Research Article

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A review of electrospinning manipulation techniques to direct fiber deposition and maximize pore size

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Abstract: Electrospinning has been widely accepted for several decades by the tissue engineering and regenerative medicine community as a technique for nanofiber production. Owing to the inherent flexibility of the electrospinning process, a number of techniques can be easily implemented to control fiber deposition (*i.e.* electric/magnetic field manipulation, use of alternating current, or air-based fiber focusing) and/or porosity (*i.e.* air impedance, sacrificial porogen/sacrificial fiber incorporation, cryo-electrospinning, or alternative techniques). The purpose of this review is to highlight some of the recent work using these techniques to create electrospun scaffolds appropriate for mimicking the structure of the native extracellular matrix, and to enhance the applicability of advanced electrospinning techniques in the field of tissue engineering.

Keywords: Electrospinning, Nanofiber, Tissue engineering, Porosity, Patterning

1 Introduction

1.1 The Extracellular Matrix

The extracellular matrix (ECM) is composed of an intricate network of interconnected macromolecules such as proteins and polysaccharides. These macromolecules are secreted by and organized by the cells in their immediate vicinity. The ECM as a whole functions as scaffolding for the tissues in the body, providing a three-dimensional structure, cell attachment sites, and a multitude of signaling pathways between cells [1–4]. The major fibrous ECM molecules are listed in Table 1 with their average fiber diameter. The molecules interlink and create a three-dimensional (3D) mesh-like structure in the space between the cells. An illustration of this mesh in relation to a mesenchymal cell can be seen in Figure 1. Interactions be-

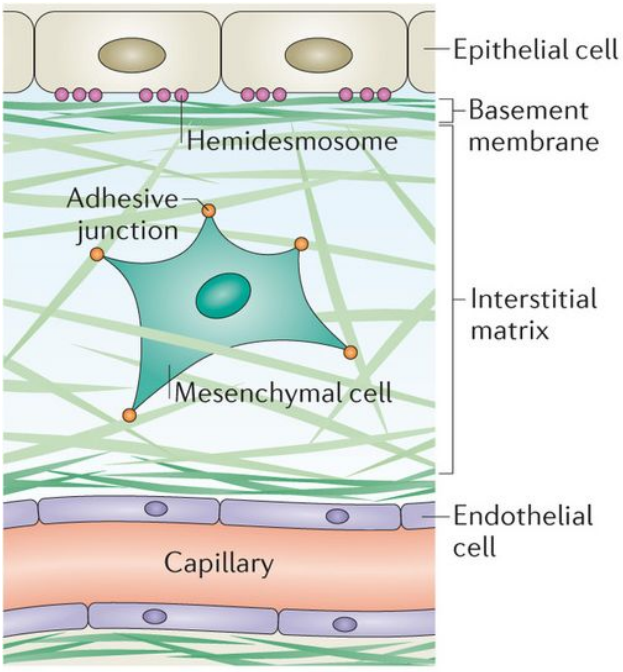

Figure 1: Skin extracellular (interstitial) matrix. Adapted with permission from Fiona Watt and Wilhelm Huck [23]. Copyright 2013 Nature Publishing Group.

Table 1: Fibrous ECM components with average fiber diameter.

Components	Average Diameter (nm)	Reference
Collagen	500	[18, 19]
Elastin	400	[4, 19, 20]
Fibronectin	2	[19, 21]
Laminin	57	[19, 22]

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tween cells and the ECM influence and characterize many bodily functions, such as homeostasis, wound healing, and aging [1, 5]. Cells migrate throughout the body by attaching and detaching to the fibers of the ECM, pulling themselves along in the direction dictated by chemotactic signals. Connective tissues such as bone, ligament, and skin have denser and more highly organized ECM than tissues such as brain or adipose due to the increased demand for mechanical support, structure, and protection [1, 6–17].

1.2 Characterization of cell-ECM interactions

Connective tissues are essential to the body's normal function, but can be targeted by several tissue-specific diseases that affect matrix protein production. Diseases such as arthritis, scleroderma, lupus, and dermatomycosis all affect the amount and structure of the ECM, causing complications throughout the body, such as fever, pain, weakness, brittle bones, and even death in some extreme cases [24]. If the ECM were to be destroyed completely, as is the case in diabetic ulcers (full-thickness wounds in diabetics caused by ischemia or injury that do not heal properly) and third degree burns, the inflammatory cells, such as polymorphonuclear (PMN) leukocytes and macrophages, needed for tissue repair would have difficulty finding and infiltrating the wound. The cells lack the guidance and initial framework of the matrix proteins, rendering regeneration of the tissue almost impossible in these types of injuries [25]. For these reasons, an understanding of the ECM and the cellular interactions within it and the development of therapeutic measures with the accumulated knowledge is of paramount importance to modern medicine. Both of these needs can be addressed with electrospinning. In order to understand the interactions between cells and ECM, an accurate, reproducible model is needed, due to the difficulty of recording the phenomena *in vivo*, as many studies do [26], or creating larger structures with which to model [27]. Electrospun scaffolds have the ability to imitate the native ECM, which allows for the creation of models and regenerative templates that are comparable to native environments, reproducible, quickly obtained, and cost-efficient. In addition, electrospun scaffolds can be developed as off-the-shelf tissue regeneration therapy due to their fibrous, porous nature, which is important for cell infiltration and motility. In order to make either of these goals a reality, the crucial next step forward in the electrospinning protocol is to create a higher degree of control in the fabrication process. Greater control leads to the ability to create tissue-specific matrix models with porosities and fiber alignment more similar to the native ECM.

