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Fabrication and Application of Nanofibrous Scaffolds in Tissue Engineering

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Abstract

Nanofibers fabricated by electrospinning are morphological mimics of fibrous components of the native extracellular matrix, making nanofibrous scaffolds ideal for three-dimensional cell culture and tissue engineering applications. Although electrospinning is not a conventional technique in cell biology, the experimental set-up may be constructed in a relatively straightforward manner and the procedure can be carried by individuals with limited engineering experience. We detail here a protocol for electrospinning of nanofibers and provide relevant specific details concerning the optimization of fiber formation. The protocol also includes conditions required for preparing biodegradable polymer solutions for the fabrication of non-woven and aligned nanofibrous scaffolds suitable for various cell/tissue applications. In addition, the information on effective cell loading into nanofibrous scaffolds is provided. Instructions for building the electrospinning apparatus are also included.

Keywords

nanofiber; electrospinning; scaffold; tissue engineering; three-dimensional culture

Introduction

Recent advances in nanotechnology and increasing recognition of the potential of nanomaterials as biocompatible and biomimetic scaffolds for cells have provided new tools for tissue engineering and the development of three-dimensional (3D) cell cultures (Christenson et al., 2007). In a native tissue environment, cells are embedded within and are in contact with the extracellular matrix (ECM), which acts both as a structural supporter as well as a regulator of cell activities. Biochemical and mechanical signals derived from cell-ECM interactions are critical to cell survival and functions. Similarly, *in vitro*, cells interact intimately with and respond to cues from their matrix environment, further highlighting that cell activities are strongly influenced by the physical and chemical properties of the culture environment (Berrier and Yamada, 2007). A 3D, highly porous substrate that mimics the native ECM can form unique spatial interactions with cells, and is thought to more accurately replicate cell-matrix interactions *in vivo*, compared to two-dimensional (2D) substrates commonly used for tissue culture (Griffith and Swartz, 2006). To meet the

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requirements for various tissue engineering applications, a variety of 3D scaffolds of different compositions have been developed. Nanofibers produced by electrospinning represent a promising candidate scaffold biomaterial for tissue engineering (Li et al., 2005). These fibers possess unique physical characteristics, such as high surface area-volume ratio and improved mechanical strength, compared to their micro-scaled counterparts. Nanofibrous scaffolds also morphologically resemble the fibrillar components of the native ECM, enhance adsorption of cell adhesion molecules, induce favorable cell-ECM interactions, maintain cell phenotype, support differentiation of stem cells, and promote cell-matrix adhesion and activate appropriate cell signaling pathways (Fig. 1) (Li et al., 2006b).

What is electrospinning?

Electrospinning technique uses a high electric field to produce ultra-fine polymeric fibers with diameters ranging from a few nanometers to a few micrometers. The mechanism of electrospinning nanofibers is based on a complex electro-physical activity between polymer solution and electrostatic force. In this procedure, a high-voltage electric field is set up between the injection needle and the collecting screen using a power supply and electrodes. When the polymer solution is extruded slowly from the syringe, a semispherical polymer solution droplet is formed at the tip of the needle. With increasing voltage, the charged polymer droplet elongates to form a conical shape known as the Taylor cone (Taylor, 1969), and the surface charge on the polymer droplet increases with time. Once the surface charge overcomes the surface tension of the polymer droplet, a polymer jet is initiated (Taylor, 1964). The solvent in the polymer jet evaporates during its travel to the collecting screen, increasing the surface charge on the jet. This increase in surface charge induces instability in the polymer jet as it passes through the electric field. To compensate for this instability, the polymer jet divides geometrically, first into two jets, and then into many more as the process repeats itself. The formation of nanofibers results from the action of the spinning force provided by the electrostatic force on the continuously splitting polymer droplets. Nanofibers are deposited layer-by-layer on the metal target plate, forming a non-woven nanofibrous mat. During the electrospinning process, both extrinsic and intrinsic parameters are known to affect the structural morphology of the nanofibers (Doshi and Reneker, 1995). Specifically, extrinsic parameters, such as environmental humidity and temperature, and intrinsic parameters, including applied voltage, working distance, and conductivity and viscosity of the polymer solution, need to be optimized to produce uniform nanofibers. The two major structures usually found in the nanofibrous mat are a uniform, continuous fibrous structure or a bead-containing fibrous structure. Variation in the relative abundance of these two structures is determined by the relative contributions of the parameters during the electrospinning process.

