Tunability of Electrospun Scaffolds for Tissue Engineering

by

Gloria Un Chyr

Submitted to the Department of Materials Science and Engineering
In Partial Fulfillment of the Requirements for the Degree of
Bachelors of Science in Materials Science and Engineering

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Abstract

Electrospinning is a cheap and quick method of creating non-woven scaffolds for tissue regeneration and growth with the proper fiber diameter for cell adhesion. However, electrospun scaffolds lack large pores between fibers and result in a densely packed mesh in which cells can adhere only to the surface of the material. Control of scaffold fiber size and porosity is critical to ensure scaffolds have a fiber diameter appropriate for cell adhesion and a high-enough porosity to allow for cell migration through the material. This thesis aims to demonstrate the tunability and control of electrospun gelatin scaffolds to make them viable for use in tissue regeneration by altering grounded collector geometry and thus the electric field that nanofiber deposition follows.

Previous electrospinning experiments show that processing parameters such as flow rate and voltage can affect fiber diameter and porosity, but are still insufficient in achieving dimensions viable for cell migration. Scaffold porosity is substantially more affected by the grounded collector geometry. By modifying collector geometry, pore size can be controlled without affecting fiber morphology and the deposition of gelatin nanofibers can be aligned or patterned to mimic natural tissue scaffolds. Introduction of a non-conductive, woven mesh in between the collector and source may allow further control of deposition patterns and thus scaffold construction. The path of electrospun fibers and the deposition patterns can be predicted by modeling the electric field.

Thesis goals

The goal of this thesis is to demonstrate the tunability and control of porosity, alignment, and fiber diameters in traditional electrospun gelatin scaffolds to make them viable for use in tissue regeneration. The deposited scaffold properties can be controlled by altering grounded collector geometry. Electrospinning as a method of quick, simple, and large-scale fabrication of tissue engineering scaffolds still lacks precision in simultaneous control of porosity, fiber morphology, and alignment. Scaffolds with a desired alignment and fiber morphology tend to close-pack because of the lack of disorder during deposition. Scaffolds with the desired alignment and porosity lose control of minimum fiber size and are not reproducible at large scales, thus limiting its production efficiency. Electric field models of various substrate geometry will provide insight for scaffold deposition patterns and proposed experimentation plans will allow fine-tuning of porosity and fiber morphology to yield large-scale scaffolds with desired properties.

The improvement of electrospinning technology will enhance its potential as a viable method of quick and cheap scaffold production for tissue engineering. Current methods lack the ability to control all three properties (fibers, porosity, alignment) in gelatin scaffolds while maintaining the benefits of cost and time efficiency. The ability to control all three properties on a large scale with traditional electrospinning may be demonstrated by modifying collector geometry and intercepting fiber deposition with a non-conductive mesh. This hypothesis will be tested by these specific experimental aims:

1. **Electric field dependence** can be determined by **modeling the electric field** around various collector geometries and comparing changes in the fields with deposition patterns in **experimental trials**.

2. **Scale dependence** of microstructure in electrospun scaffolds can be determined by **varying the diameter of spherical collectors** and observing the changes in scaffold porosity, fiber morphology, and alignment.
3. **Non-conductive geometry effects** can be determined by using grounded collectors to draw fibers in one direction and **intercepting the fibers with a non-conductive mesh** to influence scaffold growth.

Background and significance

Electrospinning

A quick and inexpensive method of producing scaffolds with fiber diameters in the ideal range for cell adhesion is electrospinning. Electrospinning uses a high voltage power supply to draw out polymer fibers from a charged melt or solution and deposits the fibers onto a grounded substrate as shown in Fig. 1a. The solution is drawn from a syringe needle tip where solution is pumped out a slow rate (<5mL/hr). The purpose of the syringe pump is to provide a steady source of material in the form of a droplet where charge can accumulate and overcome surface tension to create a jet of solution that form polymer fibers as shown in Fig. 1b. The polymer solidifies while travelling through the air by rapid evaporation of solvent and forms very long, continuous fibers that can range from 15 nm to 10 μm or more in diameter depending on the polymer solution and processing parameters. [1]

Electrospun scaffolds often exhibit desirable properties for scaffolds used in tissue regeneration. Most notably, electrospinning yields very thin fibers that provide a high specific surface area (SSA) that is optimal for cell adhesion. [2] However, a current barrier in electrospun

scaffold technology is that these thin fibers pack densely and limit cell migration. These scaffolds fail to allow cells to migrate beyond the surface and cannot support cell proliferation in a similar manner as the native extracellular matrix (ECM) as shown in Fig. 2. Therefore, this thesis aims to demonstrate that the electrospinning process can be modified to achieve scaffolds that mimic the native ECM with desirable pore sizes and maintain the high SSA of fibers.

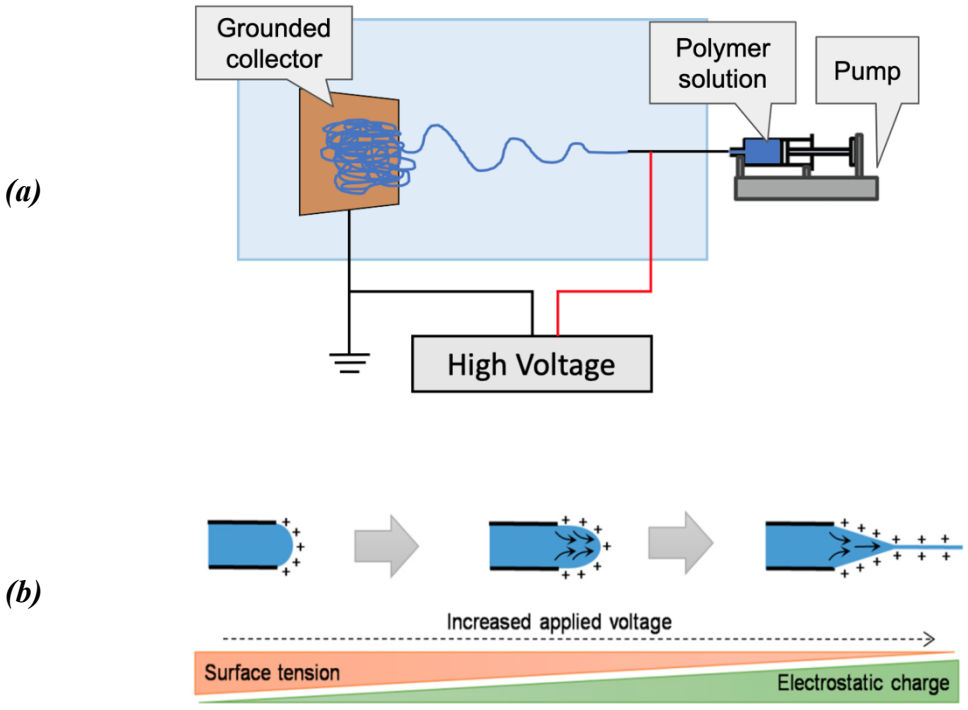


Fig. 1 (a) *High voltage power supply charges polymer solution that is ejected from a syringe by electric force. The polymer is collected as a mesh of fibers on a grounded collector, typically a flat plate of highly conductive material.* ***(b)*** *Increased applied voltage builds up charge on polymer droplet surface and eventually overcomes the surface tension of the droplet to form a thin jet of solution. (Pezeshki-Modaress, 2018)*