1.3 Electrospinning

Nanotechnology encompasses materials, structures, and devices of which at least one dimension is 100nm or less [28]. Nanofibers are applicable to biomedical technologies and especially advantageous in tissue engineering and regenerative medicine due to the size similarity with the structure of the ECM. Many techniques can be used to obtain nanofibers, such as drawing, template synthesis, phase separation, and self-assembly, but electrospinning is the only process that can control fiber size, can be scaled for larger production, and is easily replicable [28–30]. Electrospinning is a process that creates polymer nanofibers utilizing a high applied voltage and a grounded target. In this case, a fiber is defined by its geometry as a slender, elongated, threadlike object or structure [28].

Electrospinning was first patented in 1934 as an apparatus to prepare threads for textile production [31]. This device operated at 10kV and relied on grounded rotors to collect fibers as strands for making fabrics. In the later 20^{th} century, applications of electrospinning were realized as a useful production method for medical research. It has been shown that electrospun scaffolds create a good matrix and have the ability to be incorporated with growth factors, seeded with cells, and implanted into the human body to stimulate regeneration or regrowth [32, 33]. The scaffolds are porous and have fibers that can be tailored to affect cell differentiation, proliferation, and migration [34–38]. These characteristics make electrospun constructs ideal for wound dressings [39, 40] and grafts for various tissues such as skin [32, 41–46], nerves [32, 33, 47–51], vasculature [32, 33, 41, 51–66], muscle [33, 51, 67, 68], bone [15, 32, 33, 41, 51, 69–73], cartilage [32, 41, 74, 75], and even ligament [13, 32, 73, 76, 77].

1.4 Basic Electrospinning Process

To electrospin, a polymer is dissolved in a highly volatile solvent and slowly extruded from a syringe, forming a droplet at the tip. A high voltage is applied to the syringe needle, charging the polymer as it exits the syringe. A grounded target is placed a certain distance from the needle tip. This voltage differential creates a field that draws the polymer from the needle tip in the form of nanofibers,

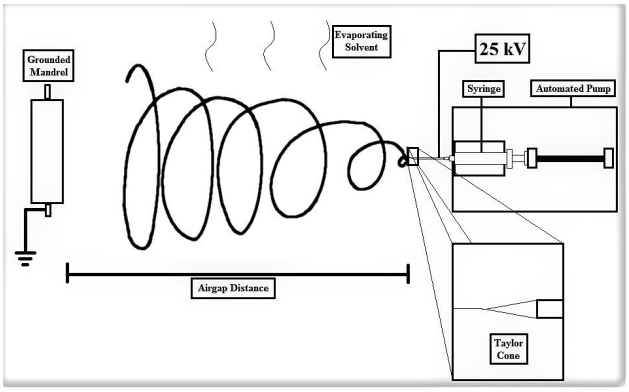

Figure 2: Basic electrospinning setup.

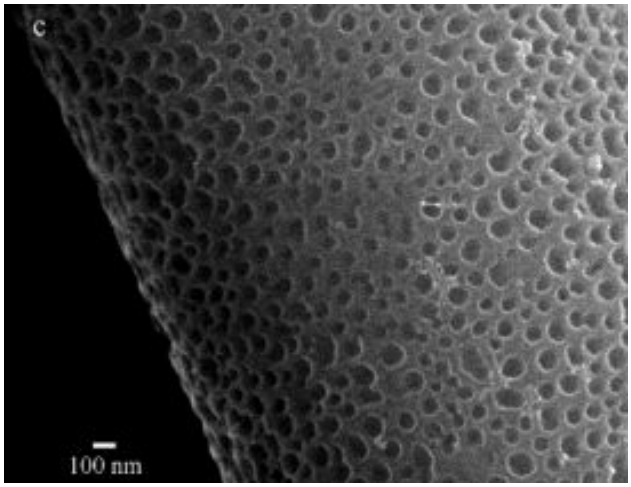

Figure 3: Porous electrospun fibers. 28 wt% polystyrene in tetrahydrofuran (15kV). Adapted with permission from Silke Megelski *et al.* [90]. Copyright 2002 American Chemical Society.

which are then deposited on the grounded target [78]. A basic illustration of this concept is shown in Figure 2. In ideal conditions, the droplet that appears at the tip of the syringe needle changes shape into a Taylor cone geometry. If this is seen, it signifies that the setup is creating quality nanofibers. If a cone appears forked, this is an indication of the presence of undesirable edge effects in the electrical field applied to the solution. The strength of the electrical field is greater closer to the needle walls and weaker at the center of the needle. Also, edge effects can be more pronounced when the field has a higher magnitude [79–81].

2 Manipulation Techniques to Control Fiber Deposition

2.1 Basic Parameter Alterations

There are many parameters that can affect the quality of the electrospinning process, such as the polymer concentration and molecular weight, chosen solvent and its conductivity, vapor pressure, surface tension, viscosity, applied voltage, extrusion flow rate, airgap distance, and ambient factors such as heat and humidity. To complicate the process further, the parameters are not independent of each other [28, 82–84].

Both the polymer concentration and molecular weight of a polymer have a direct effect on the viscosity of the solution. Chain entanglements, which are directly proportional to polymer molecular weight and/or polymer concentration, prevent the fiber-producing jet from breaking, creating longer continuous fibers. However, chain entanglements increase the viscosity, which increases the surface tension, requiring a larger force to initiate Taylor cone formation [82]. A higher concentration will result in a smaller deposition area as the viscosity of the solution discourages bending instability of the fibers in the air [85]. Concentration and viscosity can also have an effect on the surface morphology of the fibers, creating smooth or beaded fibers [86]. Heat and humidity can change the viscosity and vapor pressure of the solvent or create atmospheric effects in the voltage field, dispersing charge.