Nanofibrous biomaterials, by virtue of their structural and morphological characteristics, are well suited as tissue engineering scaffolds for various tissue types, particularly musculoskeletal tissues such as cartilage, bone, tendon, and ligament. In principle, the structural functions of ECM components, such as collagen fibers, can be fulfilled by the morphologically similar nanofibers in the engineered tissues. Nanofiber-based 3D biomaterials may also serve as biomimetic substrates to culture normal and transformed cells for cell biology research.

Strategic Planning

To fabricate nanofibrous scaffolds, an electrospinning apparatus is required. Given that this is not commercially available, one must construct the apparatus. This unit provides a straightforward, do-it-yourself protocol for constructing an electrospinning apparatus (see Support Protocol). Although relatively simple in design, the electrospinning apparatus described here is capable of producing high-quality nanofibers for experimental

applications. The nanofiber electrospinning process starts with the preparation of biodegradable or non-biodegradable, natural/synthetic polymer solutions. Polymer solutions are prepared by mixing the chosen polymer with the suitable solvent(s). The solvent can be either a single chemical component or a mixture of multiple chemicals, depending on the chemistry of the chosen polymer. In general, the solvent must be capable of completely dissolving the polymer and carrying an applied charge. This unit will focus on how to fabricate nanofibrous scaffolds with a non-woven or an aligned fiber structure, composed of clinically used, Food and Drug Administration (FDA) approved synthetic biodegradable polymers (see Basic Protocol 1).

The four intrinsic parameters mentioned above – applied voltage, working distance, and conductivity and viscosity of the polymer solution - must be adjusted and optimized to produce a uniform fiber. These parameters are dependent on the nature of the polymer used and thus vary with different polymers. After electrospinning, the finished nanofibrous scaffold needs to be sterilized before use for cell culture. Heat or γ -irradiation cannot be used to sterilize nanofibers since the fibers are likely to be damaged by these treatments. Nanofibrous scaffolds can be effectively sterilized by ultraviolet light (UV) and ethanol. The step-by-step procedure detailing effective cell seeding into nanofibrous scaffolds, including aspiration using bibulous filter paper to increase cell-infiltration efficiency, is described in Basic Protocol 2. To enhance nutrient access to cell-seeded nanofibrous constructs, rotary wall vessel (RWV) bioreactors are used to facilitate nutrient/metabolic waste exchange in and out of the 3D construct; the protocol of culturing cellular nanofibrous constructs in a bioreactor is described in Basic Protocol 2.

Basic Protocol 1

Fabrication of Nanofibrous Scaffolds

A number of polymers, both natural and synthetic, which may be biodegradable or non-biodegradable, may be used as raw biomaterials to fabricate nanofibrous scaffolds. With the discovery and synthesis of new polymers, the list of candidate polymers suitable for electrospinning continues to grow. Synthetic biodegradable polymers can be tailored to have the specified, desired properties, including degradation profile and mechanical properties, over those of natural or non-biodegradable polymers. Biodegradable poly(α -hydroxy esters) are the most widely used polymers for the fabrication of tissue engineered scaffolds, given their FDA approval and well-documented biocompatible properties (Gunatillake and Adhikari, 2003). In addition to each member of the poly(α -hydroxy esters) family having defined chemical and physical properties, the addition option of mixing or co-polymerizing two or more of poly(α -hydroxy esters) at varying ratios to generate materials with a wide spectrum of properties is another attractive characteristic of the poly(α -hydroxy esters).

There are currently six FDA-approved poly(α -hydroxy esters), including poly(glycolic acid) (PGA), poly(L-lactic acid) (PLLA), poly(D,L-lactic acid) (PDLLA), poly(D,L-lactic-co-glycolic acid 50:50) (PLGA5050), poly(D,L-lactic-co-glycolic acid 85:15) (PLGA8515), and poly(ϵ -caprolactone) (PCL), that have been successfully used to produce nanofibers. However, only PLLA and PCL have slow degradation rates suitable for cell culture applications (Li et al., 2006a). In this section, fabrication of PLLA and PCL nanofibrous scaffolds will be described in the step-by-step protocol. Besides non-woven fibrous scaffolds, the electrospinning technique is also capable of producing aligned fibrous scaffolds using an alternative setup (Theron et al., 2001). The matrix of aligned synthetic nanofibers mimics that of uni-axial collagen fibers found in native tissues and is suitable for growing of tissues with anisotropic structure, such as striated muscle and tendon/ligament. Typically, nanofibrous scaffolds are characterized on the basis of fiber morphology,

observed using scanning electron microscopy, degradation profile, porosity, and mechanical properties.