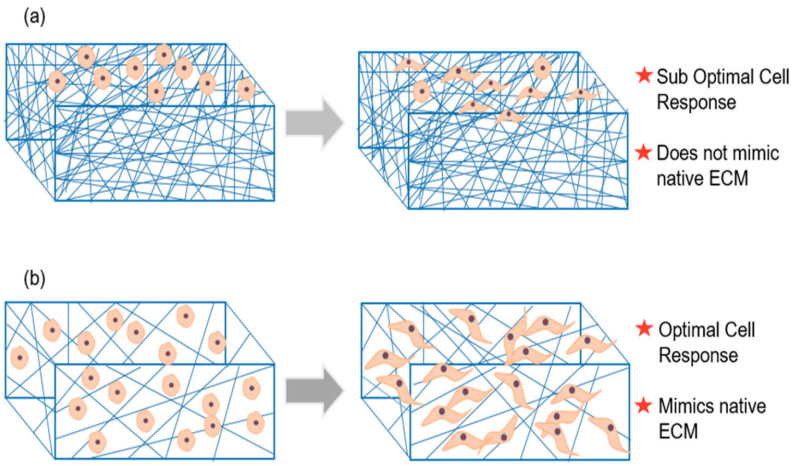


Fig. 2 (a) *Densely packed scaffolds do not provide enough space for cells to migrate between fibers and result in sub-optimal cell response.* ***(b)*** *Scaffolds with the same fiber diameters but larger pore sizes allow cells to migrate inward and proliferate like in the native ECM. (O'Brien 2005)*

Scaffolds for tissue engineering

Scaffolds for tissue engineering are porous materials that provide an environment for cells to differentiate and grow in a controlled manner to form new tissue for medical applications. In the body, a three-dimensional network of collagen, glycoproteins, and other macromolecules called the extracellular matrix (ECM) provides the support necessary for cells to grow. Scaffolds are created to mimic the native ECM both mechanically and biochemically to aid tissue regeneration. Different tissues and cells require different mechanical and chemical support but there are some characteristics that they all require from scaffolds for efficient growth: sufficient pore size for cell migration, adequate fiber diameter for cell adhesion, and durability until the cells can exist on their own.

This thesis will focus on the physical properties of scaffolds, specifically pore size and fiber diameter, rather than the biochemical properties. Pore size and fiber diameter of tissue scaffolds

are particularly important because it allows the cells to migrate through the structure and adhere to form the living tissue. Inadequate pore size and fiber diameter results in cell growth on only the surface of the scaffold and limits three-dimensional growth profiles. [3] These two scaffold properties are often studied together because it has been shown that pore size increases directly with fiber diameter. [4] This leads to conflicting results for a scaffold: the large pores allow for cell migration but the large fibers discourage cell adhesion because the specific surface area (SSA) is below the threshold of 7.13 μm^{-1}. [5] On the other hand, scaffolds with high SSA and thin fibers tend to pack densely and do offer enough space between fibers for cell migration. While desirable scaffold properties depend on the tissue of interest, the general range for desirable pore sizes is 10-100 μm and the range for fiber diameter is 100 nm-1 μm. [6]

Existing studies

Electrospinning was first developed as a variation of electro-spraying in 1902 but was not widely researched or considered until the 1990s when computer-controlled SEM became widely accessible and revealed the features of electrospun materials to be on a nanoscale. [7] Since then, numerous experiments have been conducted on electrospinning to improve the technology. In electrospinning for tissue engineering scaffolds, the most common synthetic polymers used in experiments are poly-lactic acid (PLA), poly-glycolic acid (PGA), poly-lactic-co-glycolic acid (PLGA), or poly-ε-caprolactum (PCL) because they are low-cost and biodegradable. [1] Natural organic polymers such as collagen, silk, chitin, alginate, and gelatin can also be electrospun into nanofibers when dissolved in the appropriate solvent. [8] In biomedical applications such as tissue

engineering, natural organic polymers are favorable for developing scaffolds that can be applied directly into the body because of the pre-existing biocompatibility.

Existing studies on the structure of electrospun scaffolds show that fiber morphology and processing parameters (specifically flow rate and voltage) are closely linked. [9-11]. Additionally, the pore sizes of electrospun scaffolds are significantly impacted by fiber morphology and diameter. [12,13] In a 3.042 study on electrospinning processing parameter effects on fiber morphology, it was found that higher flow rates, solute concentrations, and needle gauge resulted in higher average fiber, and higher voltages resulted in a broader range of fiber diameters. [14] This study is significant because it demonstrates that fiber morphology can be tuned greatly by simply adjusting the electrospinner set-up. Furthermore, the tunability of fiber morphology showed a corresponding control of the scaffold porosity by the packing ability of the fibers; larger fibers yielded larger scaffold pores. [15]

To address the long-standing problem of insufficient pore sizes in electrospun scaffolds, various electrospinning techniques have been pursued beyond simply adjusting the processing parameters. Pham et al. demonstrated a technique to electrospin a combination of thick and thin fibers to achieve desirable scaffold pores while keeping some areas with high SSA of thin fibers. [16] This method of layering microfibers and nanofibers increased cell infiltration into the scaffold but did not yield increased cell adhesion. The simultaneous spinning of multiple polymer solutions to increase disorder and thus porosity pursued by Theron, Zussman, and Kroll demonstrated that multiple polymer jets repel each other and result in a more non-uniform non-woven mat. [17] In this method, nine identical syringes containing identical solutions were set-up in several different matrix patterns. Syringes that were positioned closer together exhibited greater repulsion in the jet streams. Additionally, the introduction of more jets in a 2D matrix pattern was shown to reduce

some of the non-uniformity of the non-woven mats by allowing the inner jets to develop like the single jet system in traditional electrospinning.