A wide range of solvents are used to dissolve polymers, and they all have different properties. Organic and non-organic solvents can be utilized in the electrospinning process with a range of conductivities and dielectric constants that can be tailored to the specific polymer and application in order to achieve the desired fiber morphology [85, 87, 88]. The choice of solvent allows control of fiber morphology based on charge density in the solution, interactions between solvent and polymer molecules, and resulting viscosity [87, 89]. This can even result in porous fibers, as seen in Figure 3. The pores are caused by a solvent or a combination of solvents that did not completely evaporate before collecting on the grounded mandrel. After collection, the polymer solution becomes thermodynamically unstable, creating regions of highly concentrated polymer and regions of highly concentrated solvent. When the solvent completely evaporates from those regions, it creates void spaces in the resulting fibers [90].

The volatility of a solution is based on vapor pressure, boiling point, specific heat, enthalpy and heat of vaporization of the solvent, rate of heat supply, interaction between solvent molecules and between solvent and solute molecules, surface tension, and air movement above the liquid surface [28, 89]. Volatility must be controlled so that the polymer nanofibers whip through the air toward the

target and are deposited dry on the collector. This prevents fiber merging due to leftover solvent. The surface tension can be modified by adding drugs and surfactants, lowering the surface tension and reducing the diameter of the resulting fibers [91]. Conductivity of the solution can be altered as well by adding electrolytes or ionic salts [88, 92, 93].

An increase in temperature would, in general, cause viscosity of a solution to decrease, but in some cases a higher temperature can cause an increase in viscosity due to reduced enthalpic interactions, according to the Flory-Huggins solution theory [28, 88]. Humidity causes the vapor pressure to decrease, leading to increased surface tension and wetter fibers formed [28]. All of these parameters and their effects are detailed in Table 2.

Once the parameters are optimized, the fibers are collected in the geometry dictated by the grounded target. The fiber collection is focused on the grounded target, but since they are so highly charged, electrospun nanofibers typically deposit on every surface in the vicinity [28]. All surfaces are closer to the ground state than these fibers, causing the attraction. In addition, if the fibers retain even a portion of their charge after deposition, they can electrostatically repel new fibers travelling in the air, pushing fibers away and exacerbating the problem [28]. A control system must be used to increase the accuracy of the electrospinning process. There are several methods that can be used to manipulate the created nanofibers described in previous studies, such as electric field manipulation, alternating current (AC) electrospinning, and magnetic field manipulation [80, 96–112].

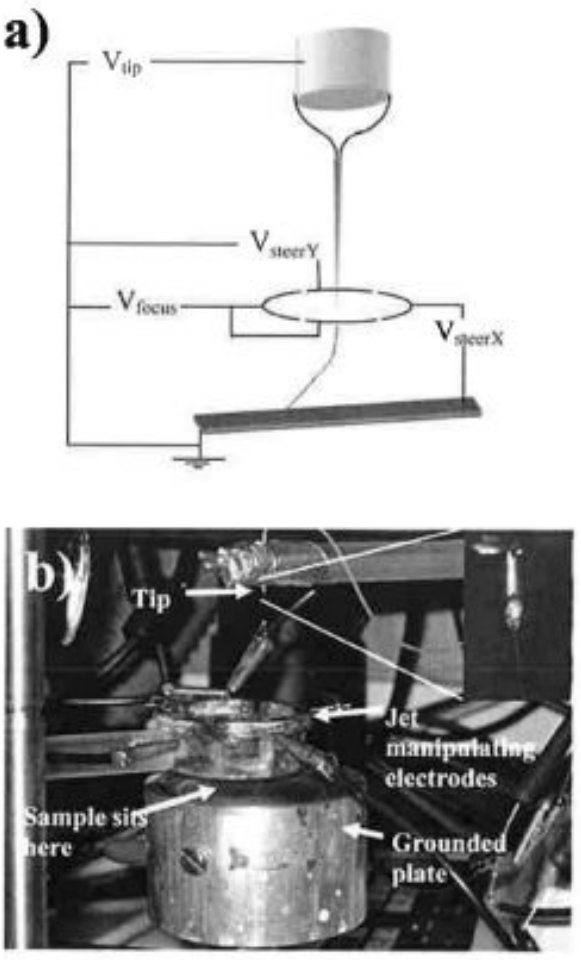

Figure 4: a) A diagram and b) a picture of steering electrodes used to control the path of electrospinning. A voltage is applied at the needle tip, as in a traditional setup, but three other voltage settings are used to control the path of the fibers before they are collected on the target. Reprinted with permission from Leon Bellan and H. G. Craighead [97]. Copyright 2006 American Vacuum Society.

2.2 Electric Field Manipulation

One technique that has been used to focus and direct fiber deposition involves the manipulation of the voltage field [80, 96–98, 101, 113–115]. Theoretically, the voltage field is a consistent gradient of electrical charge with the source at the needle tip and the sink at the grounded target, with field lines connecting the two. However, as mentioned before, the fibers collect on all surfaces of a lower charge. Thus, the process is not highly controlled and there is a significant amount of loss of material.

It has been shown that using conductive plates or rings as electrodes with a charge of similar polarity to that of the applied voltage at the needle is an effective method in controlling the electric field. These constructs can be placed as a frame around the desired path of polymer to force the fibers toward the center. As a result, the path of polymer fibers can be steered toward a target [96, 97, 99]. In the experiments performed by Bellan and Craighead, an addition of steering electrodes placed in a circle near the target of the system can reduce the resulting spot size to 5 mm in diameter (Figure 4) [97]. Similarly, according to a study done by G.H. Kim, a cylindrical electrode placed around the needle tip and given the same charge as that applied to the needle stabilizes the polymer stream and minimizes edge effects caused by a nonhomogeneous charge applied by the needle (Figure 5). This addition reduced the measured spot diameter by half compared to the spot created from the same protocol without the cylindrical electrode [80]. The steering of the polymer prevents loss of material and increases the accuracy of the resulting polymer deposition, making the process more efficient overall.