Materials

Electrospinning apparatus (See SUPPORT PROTOCOL)

PLLA (MW = 50,000) (Polysciences)

PCL (MW = 80,000) (Aldrich)

Tetrahydrofuran (THF) (Fisher Scientific)

N,N-Dimethylformamide (DMF) (Fisher Scientific)

Chloroform (Fisher Scientific)

Glass pipette

Glass threaded vial

Analytical balance

Vortex mixer

Aluminum foil

Glass slide

Vacuum desiccator

Preparation of polymer solution

- 1 Dissolve 1.6 g of PLLA polymer in 11 mL of chloroform/DMF (10:1) in a glass vial. More polymer solution can be made using the same proportions if desired.
- 2 Dissolve 2 g of PCL polymer in 14 mL of THF/DMF (1:1) in a glass vial. More polymer solution can be made using the same proportions if desired.

Storage: Raw polymers before use should be stored at 4°C in a tight container within a sealed bag. When taking out of storage, allow the container to reach room temperature before opening to prevent moisture condensation.

- 3 Screw the cap of the glass vial tight and wrap the vial cap with parafilm.
- 4 Vortex the polymer solution in the glass vial overnight.

Electrospinning of non-woven nanofibrous scaffold

- 5 Place a sheet of aluminum foil onto the fiber collection screen in the bottom of the electrospinning apparatus (see Fig. 2A).

The sheet of aluminum foil used to collect nanofibers should be wrinkle-free since the wrinkled surface results in uneven fiber deposition. The sheet of aluminum foil must be handled with care.

To directly coat nanofibers onto glass slides or coverslips, use clean forceps to pick up the slides/coverslips and place them onto the surface of the aluminum foil sheet with only a small distance separating them.

- 6 Mount the glass syringe fitted with an 18-G blunt needle vertically in the electrospinning apparatus, and transfer the homogeneously mixed polymer solution into the syringe.

- 7 Adjust the distance between the tip of the needle and the fiber collection screen to 20 cm.
- 8 Turn on the high-voltage power supply and set the voltage at 16 kV for PLLA or 12 kV for PCL.

Unwanted non-uniformed polymer droplets form before the voltage is correctly established. Use a sheet of paper towel to cover the collection screen and then remove it after the desired voltage is reached.

The fiber spinning process usually requires a period of several hours. Check the process periodically. The needle can become clogged if undissolved polymer or impurity is present. When this happens, turn off the power and then unclog the needle.

- 9 Using a sharp surgical blade, cut the nanofibrous sheet and gently remove it from the fiber collection screen.

For nanofiber-coated glass slides or coverslips, cut the fibers along the edge of the slides or coverslips. Make sure that all the excess, connected nanofibers are excised from the edges of the slides/coverslips and cut clean before they are separated from each other.

- 10 Place the nanofibrous sheet or the nanofiber-coated glass slides/coverslips in a vacuum desiccator for 2 days to remove the organic solvent residue.

Electrospinning of aligned nanofibrous scaffold

- 11 Remove the stationary copper plate collection screen and replace with the rotary metal shaft or mandrel (see Fig. 2B).
- 12 Place a sheet of aluminum foil to fully cover the shaft.

The sheet of aluminum foil used to collect nanofibers should be wrinkle-free since the wrinkled surface results in uneven fiber deposition. The sheet of aluminum foil must be handled with care.

To directly coat nanofibers onto glass slides or coverslips, use clean forceps to pick up the slides/coverslips and place them onto the surface of the aluminum foil sheet with only a small distance separating them. Secure the slides/coverslips with adhesive tape to the surface of the rotary shaft. In addition, a protective plastic shield should be set up in the front of the electrospinning apparatus to contain accidental, flying optics.