Other unique methods of electrospinning to increase scaffold porosity include spinning fibers while simultaneously depositing small particles and wet spinning. [18-21] In electrospinning with deposited particles, salt particulates are deposited simultaneously with electrospun fibers into a scaffold that is cross-linked and then salt-leached to remove the particles while maintaining the structure created with the salt. [19] This allows the scaffold to have a macro-porous and nano-porous geometry. However, scaffolds that have undergone salt-leaching have demonstrated some structural collapse even after cross-linking. [20] On the other hand, wet-spinning does not use additional solids to increase scaffold porosity. Wet-spinning is the process of electrospinning directly into a non-solvent liquid by placing the substrate behind a bath of non-solvent. In this method, the non-solvent is used to dampen the speed at which the fibers are moving when closer to the substrate and slow down the deposition of the electrospun fibers to prevent them from packing densely. [21] Wet-spinning is beneficial in improving porosity of electrospun scaffolds but introduces the scaffold to another chemical that may not be biocompatible or easily removed from the system.

Electrospun scaffold porosity may also be increased by post-processing techniques such as sonicating spun scaffolds and laser etching pores directly into the scaffold. [22,23] Sonication of electrospun scaffolds requires a set-up similar to wet-spinning where the scaffold must be submerged into a non-solvent. The scaffold is then sonicated in the non-solvent in an attempt to expand the scaffold and reduce the density of packing. [24] However, this sonication method has the same drawbacks as wet-spinning where the non-solvent becomes another chemical that may remain in the system and must be carefully selected.

Laser etching can create pores of desired dimensions, inserted directly into electrospun scaffolds. This allows the scaffold to be electrospun with optimal fiber morphology and then be optimized for porosity after fabrication. However, laser-etching may damage the scaffold by introducing thermal ablation around the pore which may negatively affect cell adhesion. [25]

Despite the many promising advances made by the above techniques, the simplest method of controlling scaffold porosity returns back to the set-up of the electrospinning system: altering the collector geometry. [26-28] Traditional electrospinning uses a simple grounded flat plate to collect ejected fibers into a non-woven mat, but any shape can be used to collect electrospun fibers as long as the geometry material is conductive. Altering the collector geometry is advantageous over other techniques such as laser-etching or spinning multiple solutions because it requires no additional materials or expensive equipment.

Importance of collector geometry

Manipulation of electric fields by modification of collector geometries can control the deposition of polymer fibers in an aligned manner with greater spacing between fibers. [27] In the last three years, greater attention has been paid to modification of collector geometries to demonstrate fine-tuned control of scaffold construction. Geometries such as beveled plates, parallel bars, E-shaped targets, and concave targets have demonstrated significant influence on the deposition patterns of electrospun fibers as shown in Fig. 3. [28, 29] In each of these geometries, the electrospun fibers are drawn between opposing edges of a geometry and are deposited according to the arrangement of edges. In the concave geometry, fibers are deposited in an aligned manner that extends radially from the center of the geometry to the edges like several lines being

drawn across a circle through the center. In the center of this radial structure, the scaffold is non-woven and exhibits a random distribution of fibers where all edges have a more equal effect on the deposition. When two E-shaped targets are used, the scaffold appears woven linearly across the beams of the E. In between the E-targets, the fibers are deposited in a crisscross pattern that shows the fibers are affected by the corners of the substrate and not just the edges. When two simple parallel bars are used as the substrate, fibers are deposited across the two bars in an aligned manner. The fibers bridge the gap between the two bars to create a linear, one-directional woven scaffold. This study demonstrates that the deposition pattern of electrospun fibers can be controlled by substrate geometry and suggests that the bridging of fibers across gaps is dictated by the divergence of the electric field surrounding the substrate.

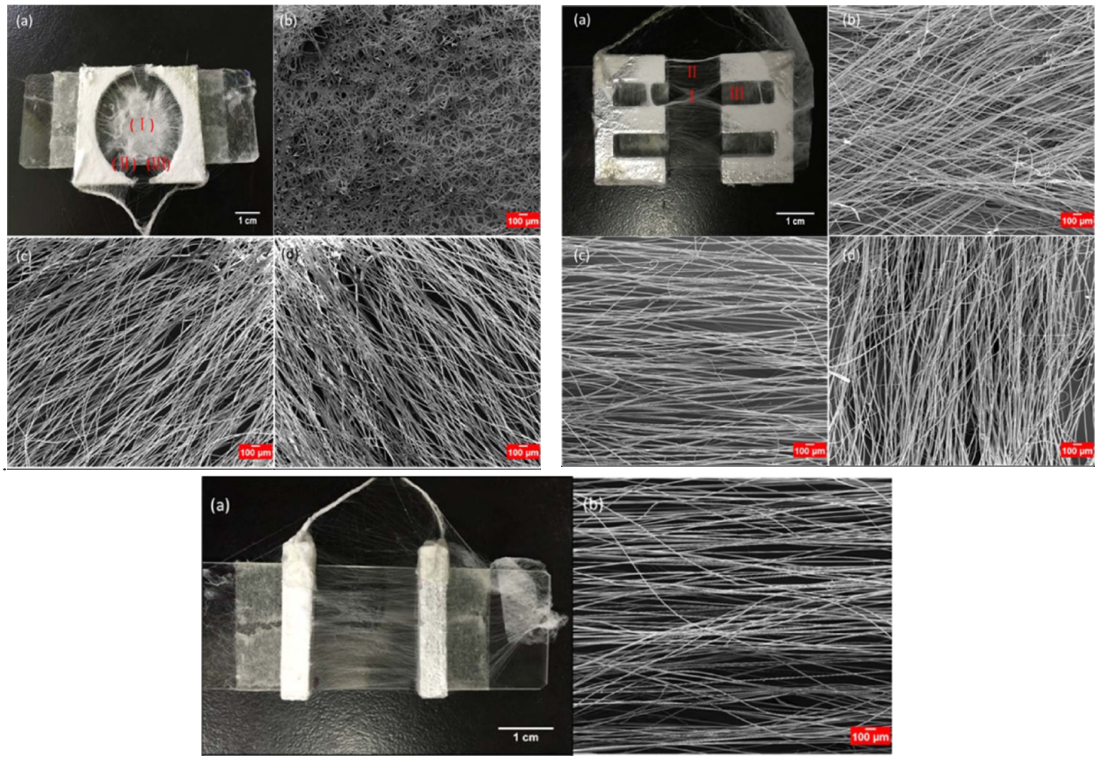


Fig. 3 *SEM images of electrospun scaffolds from modified collector geometries demonstrate fiber deposition patterns can be controlled. scale 100 µm. Concave geometry exhibits a radially woven scaffold with non-woven center, E-shaped target exhibits linear woven scaffold with across edges and corners, and parallel bars exhibit one-directional linearly woven scaffold. (Wang 2017)*

