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bFGF-containing electrospun gelatin scaffolds with controlled nano-architectural features for directed angiogenesis

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Abstract

Current therapeutic angiogenesis strategies are focused on the development of biologically responsive scaffolds that can deliver multiple angiogenic cytokines and/or cells in ischemic regions. Herein, we report on a novel electrospinning approach to fabricate cytokine-containing nanofibrous scaffolds with tunable architecture to promote angiogenesis. Fiber diameter and uniformity were controlled by varying the concentration of the polymeric (i.e. gelatin) solution, the feed rate, needle to collector distance, and electric field potential between the collector plate and injection needle. Scaffold fiber orientation (random vs. aligned) was achieved by alternating the polarity of two parallel electrodes placed on the collector plate thus dictating fiber deposition patterns. Basic fibroblast growth factor (bFGF) was physically immobilized within the gelatin scaffolds at variable concentrations and human umbilical vein endothelial cells (HUVEC) were seeded on the top of the scaffolds. Cell proliferation and migration was assessed as a function of growth factor loading and scaffold architecture. HUVECs successfully adhered onto gelatin B scaffolds and cell proliferation was directly proportional to the loading concentrations of the growth factor (0–100 bFGF ng/mL). Fiber orientation had a pronounced effect on cell morphology and orientation. Cells were spread along the fibers of the electrospun scaffolds with the aligned orientation and developed a spindle-like morphology parallel to the scaffold's fibers. In contrast, cells seeded onto the scaffolds with random fiber orientation, did not demonstrate any directionality and appeared to have a rounder shape. Capillary formation (i.e. sprouts length and number of sprouts per bead), assessed in a 3-D in vitro angiogenesis assay, was a function of bFGF loading concentration (0 ng, 50 ng and 100 ng per scaffold) for both types of electrospun scaffolds (i.e. with aligned or random fiber orientation).

Keywords

Angiogenesis; Electrospinning; Scaffolds; Cell orientation; bFGF

1. Introduction

Current approaches in therapeutic angiogenesis that involve the administration of angiogenic growth factors, cells or a combination of cells and cytokines have demonstrated promising results in a number of experimental and clinical ischemic settings [1–6]. However, the long term instability and poor organization of the newly formed microvasculature that are associated with current strategies has become a major impediment towards the development of a non-invasive clinically relevant angiogenesis approach.

The extracellular matrix (ECM) is a complex structural network of proteins and carbohydrates that provides structural support to mammalian cells and regulates a number of cellular and tissue functions (i.e. cell migration and growth, wound healing) [7,8]. Structural proteins in the interstitial ECM and basement membranes contain bioactive domains that can bind to cell surface receptors, other structural proteins, or to signaling molecules such as cytokines, chemokines, and matrix proteinases [9]. A large number of bioactive molecules (i.e. growth factors) are bound via non-covalent interactions within the ECM and are released under tissue specific conditions [10]. Furthermore, specific intracellular signaling pathways can be efficiently regulated through biological cues as well as structural interactions (i.e. mechanotransduction) between ECM and the intracellular cytoskeleton [11–14]. As a result, a number of strategies in therapeutic angiogenesis have focused on integrative approaches where angiogenic growth factors and/or cells are combined with 3-D scaffolds that mimic the extracellular matrix [5,6,15,16]. Success of these therapies relies on tailoring scaffold properties (e.g. architectural, chemical, and mechanical) in order to regulate cytokine delivery profiles and modulate cellular behavior and interactions.

Advances in micro- and nano-fabrication techniques have allowed biomaterial scientists to fabricate bioactive scaffolds with architectures similar to the native tissue [17–22]. Examples of such fabrication methods include phase inversion, foaming procedures, particle leaching, and machining/etching techniques. One scaffold fabrication method that has proven to be simple, reproducible, and allows for the formation of fibers ranging from several nanometers to several micrometers in diameter with different topographical features is electrospinning [23]. Electrospinning consists of three major components: a high voltage power supply, an electrically conductive collector, and an infusion pump to adjust feed rate of the polymer solution. Furthermore, to achieve fiber deposition a continuous jet must be generated by immobilizing charges onto the surface of a pendant droplet via five steps: (1) charging of the pendant droplet, (2) formation of the cone jet, (3) thinning of the steady jet, (4) onset and growth of jet stability, and finally (5) collection of the fibers in different deposition patterns [24]. This process yields a random fiber deposition pattern, however several methods have been developed to generate constructs with aligned fiber orientation [25,26]. Electrospinning is proven to be an effective method to fabricate highly organized tissue engineering scaffolds that can utilize a wide range of polymers (e.g. cellulose, chitin, dextrose, collagen, gelatin), provide spatial control over scaffold architecture, as well as easily tailoring cytokine release profiles [27,28].