- 13 Mount the glass syringe fitted with an 18-G blunt needle vertically in the electrospinning apparatus, and transfer the homogeneously mixed polymer solution into the syringe.
- 14 Adjust the distance between the tip of the needle and the fiber collection shaft to 20 cm.
- 15 Adjust the rotation of the rotary shaft to the desired speeds(s) with the variable rheostat transformer.

The rotation speed determines the extent of the fiber alignment. The faster the rotation speed, the greater the extent of alignment. Under the conditions described here, more than 90% fiber alignment is achieved when the linear speed of the shaft exceeds 9.3 m/s.

- 16 Turn on the high-voltage power supply and set the voltage at 16 kV for PLLA or 12 kV for PCL.

Unwanted non-uniformed polymer droplets form before the voltage is correctly established. Place a sheet of paper towel between the needle and the rotary shaft to cover the fiber collection surface and then remove it after the desired voltage is reached.

- 17 Using a sharp surgical blade, cut the nanofibrous sheet and gently remove it from the fiber collection surface of the shaft.

For nanofiber-coated glass slides or coverslips, cut the fibers along the edge of the slides or coverslips. Make sure that all the excess, connected nanofibers are excised from the edges of the slides/coverslips and cut clean before they are separated from each other.

- 18 Place the nanofibrous sheet or the nanofiber-coated glass slides/coverslips in a vacuum desiccator for 2 days to remove the organic solvent residue.

Basic Protocol 2

Nanofibrous Scaffolds for Cell Culturing

Cell seeding into a 3D, porous biomaterial scaffold is the first and critical step for the formation and growth of tissue engineered constructs *in vitro*. Because of the complex 3D structure that makes cell infiltration difficult, cell seeding into a 3D scaffold is more challenging and less effective compared to conventional monolayer culturing. For nanofiber-coated surfaces, such as glass slides or slips, cell seeding by natural gravity is a convenient and practical approach. However, cell seeding into a 3D nanofibrous scaffold constructed with interwoven ultrafine fibers requires different methods to improve efficiency. One preferred method uses bibulous filter paper to aspirate cells into the 3D scaffold in a guided manner.

Bioreactors are culture devices that circulate the culture medium to promote cell and tissue growth via enhanced nutrient transport and mechanical stimulation (Martin et al., 2004). Tissues cultured in appropriate bioreactors show increased weight and enhanced cellular phenotype, compared to tissues grown in static cultures, e.g., in tissue culture plates or flasks (Martin et al., 2000). Various bioreactor designs have been developed and used for tissue engineering applications. The rotary wall vessel (RWV) bioreactor, originally developed to simulate microgravity by randomizing gravity (Martin et al., 2004) is a commercially available device (Synthecon, Houston, TX) and is suitable for growing large-sized engineered tissue *in vitro*. Constructs cultured in a RWV bioreactor encounter low shear stress when the rotation speed of the culture chamber is set to balance the weight of the construct and the hydrodynamic force (Lappa, 2003). The randomized gravity environment inside the chamber permits the cultured tissue construct to undergo continuous free-fall with maximal enhancement of medium diffusion into the construct.

Materials

- UV light source
- 70% ethanol
- Hank's Balanced Salt Solution (HBSS)
- Bibulous filter paper (Whatman 3MM)
- Pipetter
- Multi-well culture plate
- Cell suspension in 10% serum containing culture medium (cell density = $20 \times 10^6/\text{ml}$)

Preparation of nanofibrous scaffolds for cell seeding

- 1 Using a surgical blade, cut the nanofibrous sheet (1-mm thick) into the desired shape and size.

The use of scissors should be avoided because the compressive cutting force will deform the edges of the scaffolds.

- 2 Gently remove the fibrous scaffolds from the aluminum foil and place them in the clean Petri dish.
- 3 Sterilize the nanofibrous scaffolds under UV irradiation of each side for 30 minutes in a sterile, laminar-flow hood.
- 4 Soak the nanofibrous scaffolds in 70% ethanol for 30 minutes.

Air bubbles often are trapped inside the nanofibrous scaffold. To ensure complete sterilization and hydration, trapped air bubbles must be removed, which can be done by gently rolling a sterile glass rod over the nanofibrous scaffold to squeeze out the air bubbles.