Table 2: Electrospinning Parameters with Effects

		Effect	Reference
Polymer	**Type**	Determines the size and morphology of fiber formation	[28, 82]
	Concentration **Molecular Weight**	Higher concentration and higher molecular weight increases viscosity, resulting in larger, smoother fibers and a smaller deposition area due to decreased bending instability. It also increases entanglement of molecules, resulting in longer fiber formation. Lower concentration decreases viscosity and allows for polymer congregation instead of entanglements, resulting in beading and bending instability, creating a larger spot size (area of fiber deposition).	[28, 82, 85]
	Solubility	Can prevent electrospinning process or affect fiber morphology.	[85, 87]
Solvent	**Type**	Organic solvents are typically more volatile than polar solvents such as water, speeding up the process and affecting the size (typically having smaller diameters) and morphology of fibers.	[85, 87, 88]
Solution	**Volatility**	Solvents with high volatility cause fibers to dry quicker in the air than solvents with low volatility, which can lead to a change in morphology. Low volatility can prevent the electrospinning process.	[28, 89, 91]
	Conductivity	Must be sufficient to overcome surface tension of the solution. Can be increased with addition of salts, acids, or water.	[88, 92, 93]
	Viscosity	Directly related to concentration and molecular weight of the polymer, affects size of fibers and deposition area. High viscosity can prevent electrospinning.	[28, 87, 89]
Setup	**Applied Voltage**	An increase in voltage breaks the surface tension of the solution more quickly, which can cause a change in fiber morphology and smaller fiber formation. A decrease could stop the process or form larger fibers with beading.	[28, 81, 94]
	Flow Rate	An increase in flow rate creates excess solution on the needle tip, which can result in the deposition of wet fibers on the mandrel or loss of Taylor cone formation. Wet fibers tend to fuse and flatten to create a sheet, not a mesh. A decrease in flow rate causes a disappearance of any Taylor cone, pulling non-homogenous fibers from deeper in the needle.	[28, 94]
	Airgap Distance	Larger distances prevent fiber attraction to the target, causing a greater loss of material or a halting of the process. Shorter distances cause fibers to collect on the target while still wet, resulting in fiber merging.	[28, 95]
Ambient	**Temperature**	Temperature increases cause the viscosity of a solution to decrease (in most cases) and the volatility to increase, resulting in smaller fibers. A decrease would result in larger fibers.	[28, 88]
	Humidity	Increased humidity causes the vapor pressure of a solution to decrease, resulting in increased surface tension and either the deposition of wet fibers or a stoppage of the process.	[28, 88]

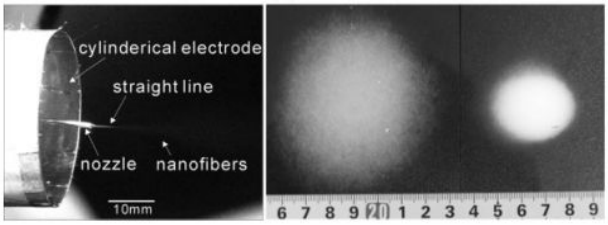

Figure 5: Cylindrical electrode used to stabilize electrospun fibers. a) Nanofibers with an auxiliary electrode surrounding the nozzle. b) Deposition of fibers without an aux. field c) Deposition of fibers with an aux. field. Shown units are in centimeters. The size of the deposition area is reduced with the application of an auxiliary electrical field. Reprinted with permission from G.H. Kim [80]. Copyright 2006 John Wiley and Sons.

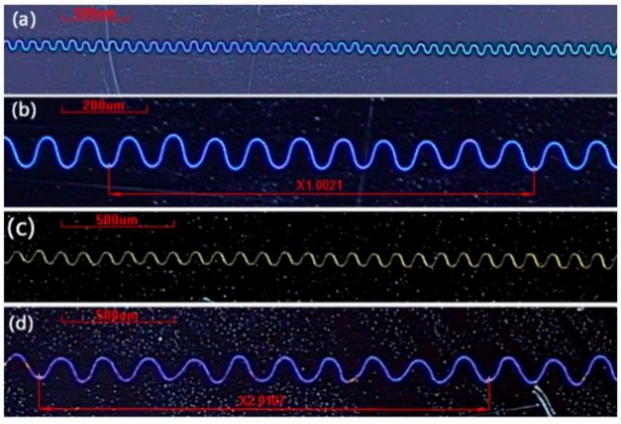

Figure 6: "Direct-write" effect when AC voltage is incorporated [110] (Open source content).

The shape of the target can affect the geometry and alignment of fiber formation. In several studies, a two-pole target was used to collect fibers between two grounded electrodes [50, 116, 117]. The result is that fibers can be collected in parallel arrays or, if a twisting motion is included, in a tight yarn-like structure that could be used to mimic native ligament or tendon.

If an electrode with a charge of opposite polarity is placed just behind the target it changes the electric field, creating a stronger pull on the fibers toward that point on the target. This technique was used by Cho *et al.* to form an electrospun nanofibrous filter within microchannels using gold electrodes embedded beneath the sides of the channels. This aligned the fibers across the channel to form a filter [101].

2.3 AC Electrospinning

The traditional setup described previously uses a high-voltage direct current (DC) power source as the driving force for the polymers. DC power gives the polymer a charge of a single polarity, and because of this, there can be undesired side-effects. These primarily involve repulsion of newly formed fibers by previously deposited fibers. This repulsion has only a small effect on the total outcome of the resulting scaffold, but it increases material loss and reduces precision. To overcome this repulsion, high-voltage alternating current (AC) power source, instead of or in combination with DC power, can be utilized to create a "direct-writing" effect (Figure 6) with electrospinning [98, 100, 102–110].