In the context of therapeutic angiogenesis, studies have attempted to design nanofibrous scaffolds that promote cellular behaviors similar to those in native revascularization processes. Ma et al. utilized gelatin grafting to modify poly(caprolactone) (PCL) nanofibers in order to improve their compatibility with endothelial cells (ECs) [29]. Gelatin grafting enhanced EC spreading and proliferation compared to PCL alone. Moreover, ECs were found to orient themselves along the direction of the gelatin grafted nanofibers and to maintain the expression of platelet–endothelial cell adhesion molecule 1 (PECAM-1), intercellular adhesion molecule 1 (ICAM-1), and vascular cell adhesion molecule 1 (VCAM-1), suggesting that the expressions of the vascular markers were directly correlated

to the architecture of the scaffold [30]. Conversely, reports suggest that in addition to influencing cell marker expression, scaffold architecture may also provide cells with specific cues that dictate directional growth. Xu et al. developed an aligned poly(L-lactide-co-caprolactone) nanofibrous matrix via electrospinning in order to evaluate smooth muscle cells (SMCs) morphology as a function of scaffold architecture [31]. Both SMCs and smooth muscle cytoskeleton proteins organized themselves along the direction of the nanofibers, thus maintaining a spindle-like structure, a key phenotypic characteristic in angiogenesis [32]. Furthermore, Zhang et al. cultured human aortic endothelial cells (HAECs) upon silk fibroin scaffolds in order to evaluate changes in cell morphology, viability, proliferation and phenotype as a function of scaffold architecture. Within seven days HAECs maintained phenotypic cell markers (i.e. CD146, VE-Cadherin, PECAM-1 and vWF) and formed short cord-like structures that began to interconnect forming a network of capillaries with identifiable lumens [33].

Herein, we describe a novel approach to fabricate bFGF-containing gelatin electrospun scaffolds with tunable fiber orientation to direct endothelial cell alignment. The scaffold's fiber dimensions and orientation were controlled by varying the concentration of the gelatin solution, the feed rate, needle-to-collector distance, the electric field potential and the polarity of the collecting electrodes. Endothelial cell migration and capillary sprouting was influenced by the architectural features of the electrospun scaffolds (i.e. fiber dimension and orientation) and the loading concentration of the immobilized bFGF.

2. Materials and methods

2.1. Scaffold fabrication and crosslinking

Gelatin B (Sigma Aldrich USA, Gelatin Type B from Bovine Skin G9391-500G) was dissolved at 10% w/v in 1,1,1,3,3,3 hexafluoro-2-propanol (Sigma Aldrich USA, Fluka HFIP 52,512-100 mL), the solution was loaded into a 5 mL syringe (Thomas Scientific USA, Henke Sass Wolf Norm-Ject 8939N52) and electrospun at specified parameters from a 22G blunt needle (Table 1). A total of 500 μ L was deposited onto aluminum covered glass slide and left to dry for 1 h under vacuum (25 in Hg) at room temperature. Aligned fiber orientation was achieved by controlling the polarity of the collecting plates of our custom made electrospinning apparatus (Fig. 1). The device consists of a series of linear actuators and a high voltage power supply (Spellmann HV, model: 230R, UK) connected to a high voltage relay switch. The actuators control needle-to-collector (N-t-C) distance and feed rates while the high voltage supply provides the necessary electric field for fiber formation. In contrast to current electrospinning techniques where the deposition pattern of the resulting fibrous scaffolds is passively modulated by controlling the collector's architecture [34–39], our design allows for a dynamic control of the electrostatic field by alternating the polarities of collecting electrodes using a DPDT relay switch. All of the device components are automated and controlled via a Graphical User Interface (GUI) program (LabView 8.2) (Fig. 1B). Interfacing was achieved through a USB-6009 NI DAQ card (National Instruments). This set-up allows us to fabricate random, aligned, and gridded scaffolds (Fig. 1). Additionally, we have also constructed a rotating mandrel with variable speed, which allows us to create tubular scaffolds with nanofibrous topographical features (Fig. 1F). Crosslinking of gelatin B nanofibrous scaffolds was performed with glutaraldehyde vapor (Sigma Aldrich USA, Glutaraldehyde Solution Grade II 25% in H₂O G6257-1L). Briefly, 2 mL of 25% glutaraldehyde solution per scaffold was deposited in an airtight container that contained the electrospun scaffold still attached to the aluminum collector. Scaffolds were crosslinked at various times (15 min to 2 h) in room temperature and then washed for 1 h in 100 mM glycine solution (Sigma Aldrich) to block any unreacted aldehyde groups.