In 3.042, manipulation of electric field was tested with collector geometry modified to two separated plates at varying angles, concentric bands, and a curved plate resembling a hemispherical shell as shown in Fig. 4. Polymer fibers ejected from solution were deposited on the collector in patterns that resembled the electric field of the respective collectors. This suggested that the fibers follow the electric field created by the grounded collector geometry and high voltage source. [14]

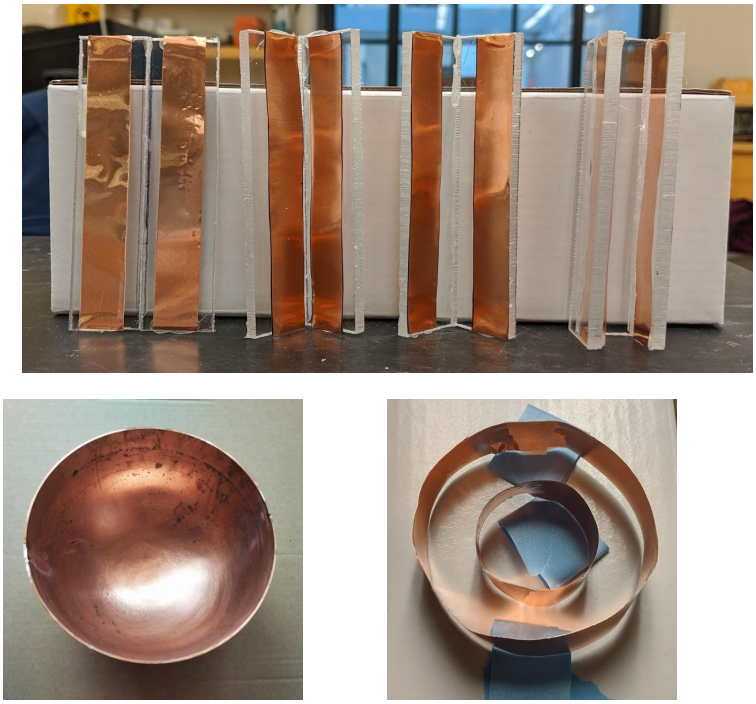
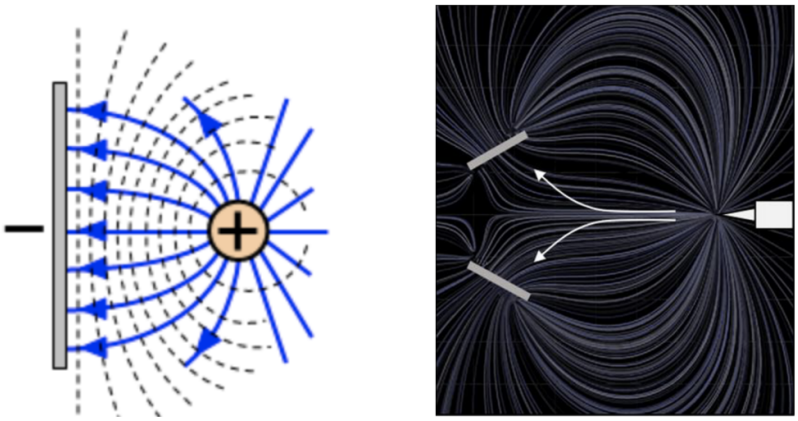


Fig. 4 *Modified collector geometries used in 3.042: two separated plates at various angles, curved plate from spherical shell, and copper foil fixed as concentric circles.*

Traditional single plate geometries demonstrate a randomly deposited mesh of fibers that follow circular pattern created by the chaotic plume of a jet of solution. [30,31] The introduction of two separate plates creates a divergence in the electric field and path of deposition for the fibers as shown in Fig. 5 and leads to alignment of the scaffold. [27] Varying the angle between two separated plates demonstrates that the visible alignment of electrospun fibers can be controlled as

shown in Fig. 6. Additionally, the porosity can be significantly improved by creating more divergence in the electric field as seen in Fig. 7. [14] Fiber diameter in scaffolds spun onto the simple flat plate and various modified geometries showed no significant difference (Table 1) but the average porosity increased dramatically with geometries that have more divergent electric fields such as the parallel plates and concentric circles. [14]



(a) **(b)**

Fig. 5 *Electric fields in electrospinning modeled with a single source point and (a) simple flat plate and (b) two separated plates at an angle (Zhou 2019)*

Fig. 6 *Electrospun gelatin scaffolds on two separated plates show that fibers can be visibly aligned by varying the angle between the plates.*

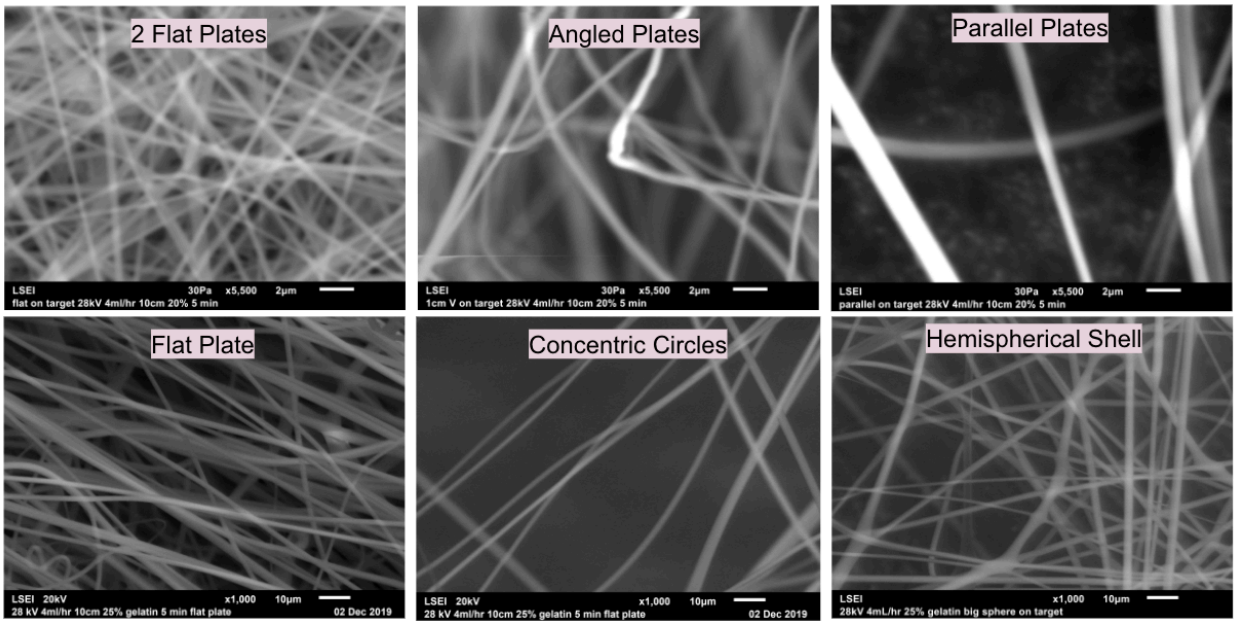


***Fig.** 7 SEM images of electrospun scaffolds deposited on various collector geometries. Porosity was greatest in scaffolds from the two parallel plates and the concentric circle.*

