

150 kDa

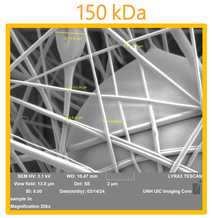

Fig. 14: SEM Image of 4 mL Dex to 1 mL Silk Sample

1. Cells Growth Screening

Sample of Nanofiber	Dextran Only	3:1 Dex - DexMA	1:1 Dex - DexMA
Cell Compatibility	✓	✓	X

Table 1: Analysis of are Structure and How Well it Can Grow Cells

2. Spinning Distance Screening

Concentration (mg/mL)	Distance (cm)	Average Diameter (µm)
0.7	10	0.745
0.7	12	0.625
0.7	14	0.565

Table 2: Average Widths of Fibers Derived from SEM Imaging Analysis

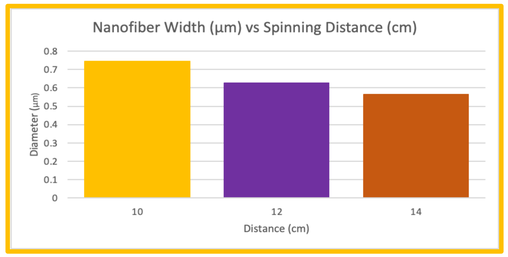

Fig. 15: Nanofiber Widths at Spinning Distances

3. Nodules Screening

Distance for creation of 3:1 Dex : Dex-MA	10 (cm)	12 (cm)	14 (cm)
Nodules Prevented	X	✓	X

Table 3: Tested Distance for Where no Nodules form

Parameter Quantification

Sample images were obtained via scanning electron microscopy (SEM) for nanofiber analysis. Preparation for cross-sectional imaging required processing samples in liquid nitrogen to prevent fiber deformation. Due to the high concentrations of energy directed at the sample to image, **nanofibers experienced thermal-induced morphing.**

Cell Culture

Fig. 16.1: 0.7 mg/mL Dex + DexMA (50%) (1:1) + 0.5 mL

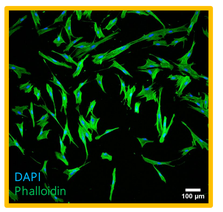

Fig. 16.2: 0.7 mg/mL Dex + DexMA (50%) (3:1) + 0.5 mL

The cell study **indicated successful growth and cell attachment to nanofiber scaffolding.** Confocal microscopy results portray cells oriented in a linear pattern, along the length of a fiber. The growth and attachment of these cells to the scaffolding indicates **electrospun scaffolding produced a viable environment for cell growth.**

Microstructures and cells are shown in green, while the background is in black.

However, due to issues with the **weakness** of our material, the first setting was rendered unsuitable for cell growth. The gel fell apart. However, **microstructures** are seen on the setting, which is rare for these concentrations of dextran.

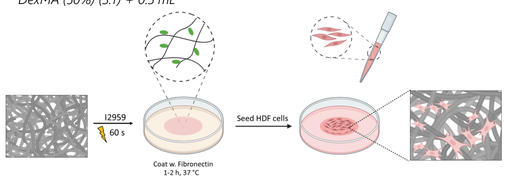

Fig. 17: UV Crosslinked Hydrogel

References

[1]Akiyama, S. K., Yamada, K. M., & Hayashi, M. (1981). The structure of fibronectin and its role in cellular adhesion. Journal of supramolecular structure and cellular biochemistry, 16(4), 345-358.
[2]Agrahari, V., Agrahari, V., Meng, J., & Mitra, A. K. (2017). Electrospun nanofibers in drug delivery: fabrication, advances, and biomedical applications. In *Emerging nanotechnologies for diagnostics, drug delivery and medical devices* (pp. 189-215). Elsevier.
[3]Madhusoodanan, J. (2019). Matrix mimics shape cell studies. *Nature*, *566*(7745), 563-565.
[4] Möller, S., Weisser, J., Bischoff, S., & Schnabelrauch, M. (2007). Dextran and hyaluronan methacrylate-based hydrogels as matrices for soft tissue reconstruction. Biomolecular Engineering, 24(5), 496-504.
[5] Pereira, R. F., & Bártolo, P. J. (2015). 3D photo-fabrication for tissue engineering and drug delivery. Engineering, 1(1), 090-112.[6]Abdulaziz R. Alharbi, Ibrahim M. Alarifi, Waseem S. Khan, Ramazan Asmatulu, (2016)
[7] Highly Hydrophilic Electrospun Polyacrylonitrile/ Polyvinypyrrolidone Nanofibers Incorporated with Gentamicin as Filter Medium for Dam Waterand Wastewater Treatment, 40, 38-56