2.2. Scaffold characterization

2.2.1. Assessment of fiber morphology as a function of electrospinning parameters

Fiber orientation and morphology of the electrospun scaffolds were assessed as a function of electrospinning parameters using Scanning Electron Microscopy (SEM). Specifically, N-t-C (cm), electric potential amplitude (kV), feed rate (ml/h), electrode polarity (+/−) and polymer concentration (wt.%) were varied and 500 μL of electrospun polymer collected, cut to 1 cm × 1 cm (dimensions) and imaged with a scanning electron microscope (SEM) at 12 kV 6000X. The dimensions of the fibers were assessed by evaluating 20 random individual fibers from 3 SEM images using ImageJ software (Table 2, Fig. 2). Utilizing edge-based image processing techniques, both fiber alignment and orientation were modeled (Figs. 3 and 4). Briefly, the input SEM images were converted into binary edge maps (Fig. 4: A2–D2), where edges correspond to the fiber boundaries and the orientation distribution of the edges indicates the fiber alignment. Using the Hough transform for the edge maps, we calculated the features of all lines (edges) detected, specifically, the location (distance from an origin) and orientation. The orientation histogram of a scaffold edge map indicates alignment (Figs. 3 and 4III), while the covariance and Chamfer distance between the edge orientation histograms of different scaffold fibers indicate similarity with respect to fiber alignment (Figs. 3 and 4II).

2.2.2. Effect of crosslinking on scaffold morphology, swelling and degradation

The topographical features of the nanofibrous scaffolds were also assessed as a result of the crosslinking technique. SEM images were evaluated using ImageJ for changes in diameter and morphology. Briefly, both aligned and random scaffolds were electrospun under parameters B₁₅ (Table 1) and crosslinked with glutaraldehyde vapor for one hour. Following crosslinking and washing, 3 SEM images per group were assessed for fiber morphology and dimensions.

The degree of swelling of scaffolds was determined as a function of crosslinking time. Electrospun scaffolds were crosslinked with glutaraldehyde vapor, washed with glycine and PBS and then submerged in ddH₂O for 24 h to allow for complete swelling to occur. The scaffolds were then weighed and their wet weights were recorded (W_w). The wet scaffolds were placed in a vacuum oven and allowed to dry for 1 h at 25 in Hg and 60 °C. After complete drying, the scaffolds were weighed and their weight recorded as dry weight (W_d). All samples were performed in triplicates for statistical analysis. Degrees of swelling were calculated with the following formula:

$$DS = \frac{W_w - W_d}{W_d}$$

The degradation of the electrospun gelatin scaffolds as a function of preparatory conditions was assessed in a simulated biological fluid over a period of one week. Glutaraldehyde vapor crosslinking ranged from 1 to 24 h. Crosslinked scaffolds were washed with glycine and PBS and their initial dried weight was recorded following vacuum drying (25 in Hg, at 50 °C) for 1 h. The dried samples were then incubated in 2 mL of 0.1 mg/mL collagenase solution (Collagenase type I Calbiochem Cat# 234153) and at specified times the scaffolds were removed, vacuum dried and their dry weight was recorded. Scaffold weight loss was plotted against the incubation time for each crosslinking condition.

2.3. Cell proliferation studies

HUVECs (Lonza AG, Rockland, ME) were routinely cultured in endothelial growth media-2 (Lonza AG, Rockland, ME) at 37 °C and 5% CO₂. Passages 2 to 4 were utilized for all

experiments. All other materials used such as Calcein AM (C3099 1 mg/mL solution), Medium 200PRF (M-200PRF-500), trypsin/EDTA 0.025% solution (R-001-100), trypsin neutralizer (R-002-100), and low serum growth supplement (S-003-10), were also purchased from Invitrogen.

bFGF was physically immobilized onto electrospun scaffolds and its effect on HUVEC proliferation was assessed as a function of scaffold architecture and growth factor loading concentrations. Gelatin B scaffolds were cut into 10 mm diameter discs, sterilized in 70% isopropanol solution overnight, washed in PBS three times for 15 min, transferred to 24 well plates and allowed to dry in a biological fume hood for 15 min. Subsequently, 10 μ L of bFGF solution, at three different concentrations, was pipetted onto the dehydrated scaffolds and allowed to be completely adsorbed (final bFGF concentrations: 10, 50 and 200 ng of bFGF per scaffold). HUVEC cells (10,000 viable cells per scaffold) were then seeded into the middle of the scaffolds in 10 μ L aliquots, and incubated for 30 min prior to the addition of medium. Following cell attachment, each well was supplemented with 2 mL of low serum media thus bringing the final concentration of bFGF per well to 5, 25, and 100 ng/mL. Seeded scaffolds were then incubated at 37 °C and 5% CO₂ for 5 days after which they were collected and assessed. Subsequent to incubation, live cells were stained with 100 μ L per well of 0.1 mg/mL calcein AM in PBS for 30 min at 37 °C and 5% CO₂ atmosphere. After cell staining, representative pictures of each scaffold were taken at 4x magnification with an Olympus model BX51 microscope. The images (five images per scaffold representing approximately 80% of the total scaffold area) were then processed utilizing ImageJ and an add-on (Cell Counter) software.