***Table 1.** Collector plate effects on average fiber diameter. Experiments were performed using 28kV voltage, 4mL/hr flow rate, 10cm distance, 20-gauge needle, and 25% gelatin solution.*

Geometry	**Average Fiber Diameter (µm)**	**Standard Deviation (µm)**
Flat Plate	1.70	0.39
Concentric Circles	1.90	0.44
Hemispherical Shell	1.80	0.36

Extremely fine control of fiber deposition can be achieved by direct-wire electrospinning where collector geometry is modified to a "sharp-pin" electrode and moves to create desired patterns. [32] This allows the deposition of fibers to be controlled to a point and is comparable to a reverse-3D printing. Direct-wire electrospinning seems to be a promising new approach to additive manufacturing which would be useful in achieving tissue scaffolds with high porosity and thin fibers, but it is not robust enough to stand on its own without combination with other additive

manufacturing processes. [33] Similarly, near-field electrospinning (NFES) can be used to create fine patterns in scaffolds while still maintaining a reasonable fiber diameter as seen in Fig. 8 but has its own drawbacks. [34] With NFES, the electrospinning process is limited to a very small area for precise control and lacks 3D construction capabilities.

Both direct-wire and NFES methods of electrospinning may allow greater control of scaffolds and therefore potential to increase scaffold porosity, but they also pose several disadvantages over traditional electrospinning. Most notably, direct-wire electrospinning and NFES are only effective at small-scale fabrication, tend to have larger fiber diameters, and until very recently have been limited to 2D applications. [35] These methods also complicate the electrospinning set-up as compared to simply modifying collector geometries.

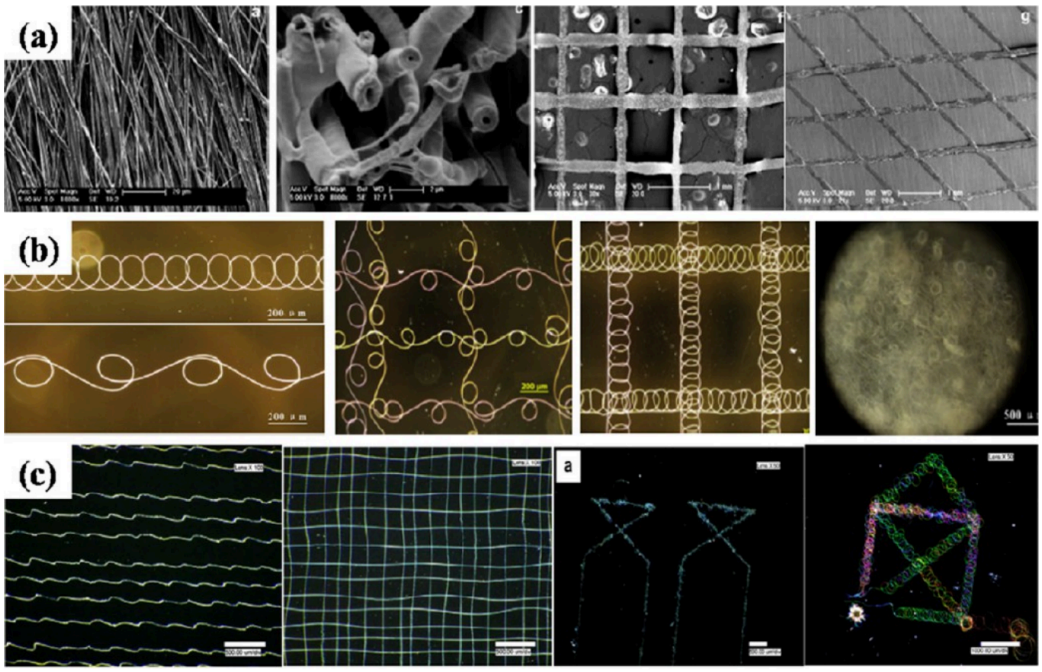


Fig. 8 (a) *Aligned and patterned core−shell structured fibers via NFES. (Zhou, 2011)* ***(b)*** *Buckled patterns of PS fibers and fiber balls consisted of stacked coils. (Xin, 2012)* ***(c)*** *Nanofiber patterns of low-voltage high precision deposition via a counter electrode/substrate technique. (Hellman, 2009)*

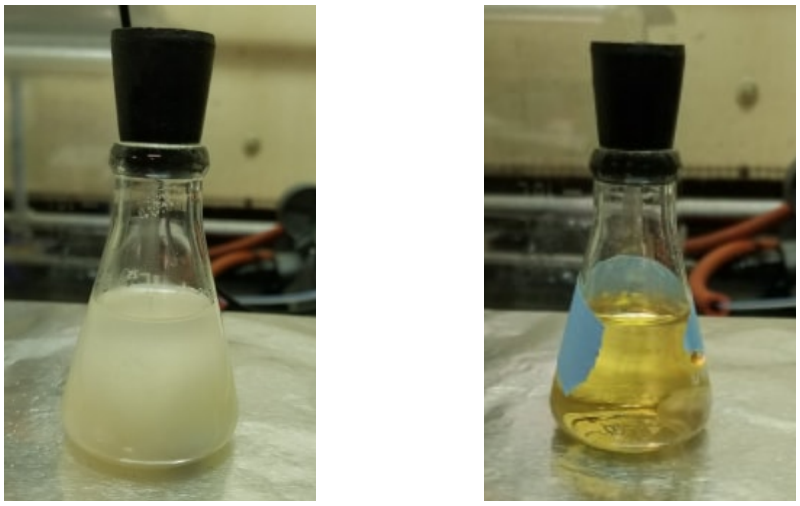
Experimental Plan

Materials Selection

Gelatin is an ideal candidate for electrospinning solutes because it is much cheaper than other synthetic polymers, and electrospun gelatin structures are transferable to other natural materials such as collagen. [36,37] Another advantage of gelatin over other structural proteins is that it does not denature at high voltages that are used in electrospinning. [38] Glacial acetic acid is chosen as the solvent for this experiment because it can be used to fully dissolve the gelatin into a homogenous solution without the toxic risks with other previously explored solvents such as hexafluoro-2-propanol (HFP) or 2,2,2-trifluoroethanol (TFE). [39, 40]