- 5 Remove the 70% ethanol and hydrate the nanofibrous scaffolds with sequential washes of 50% ethanol, 25% ethanol, sterile distilled water, and finally HBSS for 30 minutes each.
- 6 Store the nanofibrous scaffolds in the HBSS while preparing cells for seeding.
- 7 Place the nanofibrous scaffold onto a sterile piece of bibulous filter paper.
- 8 Seed the desired number of cells by quickly pipetting the cell suspension onto the nanofibrous scaffold, as the solution is being aspirated into the scaffold.
- 9 Place the cell-loaded nanofibrous scaffold into wells of the multi-well culture plate.

The nanofibrous scaffold may become dry if left on the filter paper too long. Cell seeding into the scaffolds should be done one at a time, with other scaffold kept in HBSS.

Replace the wet filter paper as needed to maintain an efficient absorption capability.

- 10 Maintain the culture plate in the incubator. Add a small volume of additional cell medium periodically during the next 2 hours to prevent desiccation.
- 11 Add appropriate amount of culture medium into the wells for regular static culture. For cultures to be maintained in the RWV bioreactor, the cell-seeded scaffolds should be cultured in culture plates for 3 days before transferring to the bioreactor.

Culture of cellular nanofibrous constructs in RWV bioreactor

- 12 Gently transfer the cell-based nanofibrous constructs to the sterilized RWV bioreactor chamber and reassemble the chamber.

The choice of the RWV chamber size is based on the dimension of the samples being cultured. The internal space of the chamber should be large enough for the samples to move freely. Multiple samples may be placed in a single chamber, provided that the constructs do not collide with one another when the chamber is rotating.

- 13 Fill the chamber completely with culture medium and purge all air bubbles.
- 14 Mount the chambers to the bioreactor base and adjust the rotation speed until the samples appear to be suspended in the culture medium.

Support Protocol

The construction and set-up of an electrospinning apparatus is presented here. The electrospinning apparatus is composed of three major components: a high voltage power supply, a polymer solution reservoir (e.g., a syringe, with a small diameter needle) and an accessory flow control pump, and a metal collecting screen. The high voltage power supply with several independent adjustable controls and independently functioning DC outputs should be capable of providing multiple up-to-50-kV. A syringe is used to store the polymeric solution and a metal needle is connected to the electrodes of the power supply to deliver a charged polymer jet. Polymer flow can be driven by gravity when the syringe is placed vertically. However, a syringe pump is usually used for precise control of flow rate. The fiber-collecting screen should be conductive and can be either a stationary plate or a rotary platform. As described above, the plate is used to obtain non-woven fibers, while a rotary platform is used to obtain aligned fibers.

There are two basic electrospinning set-ups, vertical and horizontal. In the former, nanofibers move downwards vertically to the fiber collection screen, whereas in the latter, nanofibers travel horizontally to the target plate. Both set-ups function well in the production of nanofibers, and the selection of the set-up is a matter of preference. With computer-controlled automation, the set-up can be transformed into a more sophisticated one capable of fabricating complex nanofibrous structures. For example, controlled delivery using multiple jets can be used to fabricate a single nanofibrous scaffold that is composed of multiple layers, with each layer derived from a different polymer. In addition, the multiple jet set-up is more efficient for the production of a large quantity of scaffolding material. Generally, a basic set-up is sufficient for the purpose of making nanofibrous scaffolds for tissue engineering and 3D culture experiments in the laboratory. The protocol described here is for constructing a basic model of the vertical electrospinning apparatus.

An isolated or low-traffic space in the laboratory is needed to set up the electrospinning apparatus, since a high voltage charge is utilized during electrospinning, and is potentially hazardous. To enhance safety, a protective casing, made of non-conductive plastic, is commonly used to enclose the entire electrospinning apparatus. The enclosure also minimizes variations in environmental factors, such as temperature and humidity, which affect nanofiber formation. In addition, proper venting via a chemical hood is required to remove vapor from volatile chemical solvents.

Materials

- High voltage DC power supply
- Glass syringe
- 18G blunt metal needle
- Teflon plate
- Copper plate
- Aluminum foil/plate
- High-density polyethylene (HDPE) plate
- Polycarbonate plate
- HDPE square rod
- Banana electrical clips

Construction of closed apparatus box

- 1 Build the electrospinning apparatus box (20 in × 20 in × 30 in) (Fig. 3) by assembling the top (A), the bottom (B), the back (C), and the two side (D&E) HDPE panels.