The release profile of bFGF from the gelatin electrospun scaffolds was assessed as a function of fiber orientation (aligned vs. random) using an ELISA assay (Invitrogen Corporation Camarillo California Cat# KHG0022). bFGF was physically immobilized within the gelatin scaffolds (200 ng per scaffold), the growth factor-loaded scaffolds were then placed in culture plates containing phosphate buffer solution (PBS, pH of 7.4) and aliquots of the releasate were periodically analyzed using a Beckman Coulter Plate Reader at a wavelength of 450 nm according to the manufacturer's protocol. All cell and release studies were performed in triplicates and the data was represented as the average $\pm$ the standard deviation (SD).

2.4. Cell morphology studies

To assess cell morphology as a function of nano-architectural cues HUVECs were seeded on both aligned and random fiber oriented scaffolds prepared under the same conditions as in the proliferation experiment. Briefly, aligned or random 10 mm diameter disc scaffolds, were carefully placed at the bottom of each well and left to dry for 15 min under sterile conditions in order for the scaffolds to adhere to the bottom of the well. Subsequently, a 10 μ L aliquot containing approximately 10,000 viable cells, as per trypan blue assay, was deposited on the center of each scaffold and on three empty wells (controls) followed by incubation at 5% CO₂ and 37 °C for five days. After the incubation period, scaffolds were removed and stained for actin filaments utilizing Alexa Fluor 488 Phalloidin and 4-6-diamidino-2-phenylindole (DAPI). Imaging processing techniques to segment differences in color of the nucleus and cytoplasm were used to determine the orientation of the seeded cells as a function of scaffold architecture.

2.5. 3-D in vitro cell assay

HUVECS were combined with dextran-coated Cytodex -3 beads (Sigma-Aldrich, Saint Louis, MO) at a concentration of 2×10^6 HUVEC per 2500 beads in 1.5 mL of EGM-2 medium (Lonza AG, Rockland, ME). Bead-cell solution was tapped and mixed every 20

min for 4 h at 37 °C and 5% CO₂. Subsequently, the bead-cell solution was transferred to a T25 culture flask and incubated overnight in 5 mL of EGM-2 at 37 °C and 5% CO₂. The next day, HUVEC coated beads were washed thrice with EGM-2 and resuspended at a concentration of 500 beads per ml in a 2 mg/mL fibrinogen and 0.15 U/ml aprotinin solution. An aliquot of 0.5 ml of bead/fibrinogen solution was combined with 0.625 U/ml of thrombin in one well of a 24 well plate. This solution was allowed to clot for 5 min at room temperature and then incubated at 37 °C and 5% CO₂ for 20 min. Fresh EGM-2 was added to each well followed by the addition of the bFGF-containing scaffolds. All gelatin scaffolds were prepared under the B₁₅ electrospinning conditions (Table 1) and crosslinked for 1 h with glutaraldehyde vapor.

Bead assays were assessed at days 0, 3, 6 and 9. Images of each well were captured utilizing a Motic AE 20/21 microscope (VWR, Radnor, PA) with a 4X objective. To ensure each well was observed in its entirety 25 images were taken per well per group. The number of images per well was defined as a function of total well area (2 cm²) and microscope field of view (diameter 0.26 cm). Of these 25 images, 10–15 were selected to evaluate both sprout length and sprout number per bead. Briefly, sprouts extending from 3–5 beads per image were measured in length and then counted. The total number of measurements were then averaged to determine the overall length and sprout number per well. Both length measurements and sprout number were processed utilizing Motic Images Plus 2.0 (Motic Group Co Ltd.). The software was calibrated based on the working magnification, which allows precise measurement of sprout length. Furthermore, a counting function included in the software, permitted the calculation of sprouts per bead per image. Results were then exported to Microsoft Excel for statistical analysis utilizing a paired t-test. All 3-D in vitro studies were performed in triplicates.