Solution preparation

Solutions for electrospinning are prepared by heating glacial acetic acid to 70°C and stirring in 300-bloom food-grade gelatin to desired concentrations. The solution must be kept agitated at 70°C for several hours to prevent gelling at room temperature. After approximately 18 hours of heated stirring, the solution becomes stable as a transparent, viscous liquid at room temperature. During the mixing process, the heat can degrade the gelatin which is signaled by the darkening of the solution from an initially white, semi-clear solution to dark yellow as shown in Fig. 9. Mixing at higher temperatures will lead to further gelatin degradation and possibly denaturation. Differential scanning calorimetry (DSC) of the gelatin powder used in this experiment shows that the temperature used for mixing is well below the denaturation temperature of 175.9^{o}C as shown in Fig. 10 as well as the glass transition temperature for commercial gelatin. [41]



(a) **(b)**

Fig. 9 *20% w/v gelatin in acetic acid* ***(a)*** *just after combining and* ***(b)*** *after heating and stirring for 18 hours and allowing to cool to room temperature.*

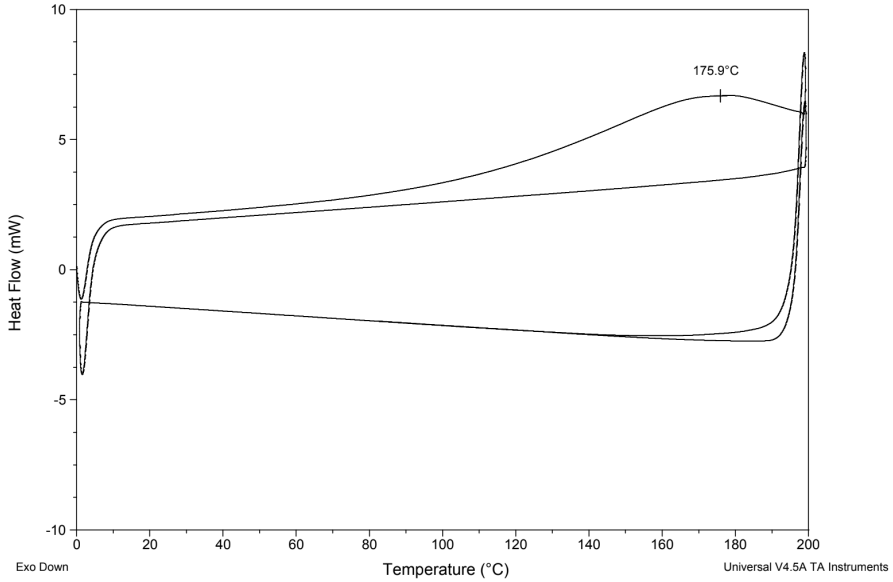


Fig. 10 *DSC graph of 300-bloom food grade gelatin shows only one peak at 175.9°C with no earlier thermal transitions that could signify thermal denaturation. Thus, it is unlikely that heating to 70°C for dissolution denatures the gelatin*. [14]

Electrospinning set-up

Electrospinning requires four main components: a high voltage power supply (HVPS), syringe pump, needle-tipped syringe, and a grounded collector. In these experiments, the distance between the tip of the syringe and grounded collector will be set to 10cm so that the electrospun fibers can be kept electrically insulated from the surroundings inside a polycarbonate box as shown in Fig. 11. The HVPS will be set to 28 kV to maximize voltage while remaining safely under the dielectric breakdown of air (300kV/m). [39] As found in 3.042, higher voltages yield a broader range of fiber diameters which can be beneficial in achieving scaffolds with larger pores dictated by larger fibers but maintaining good cell adhesion with the smaller range of fibers. Solution will be flowed at a rate of 4 mL/hr through a 20-gauge needle tip for 5 minutes as done in 3.042 for distinct fiber morphology and sufficient fiber collection for SEM imaging. Electrospinning will occur under ambient conditions (room temperature, relative humidity of fume hood) such that any techniques developed will not require extensive changes to the electrospinning environment.

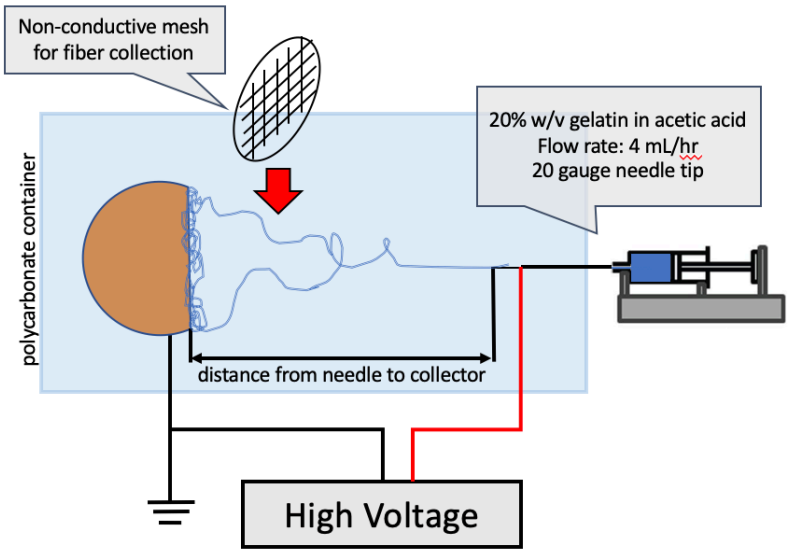


Fig. 11 *Electrospinning set-up with copper half-spheres. A non-conductive mesh is inserted between the syringe needle and grounded half-sphere to collect fibers. Deposition is influenced by the paths from electric field and may be modified by introducing obstacles (mesh) that begin to collect fibers in mid-flight.*

Collector Geometries

Three copper half-spheres were prepared by New England Copperworks with a thickness of 0.032 in (0.08cm) and diameters of 3 in (7.62 cm), 4 in (10.16 cm), and 5 in (12.7 cm) as shown in Fig. 12 below. A stainless-steel bolt is soldered onto the back of each copper sphere so that it can be fixed to a stand. Non-conductive meshes will be made by weaving together suture threads into a net that can collect fibers mid-flight without interrupting the electric field as shown in Fig. 13. Four separate meshes will be made using commercial nylon, silk, polyester, and polypropylene suture threads. The different thread materials are not expected to impact the structure of the scaffold but may provide different degrees of adherence for the fibers and durability against residual solvent in the fibers. The spacing between threads in the mesh will be square and varied between 0.25in, 0.5 in, 0.75 in, and 1 in. This will allow us to determine if the mesh density influences the flight patterns or deposition of fibers ejected from the syringe. The four variables in this experiment will be the size of copper substrate, presence of non-conductive woven mesh, mesh material, and tightness of weave in the mesh.