Direct-writing refers to the ability that some researchers have obtained to collect single looping nanofibers and move either the target or the nozzle to "write" the pattern they desire. Direct-writing is a cross between electrospinning and 3D printing, combining the nanofiber formation by electrostatic manipulation of electrospinning with the accurate material placement of a 3D printer; it is only possible through the charge polarity switching of AC electrospinning. The voltage polarity in an AC power source allows negatively charged fibers to attract positively charged fibers, alternating back and forth throughout the process, creating a more charge-neutral construct overall.

2.4 Magnetic Field Manipulation

Another method for manipulating the fiber path through the air is manipulating the magnetic field within the system. Essentially, when a charged particle enters a magnetic field, a force is generated that deflects that particle in the intended direction. This force is generated orthogonal to both the velocity of the charged particle and the direction of the magnetic field line it intersects, following the right hand rule [118]. Past studies have used magnetism to align fibers as a way to make them easier to gather for use in electronic or photonic devices in which precision, accuracy, and careful assembly is required. Yang *et al.* and Liu *et al.* both used permanent magnets to induce the polymer to form parallel nanofiber assemblies that can be lifted off and deposited directly on a device or substrate (Figure 7) [111, 112].

There are other potential approaches to utilizing magnetism for electrospinning. Electromagnets can be used as an advantageous replacement for permanent magnets, the advantage of which being that the magnetic field created by electromagnets can be tuned to the correct magnitude by modifying the applied current. Electromagnets are created by winding wire into a coil, or solenoid. The strength

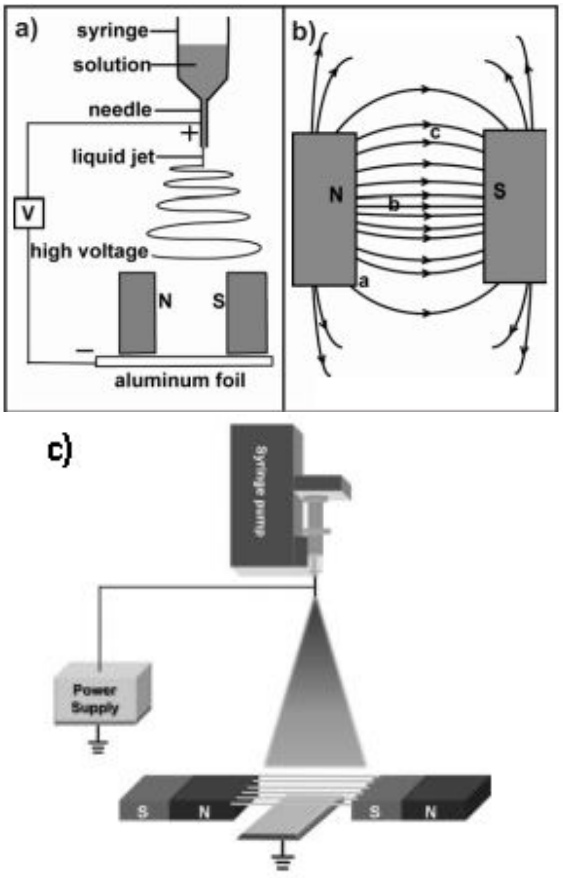

Figure 7: Alignment of electrospun fibers using magnetism [111, 112]. In both examples, two permanent magnets were used to collect parallel nanofibers on the target.
(a,b) Reprinted with permission from D. Yang *et al.* [112]. Copyright 2007 John Wiley and Sons.
(c) Reprinted with permission from Y. Liu *et al.* [111]. Copyright 2010 John Wiley and Sons.

of the magnetic field produced by a solenoid is given by Equation (1) [119]:

$$B_x = \frac{\mu_0 nI}{2}\left\{\left[\frac{x - x_1}{\sqrt{(x - x_1)^2 + R^2}}\right] - \left[\frac{x - x_2}{\sqrt{(x - x_2)^2 + R^2}}\right]\right\} \quad (1)$$

B_x is the magnitude of the magnetic field in the x-direction at a specific point in the center of the solenoid, μ_0 is the permeability constant, n is the number of loops in the solenoid, I is the magnitude of the current in the wire, R is the radius of the loops, x is the length of the solenoid in the x-direction, x_1 is the distance from the start of the solenoid to the point at which the system is being analyzed, and x_2 is the distance from that point to the end of the solenoid's length. To calculate the total strength of the

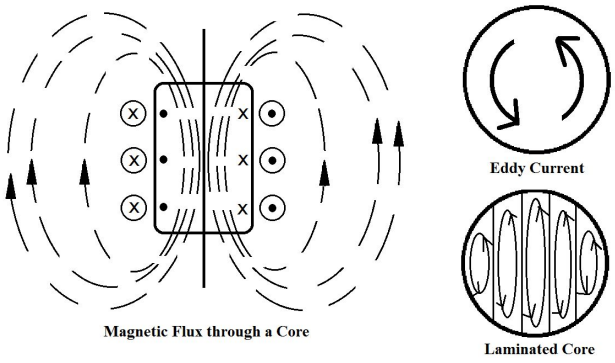

Figure 8: Magnetic flux and eddy current visualization through core (side and bottom). The eddy current causes a decrease in magnitude of the magnetic field. Laminating the core prevents this effect.

magnetic field inside a solenoid, the field is assumed to be uniform throughout the interior of the wire loops. This eliminates the length dependency, and the equation can be simplified to Equation (2) [120]:

$$B = \mu_0 nI \quad (2)$$

The field can be further stabilized by wrapping the solenoid around a ferromagnetic core such as iron or 1018 steel. This allows the magnetic field lines to concentrate within the core and become more focused. The drawback to using a stabilizing core is that solid iron or steel draws charge into itself, creating "eddy currents," similar to turbulence in an electromagnetic field. These currents create a second magnetic flux within the core that is in opposition to the desired primary flux. This reduces the magnitude of the total magnetic field and lowers the efficiency of the magnet (Figure 8) [121]. To overcome this drawback, a core can have layers of ferromagnetic material and insulation (also called lamination), to prevent the secondary magnetic field from forming by disrupting the induced current flowing through the core, but still allows the primary magnetic field to flow freely. A further concern with electromagnets is that at higher power, energy is released as heat instead of producing the electromagnetic field. To overcome the potential overheating while maintaining field strength, multiple magnets may be used.