Black or dark color plastic is preferred to construct the back panel of the enclosure box, to provide better observation of fiber formation, since electrospun nanofibers are seen as white ultra-fine fibers.

- 2 Install the transparent polycarbonate front door (F).

The transparent door protected observation of the electrospinning process.

- 3 Attach the two adjustable-length HDPE arms (G) onto the inside of the top panel of the box. The free end of the arms has a drilled hole to function as the syringe holder.

The length of the arms should be adjustable to permit a distance range of 5 to 30 cm between the tip of the needle and the fiber collection screen.

- 4 Place the wooden plate (H) on the bottom plastic plate. The wooden plate has a 5-cm hole at the center to allow the negative/ground wiring to pass through.
- 5 Place the copper plate (I), which serves as the fiber-collecting screen, on top of the wooden plate.

The copper plate functions as the negative/ground target that needs to be connected to the negative charge of the power supply during electrospinning. A connector, such as a banana socket, may be installed at the center of the copper plate to connect with the negative/ground charge.

- 6 A Teflon plate (J), the same size as the wooden plate and with a square opening in the center, is placed on top of the copper plate,

The Teflon plate is non-conductive and is used to electrically mask the copper plate underneath, such that nanofibers are only allowed to deposit on the exposed area of the copper plate defined by the opening. The area of the opening thus determines the size of the finished electrospun nanofibrous sheet.

- 7 Place the needle-attached syringe in the holder.
- 8 Connect the positive charge output to the needle and the negative charge to the copper plate.

Reagents and Solutions

Graded ethanol series

25% ethanol in sterile, distilled water

50% ethanol in sterile, distilled water

70% ethanol in sterile, distilled water

100% ethanol

Store at room temperature

Serum-containing culture medium

DMEM culture medium containing 10% fetal bovine serum

Store at 4°C and warm to 37°C in water bath before use

Commentary

Background Information

Electrospinning was developed based on the “electrospray” phenomenon first described by Lord Rayleigh in 1882 (Rayleigh, 1882). He discovered that a highly charged droplet would break down into smaller droplets when passing through a voltage gradient, as the result of Coulombic repulsion disrupting the droplet surface tension. Instead of producing small droplets in the electrospinning process, electrospinning produces continuous long fibers. In 1934, Formhals was granted a series of U.S. patents on the applications of using the technique to make fine fibers from a cellulose acetate solution (Formhals, 1934). Recently, the surging interest in nanotechnology and tissue engineering has engendered renewed attention to this technology.

Critical Parameters and Troubleshooting

Electrospinning is a complex electrophysical process in which both extrinsic and intrinsic parameters affect nanofiber formation (Doshi and Reneker, 1995). Uniform nanofibers are produced only under optimal conditions where both extrinsic and intrinsic parameters work together seamlessly. In general, the intrinsic parameters are more critical in determining the nanofiber structure than the extrinsic parameters.

Polymer solution viscosity—Viscosity of a polymer solution, directly proportional to polymer concentration, is the most critical parameter influencing nanofiber uniformity and size (Fong et al., 1999). Every polymer solution has its specific viscosity range in which uniform nanofibers can be produced, and the range varies with the polymer or solvent types (Liu and Hsieh, 2002). Therefore, each polymer solution needs to be individually characterized to assess the optimal viscosity range. In addition, viscosity affects fiber size; a more viscous polymer solution makes larger fibers (Deitzel et al., 2001).

Applied voltage and working distance—Applied voltage is the electro-driving force for nanofiber spinning, and usually a voltage of at least 5 kilovolts DC is required for electrospinning. Working distance is defined as the distance between the syringe tip and the fiber collection screen, and together with applied voltage, can determine the strength of applied charge in the electrostatic field. Similar to polymer solution viscosity, charge strength also has to be optimized to a range appropriate for uniform nanofiber formation.