Fig. 12 *Sample image of copper half-spheres from New England Copperworks. Images of actual samples were not taken before MIT campus closed.*

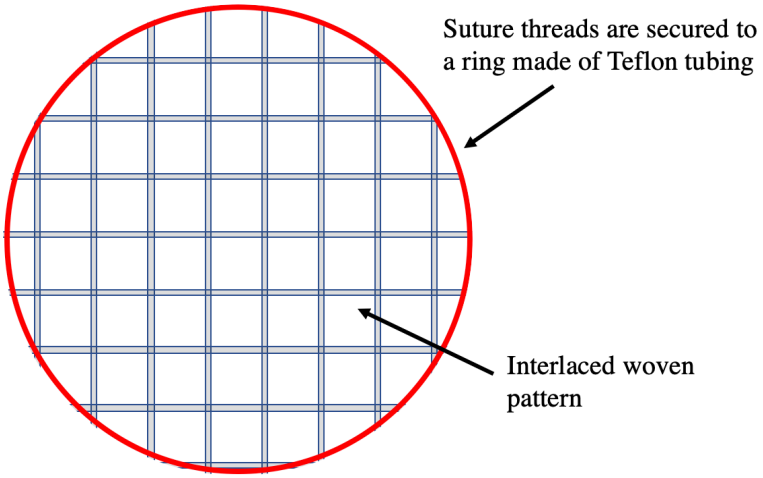


Fig. 13 *Diagram of non-conductive mesh made from interlacing suture threads in a woven pattern. Spaces in between threads is square at distances of 0.25in, 0.5 in, 0.75 in, and 1 in.*

Data Collection & Analysis

Gelatin scaffolds will first be spun using each of the three copper half-spheres to determine the effects of collector scale of the distribution of fibers during collection as well as scaffold porosity, fiber morphology, and alignment. Then, the non-conductive suture meshes will be inserted between the spheres and syringe and the electrospinning process will be repeated to intercept fibers before they reach the sphere and begin scaffold growth on the mesh.

Scaffold porosity, fiber morphology, and alignment will be determined by SEM imaging and optical microscopy of each sample at various positions. Porosity is taken to be the average distance between fibers by using both SEM and digital scanning optical microscope. It is beneficial to use both techniques in measuring porosity because it allows vertical distance to be estimated through changes in depth of field in optical microscope and x-y distance to be estimated in clear 2D SEM images. Fiber diameter and porosity will be estimated by imageJ analysis of SEM images. Alignment is a qualitative property that can be described by the deposition patterns as viewed with and without SEM. An example of scaffold measurement technique is provided in Fig. 14.

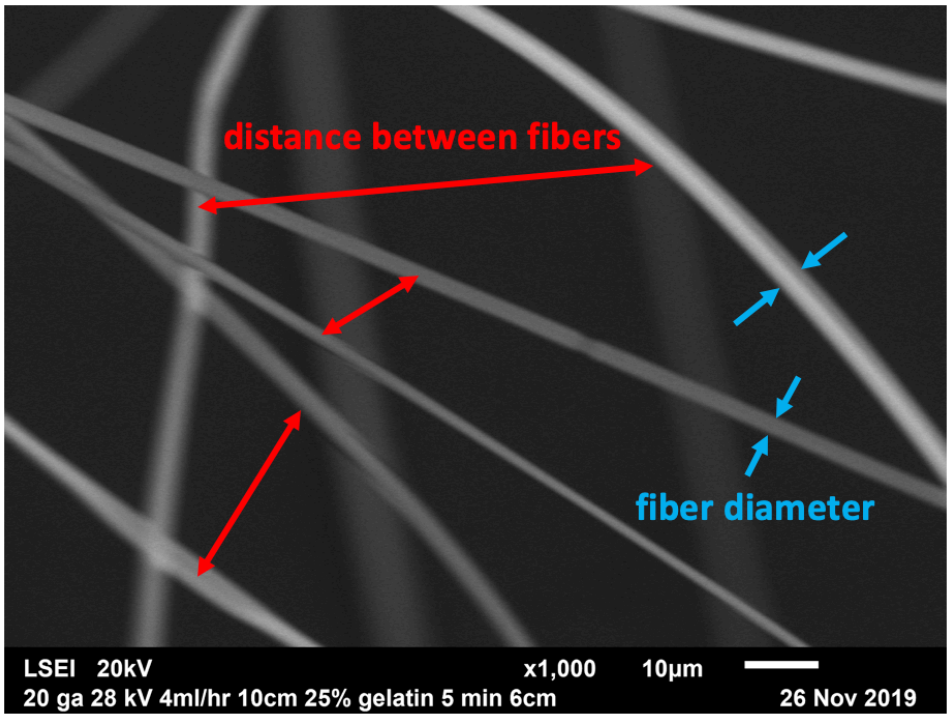


Fig. 14 *SEM image of electrospun gelatin scaffold from 3.042 with red arrows indicating example measurements of distance between fibers to estimate porosity and blue arrows indicating example measurements of fiber diameter.*

Expected Experimental Results

Scaffolds electrospun directly onto the 4-inch half-sphere will be considered the control substrate for this experiment. It is expected that these scaffolds will resemble those from the curved plate experiments in 3.042 because the main differences between these substrate geometries is the curvature and depth of the sliced sphere, and the uniformity of the substrate. The four main variables that will be tested are listed below with the expected experimental results and corresponding explanations.

1. **Size of substrate**

   Increase in diameter of the half-sphere substrates is expected to yield scaffolds with lower porosity because the degree of curvature is larger and

the edge effects of a sphere will be less apparent. Since the edges of the sphere will be farther apart, it's possible that the fibers that are spun along the circumference of the collector will have less interaction with the opposite edge.

2. **Introduction of non-conductive mesh**

   The insertion of a non-conductive, woven mesh between the charged needle and the grounded substrate is expected to impact the overall structural alignment of the deposited fibers by making the scaffold woven at a macro-level and non-woven in microscale. The jet solution will continue to follow the path of the electric field as dictated by the collector geometry but the mesh will collect the fibers at a different point in its path than the substrate.