Using solenoids to direct charged particles is not a new concept. This technique creates an effect similar to that used in television steering coils or electron microscopes. In steering coils, a beam of light is directed vertically and horizontally to different points on the screen to create the image on older television [122]. Electron microscopes most closely display the effect. The charged electrons generated at the filament are centered to a specific point by electromagnets, termed "lenses," for the purposes of creating a highly detailed image. The magnets are called lenses be-

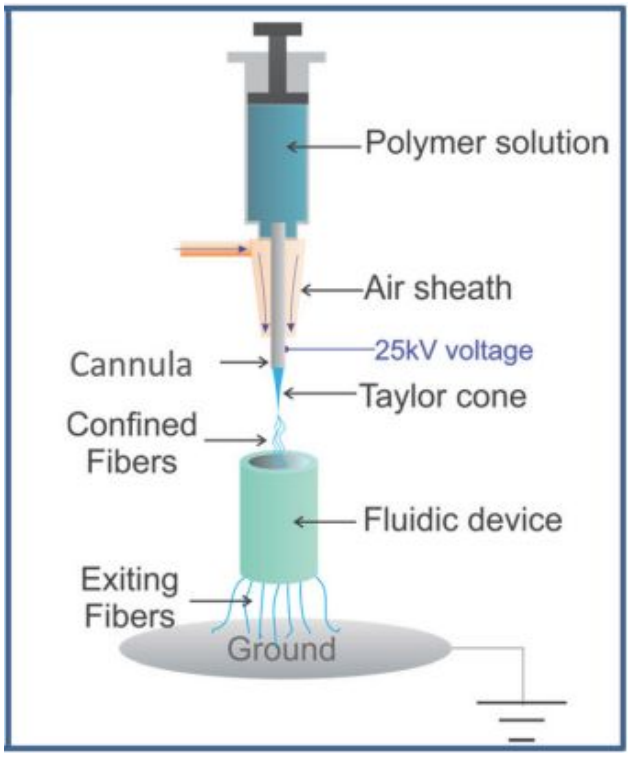

Figure 9: Diagram for the setup of an air-based fiber direction system. Reprinted with permission from Chen *et al.* [124]. Copyright 2016 The Royal Society of Chemistry.

cause they focus electrons in a highly accurate way, similar to the way glass lenses focus light in a standard light microscope [123].

Using the concept behind the electron microscope and the television steering coils as guides, a similar guidance system can be constructed for the control of electrospun nanofibers. The calculations to describe this process are more complex as the charge states of the nanofibers are not constant, varying along the segments of the polymer chain.

Using a setup of three concentric rings of electromagnets with decreasing inner diameter, the primary author of this review obtained some limited success in reducing the deposition area of electrospun fibers to a 1 cm diameter spot (unpublished data from our lab). The electromagnet device, while creating a small spot size, also incurred a large loss of polymer. The loss could be attributed to an attraction to the charged wires of the magnets stronger that the forces exerted by the magnetic field. There is potential to overcome this shortcoming by using permanent magnets in future studies.

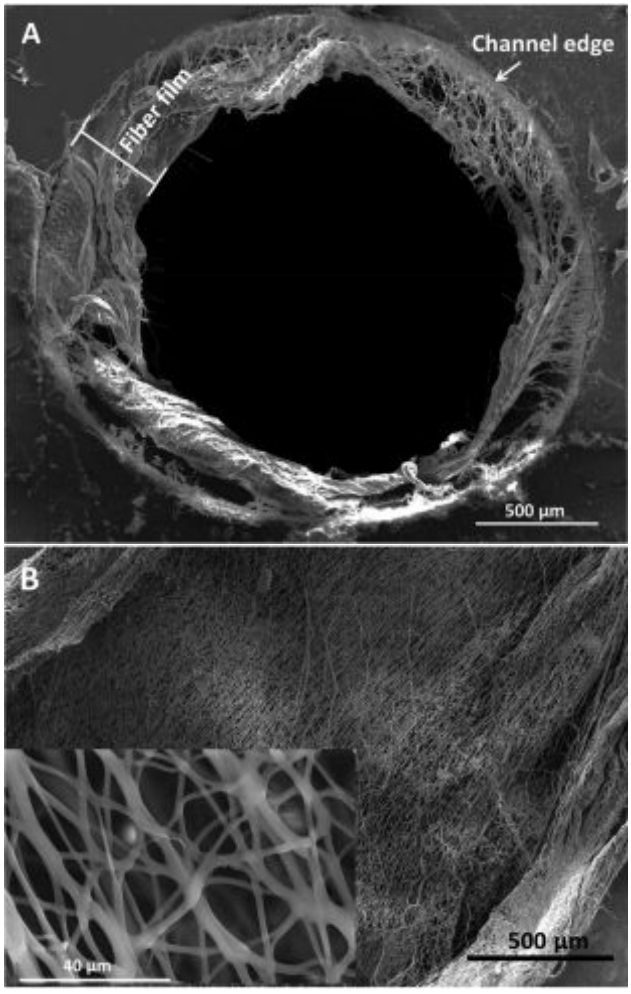

Figure 10: SEM images of a fiber coated channel looking A) through the opening and B) along the channel. Reprinted with permission from Chen *et al.* [124]. Copyright 2016 The Royal Society of Chemistry.