Polymer solution conductivity—Polymer solution conductivity is primarily determined by the nature of the polymer and solvent, and the availability of ionizable salts. It has been shown that a more conductive polymer solution carrying more electric charge during electrospinning, with the as-spun fibers generating a stronger repulsive force, tends to produce more uniform nanofibers. Different approaches, such as using dipolar aprotic solvents (Lee et al., 2003) and adding conductive agents (Zong et al., 2002) in the polymer solution, are applied to enhance polymer solution conductivity.

The biggest challenge with electrospinning is finding the optimal combination of polymer solution viscosity and conductivity, applied voltage, and working distance for electrospinning. If the optimal combination is not reached, the electrospun structure turns into beads or bead-strings, instead of fibers. When bead-containing structures occur, increasing polymer solution viscosity should be the first parameter to vary in order to improve fiber formation. Increasing polymer solution conductivity can also be tested for the purpose of reducing bead formation.

Anticipated Results

Support Protocol—A basic, functioning electrospinning apparatus box should be assembled for electrospinning non-woven or aligned nanofibers.

Basic Protocol 1—After overnight mixing by vortexing, the polymer solution turns into a homogeneously viscous and transparent liquid. During electrospinning, the researcher should observe that the polymer solution comes out the needle, transforms into nanofibers, and deposits onto the fiber collection screen. A white nanofibrous mesh spot should be observed on the screen, gradually increasing its dimensions and eventually covering the entire screen. After electrospinning, a thick fabric-like nanofibrous mat is produced.

Basic Protocol 2—Both PLLA and PCL nanofibrous scaffolds are hydrophobic but should be completely hydrated by immersion in a graded series of ethanol solutions. After the hydration step, the researcher should observe that the nanofibrous scaffolds are easily hydrated in HBBS. Using labeled cells and subsequently microscopy, cells should be observed adhering to nanofibers after cell seeding and culturing in serum-containing medium.

Time Considerations

Support Protocol—The electrospinning apparatus box can be constructed by a skilled mechanic in a few days.

Basic Protocol 1—It takes less than 10 min to prepare the polymer solution for vortexing and both PLLA and PCL can be completely dissolved overnight. The time needed for electrospinning nanofibers depends on the amount and the conductivity of polymer solutions. In general, it takes 8 hours for PLLA and 35 hours for PCL. After a nanofibrous mat is produced, placement for 2 days in a vacuum desiccator is preferred for the complete removal of harmful chemical residue.

Basic Protocol 2—The preparation of nanofibrous scaffolds for cell seeding takes about 4 hours, including 1 hour of sample cutting and 3 hours of sterilization using UV irradiation and ethanol. The time needed for cell seeding varies, depending on sample number, but should be completed expeditiously to maintain cell viability.

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Figure 1.

Application of electrospun poly(L-lactic) acid (PLLA) nanofibrous scaffold seeded with chondrocytes for cartilage tissue engineering. (A) Scanning electron micrograph (SEM) of electrospun PLLA nanofibers showing interconnecting pores defined by randomly oriented ultrafine fibers with diameters ranging from 500-900 nm. (B) SEM view of aggregates of globular cells growing in chondrocyte-seeded nanofibers on culture Day 28 (with permission from Mary Ann Liebert, Inc.). (C) Confocal laser scanning microscopy showing punctate, disorganized actin cytoskeleton distributed cortically in cells after 28 days of culture (red: TRITC-phalloidin labeled actin filaments; blue: DAPI labeled nuclei). (D) Hematoxylin-eosin histology of chondrocyte-nanofiber composites on culture Day 28 showing oval or round cells encased in extracellular matrix-rich lacunae, characteristic of hyaline cartilage. Bar: (A, B) 5 μ m, (C) 10 μ m, (D) 50 μ m.

Figure 2.

Two basic set-ups of electrospinning apparatus. Non-woven nanofibrous scaffolds can be produced and collected on a flat metal plate (A), whereas aligned nanofibrous scaffolds can be collected onto a rotary metal shaft (B).

Figure 3.

Schematic of electrospinning apparatus box. The box is constructed with HDPE plates representing the top (A), bottom (B), back (C), and two side (D&E) panels, and the front polycarbonate door (F). Each of the HDPE arms (G), with one end attached to the top, is composed of three inter-connecting square rods, and is flexible for height adjustment of the syringe holder. Inside the box, a Teflon plate (J) is placed on top of the copper plate (I) that is on top of the wooden plate (H).