3. **Mesh material**

   The various materials used for suture threads is not expected to have a significant impact on the alignment of the scaffolds or the fiber morphology. However, the ability for fibers to adhere to the mesh may vary between materials and the trace amounts of acetic acid that may still be present in the solution jet may damage some meshes.

4. **Mesh dimensions**

   The dimensions of the lattice in the mesh will impact the deposition of fibers. A mesh with larger openings may allow some fibers to travel between threads and collect and cause bridging between fibers deposited on the mesh and the collector. Smaller openings on the mesh will allow less fibers to

reach the collector and thus create a new flat area of fiber collection as the scaffold builds up.

Electric Field Modeling

To confirm the relationship between substrate geometry, electric field, and fiber deposition pattern, the electric field between a charged syringe needle and various substrate geometries will be modeled using Autodesk Inventor EMS. Other software that supports modeling of DC circuits such as COMSOL Multiphysics may be used if a license could be procured. Electric field will be solved for the following specific models:

1. Simple flat plate 12cm x 15cm at a distance of 10 cm from needle
2. Two plates 12cm x 1cm angled at 90 degrees with separation of closest edge of 0.2cm and a distance of 10 cm from needle
3. Half-spheres of diameters 3 in, 4 in, and 5 in (7.62cm, 10.16cm, and 12.7cm) with thickness 0.032 in (0.08cm) at a distance of 10 cm from needle

The models should generate a 3-dimensional map of electric field created by the positively charged needle and grounded substrate similar to those generated by Smółka, Firych-Nowacka, and Lefik in Fig. 15. below. [42] The electric field models generated by Smółka et al include a variety of grounded substrates including different arrangements of bars, hoops, and drums. However, there are no models generated for half-spheres and thus this step of the experimental plan is still novel work.

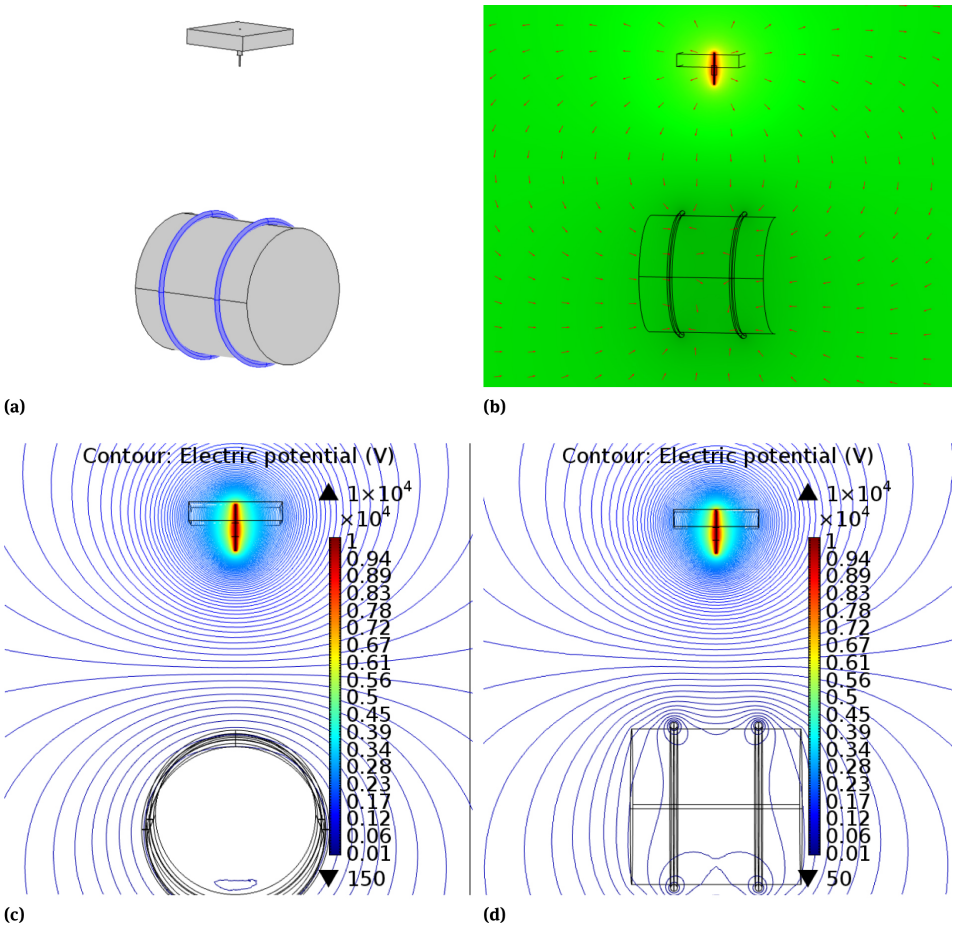


Fig. 15 Electric potential field generated by a positively charged needle and a grounded drum collector with wire wound around it. (a) electrospinning set-up and geometry model (b) distribution of electric field vector (c - d) electric potential contour lines from two perspectives showing how the potential changes according to both the shape of the drum and the position of wires.

Further Research

If the experiments outlined in this thesis are executed successfully and scaffolds can be constructed with desirable porosity, fiber morphology, and alignment, the next steps would be to determine a viable cross-linking method for the gelatin or apply the techniques with a different electrospinning material. Gelatin is an extremely hygroscopic material and will quickly lose its structural integrity in an aqueous environment. Thus, it is necessary to cross-link the electrospun scaffolds to improve its mechanical and chemical stability to make it durable enough to allow cell proliferation when in contact with the body. The most common cross-linking agents for electrospun gelatin and collagen scaffolds have been genipin, glutaraldehyde, N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC), and EDC with N-hydroxysulfosuccinimide (EDC-NHS). [43] In cross-linking gelatin scaffolds, there is a current trade-off between strength of cross-links and the toxicity of the agent. Genipin and glutaraldehyde are low-toxicity cross-linking agents but are not as effective as the highly toxic EDC or EDC-NHS. [44] Traces of cross-linking agents can be found in electrospun scaffolds even after rinsing the scaffold so it is crucial to develop a cross-linking method that yields desirable mechanical and chemical properties without posing further risk to patients needing tissue regeneration. Alternatively, the electrospinning techniques discussed in this thesis may be applied to other materials with sufficient strength and durability that may not need to be cross-linked.

If an electrospun scaffold with viable mechanical and biochemical properties can be constructed and crosslinked, the next step could be to grow cells and record the cell adhesion and proliferation rate. A cell proliferation study would be performed by culturing human dermal fibroblasts on the electrospun scaffold for 1, 3, 5, and 7 days as done in a study by Zhang et al on cell proliferation of cross-linked gelatin scaffolds. [45]

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