2.5 Air-Based Fiber Focusing

Electrospun fibers can be focused directly onto targets or into a small, enclosed space by using specialized air jackets, as shown by Chen *et al.* [124]. The air jacket or "sheath" surrounds the charged syringe needle and focuses air parallel to the stream of dissolved polymer (Figure 9). The application of focused air through the air sheath at an optimized pressure of 10-15 psi reduces the loss of polymer fibers to surrounding surfaces and focuses the deposition of fibers into the 2 mm diameter channel of a 3D-printed fluidic device (Figure 10). In this protocol, it is essential that the fibers are alternatively deposited and dried with air to completely evaporate the solvent and prevent the fusing of fibers. To perform this step, the air jacket is kept on while the extrusion of the polymer solution is halted. If the drying step is not included, the fibrous coating be-

comes fused and more sheet-like, losing the desired mesh structure.

3 Modifications to Increase Porosity

Functional regeneration requires proper distribution and rapid invasion and alignment of cells within the 3D environment of the electrospun scaffold. Researchers have developed methods to modify the electrospinning process to mimic native tissue properties in order to promote intracellular response and duplicate intercellular reactions that conform to the reactions seen with native ECM structure. General electrospinning parameters adjust fiber size and deposition, but do not adequately adjust the sizes of the space between the fibers, otherwise referred to as pore size. Multiple techniques have tailored the pore size to mimic native ECM without significantly compromising mechanical integrity in order to promote full depth cellularization of electrospun scaffolds, including the addition of back pressure to the mandrel and using a yarning technique to control deposition.

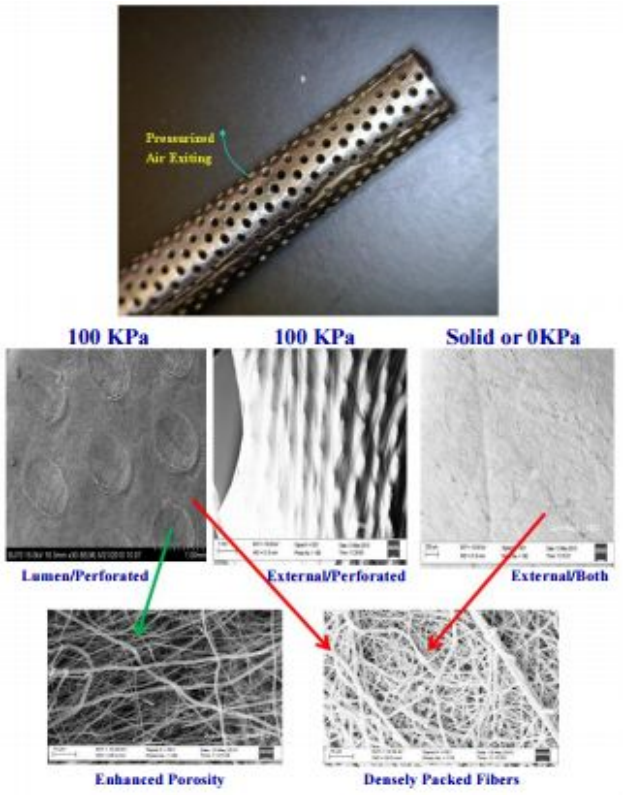

Figure 11: Air impedance mandrel [125]. (Open source content).

3.1 Air impedance electrospinning

In air impedance electrospinning, highly pressurized air flows through defined pores in a hollow electrospinning mandrel to disrupt fiber deposition and compaction, but only in the mandrel pores (Figure 11) [125–127]. The lumen of the mandrel pores increase cell penetration up to the point where the pressure of the fibers overcome the air pressure; both the outside of the lumen and between the mandrel perforations show very little cell infiltration similar to that of a solid mandrel [126, 128]. This method is effective in increasing the pore size in the resulting scaffold but requiring little sacrifice in overall mechanical strength, possibly due to the dense fiber regions providing stability and strucural support. A variation in the void space to solid metal ratio in the mandrel surface could, therefore, have effects on overall porosity and mechanical strength of the resulting scaffold.

3.2 Porogen and sacrificial fiber incorporation

The addition of porogens such as salt or water soluble polymers within an electrospun scaffold is a modified protocol of standard porogen leaching from a polymer; porogens of a distinct and uniform size are incorporated within an electrospun scaffolds and dissolved in a solvent after the electrospun scaffold has cured. The porogens leach from the scaffold, leaving uniform pores dependent on the concentration and crystal size of the porogen [129]. Electrospun scaffolds created with the porogen leaching technique show significant cellular infiltration [64, 130–132]. Similar to porogen leaching, a soluble polymer and an insoluble polymer can be co-electrospun onto a central mandrel, followed by a rinse in dissolution media to dissolve the soluble fibers. This leaves the insoluble fibers and causes a higher porosity within the scaffold [133–135]. For example, poly(ethylene oxide) (PEO) is a polymer that can be used as either electrosprayed beads or as secondary fibers in combination with silk fibroin (SF), a protein usually extracted from silkworm cocoons, to create a composite scaffold. The PEO is then removed from the scaffold through graded ethanol and deionized (DI) water washes or just DI water washes, leaving a microporous SF scaffold that was more susceptible to cell infiltration [136–138]. Another example is the incorporation of sodium chloride crystals in poly(L-lactide) electrospun scaffolds. The salt can be leached out with DI water, leaving larger pores in

the scaffold [139]. While the porosity of these scaffolds increases, the potentially undesirable effect of using a sacrificial material is that the mechanical strength is also decreased [138, 140].

3.3 Dynamic liquid electrospinning

Dynamic liquid electrospinning has been successfully used to create highly porous scaffolds of aligned nanofibers [141–144]. These scaffolds, called nanoyarn scaffolds, are created by collecting fibers in a water reservoir instead of a target. The reservoir drains through a small central drain hole (d = 8 mm), creating a vortex that twists and organizes the fibers on the surface of the water into nanoyarn structures. The nanoyarn is then collected on a rotating mandrel directly under the drain hole. The completed scaffold is then removed from the mandrel, frozen at −80°C for 2 hours and then lyophilized overnight, creating a highly porous sheet of aligned nanoyarn [141]. The scaffolds created with this technique have applications with tissues, such as tendons, that have a naturally aligned ECM structure [143, 144].

3.4 Cryo-electrospinning and alternative porosity manipulations

Higher porosities over traditional electrospinning have been achieved using cryogenic electrospinning procedures, where fibers are formed either into solutions or onto mandrels held at very low temperatures [145]. As the mandrel or solution is super cooled, crystals are formed and polymer solutions are electrospun around and throughout the crystals [145–148]. Once the scaffold is finished, the embedded crystals are removed by sublimation with a lyophilizer. This leaves void spaces in place of the crystals without damaging the surrounding fibers [145, 146]. The pore size can be controlled by adjusting the relative humidity in the electrospinning chamber, allowing for increased flexibility in pore size [146].

Alternative techniques have been utilized to increase porosity in electrospun scaffolds, including the creation of macropores via laser cutting, and spinning of low density scaffolds [149, 150]. Laser ablation involves rapid precise, intense heating of electrospun scaffolds to create controlled patterns and pores [151]. Variations and patterns can be created by controlling power, pulse, and orientation of ablation, which can be then optimized for cell infiltration [150, 152–154]. Cell infiltration can be significantly enhanced using a method of electrospinning that spins a polymer around a needle array as opposed to a solid or hollow mandrel and creates a focused, low density, uncompressed three-dimensional electrospun nanofibrous scaffold [155]. This scaffold, known as FLUF (Focused, Low density, Uncompressed nanoFiber), provides deep interconnected pores and a stable, ECM-mimicking scaffold (Figure 12).

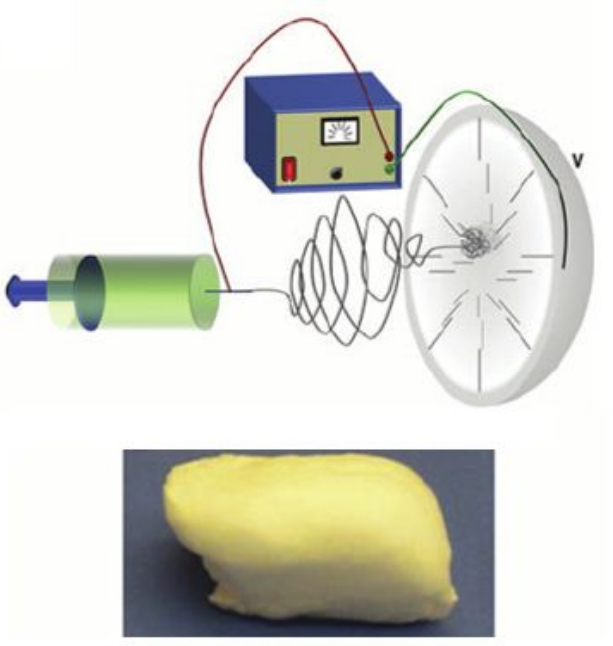

Figure 12: Creation of the Focused, Low density, Uncompressed nanofiber (FLUF). Top: FLUF electrospinning setup using a plastic hemisphere with grounded needles placed at regular intervals throughout the interior to collect the fibers; Bottom: finished FLUF scaffold. Modified and reprinted with permission. Copyright © 2011 Elsevier Publishing [155].

3.5 Post-production processing of electrospun fibers to create a 3D matrix

Three-dimensional macroporous scaffolds with increased elasticity and absorbency have been created using sheets of traditionally-processed electrospun nanofibers as the structural material [156, 157]. The nanofiber sheets are cut into small pieces and dispersed in *tert*-butanol. The dispersion is poured into a mold, frozen at −80°C for two hours, freeze dried, and then cross-linked via chemicals or heat treatment [156, 157]. The resulting scaffolds exhibit retained absorption capacity after repeated compression and drying and have the ability to recover their original shape without loss of strength after 80% compressive strain or 100 cycles of 60% strain. In addition to the

mechanical and absorbent properties, the scaffolds contained pores large enough for cell infiltration, allowing for viable cell growth deep inside the scaffolds [157].

4 Conclusion

The process of electrospinning has been in use for many years, but it is still being perfected, changed, and molded to new purposes. Its natural flexibility allowed it to change from a textile fabrication technique to a preferred technique for the creation of nanofibrous tissue engineering structures by researchers around the world.

The mutability of the technique is further seen in the research covered by this review. Control of the electrospun nanofibers has become increasingly important as certain projects require precise placement of fibers to obtain favorable constructs. Manipulating the parameters such as the polymer type, solvent type, concentration, flow rate, applied voltage, target geometry, temperature, and humidity begins this process of optimization, but further control is needed. This control must happen during the nanofiber formation process, controlling the deposition and alignment of the fibers. Altering the voltage field with auxiliary electrodes, using alternating current instead of direct current, utilizing a magnetic field to induce a force on the charged fibers, and using an air sheath to focus the fibers have shown to be effective methods, but there is room for improvement.

Further investigation with the techniques mentioned in this review is needed to determine the most effective method or combination of methods. Even after the design of a deposition control system, characterization of its effects on fiber size and morphology will be important, especially in the context of tissue engineering and regenerative medicine research. In order to develop therapies and dressings for wounds, comparable structures are essential. In addition, each polymer will likely respond differently to the control system. Choosing the correct polymer/solvent combination will become more important in this context, as the material must be effective in the system, but it also must be viable as a treatment in the human body.

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