3. Results and discussion

3.1. Characterization of electrospun scaffolds

3.1.1. Scaffold morphology as a function of electrospinning parameters—Fiber diameter and morphology of tissue engineering scaffolds can significantly affect cell adhesion, proliferation, and differentiation [31]. Several studies have shown that cellular attachment and infiltration are predominantly affected by the scaffold's pore size and individual fiber morphology [33]. Herein, we examined the effect of electrospinning parameters (i.e. voltage, needle to collector distance, etc.) in preparing scaffolds with controlled fiber dimensions and orientation. Scaffolds were electrospun under a set of conditions in which gelatin concentration (5% or 10%), voltage amplitude (15 kV or 20 kV), needle-to-collector distance (10 cm or 15 cm), and feed rate (1, 4, or 8 mL/hr) were varied in all possible combinations, and fiber dimensions were determined using scanning electron microscopy (Table 2). Fiber diameters ranged from 0.15 μ m to 2 μ m, but most importantly, uniform nanofiber distribution was only achieved at specified fabrication conditions. Specifically, electrospun scaffolds prepared under parameters B₁₅ (15 kV, 10 cm N-t-C, 4 mL/hr feed rate) were highly uniform with 70% of their fiber diameter ranging between 750–850 nm (Fig. 2A and B). On the other hand, when the N-t-C was increased to 15 cm and the feed rate to 8 mL/hr (Table 1, F15) the fiber diameters of the electrospun scaffolds ranged between 150–2000 nm (Fig. 2C and D). Scaffolds prepared under the conditions described in B₁₅ (15 kV, 10 cm N-t-C, 4 mL/hr feed rate) were utilized for the remainder of the described studies.

3.1.2. Deposition patterns and effects on fiber diameter and morphology—A number of studies have demonstrated the ability of nanoscale topographical features to promote cell differentiation, attachment and growth [25,40]. Fiber alignment and orientation was assessed by image processing techniques. Fig. 3 illustrates an example of the

computational approach that was used to assess the fiber orientation. Essentially, brighter regions in these histograms correspond to orientations and distances from an origin under which large numbers of edge pixels were found. These 2D histograms (Fig. 3I) have smooth intensity variations and high contrast with surrounding “orientation-distance” regions indicating alignment. We calculated the distribution of the lines (over all positions on the image plane) with respect to orientation (Fig. 3III), and determined the dominant orientations (peaks) and orientation variances in the “model” scaffolds. Furthermore, fiber alignment or “randomness” between two scaffolds was also estimated, by comparing their orientation distributions with respect to their covariance and a distance metric (Chamfer distance) (Fig. 3II). In the “model” example of Fig. 3, there is an increasing variance from (A1) to (C1), and the three distributions indicate a dominant fiber orientation around 90° (Fig. 3III). In contrast, (D1) shows the lowest variance and its values are distributed more equally to several orientations, which indicates randomness.

Using the same techniques, we compared the SEM images of our electrospun scaffolds (aligned and random fiber orientation) in two different image resolutions, 1500× and 300× (Fig. 4). In Fig. 4III, the orientation distributions calculated from the Hough Transform indicate the similarities and differences between scaffolds, which are quantified with the covariance matrix and the chamfer distances. Scaffolds (A2) and (C2) are similar with 90° dominant fiber orientation, and are different from (B2) and (D2). (D2) corresponds to scaffolds with the most randomly oriented fibers, while the difference between (B2) and (D2) is due to the image resolution (scale) difference. The mode of fiber deposition (aligned vs. random) did not significantly affected fiber dimensions: fiber diameters ranged from $0.83 \pm 0.14 \mu\text{m}$ to $1 \pm 0.27 \mu\text{m}$ under the same electrospinning conditions with the only variable being the electrode polarity.

3.1.3. Effects of crosslinking on scaffold morphology—Crosslinking techniques used to control the mechanical properties of tissue engineering scaffolds can significantly affect fiber morphology as well as overall scaffold architecture which in turn can influence cellular behavior [41]. We assessed glutaraldehyde vapor (GA) as a crosslinking agent at variable concentrations and exposure times. As a result, the physicochemical properties of gelatin scaffolds can be easily tailored by controlling the GA cross-linking conditions. Within one hour of GA crosslinking, scaffold architecture and fiber dimensions for either aligned ($1 \pm 0.3 \mu\text{m}$ to $1 \pm 0.2 \mu\text{m}$) or random ($0.58 \pm 0.15 \mu\text{m}$ to $0.83 \pm 0.14 \mu\text{m}$) configuration were not significantly affected (Fig. 5). The degree of swelling of our scaffolds was determined as a function of crosslinking time in a 25% glutaraldehyde solution. There was a linear relationship between scaffold swelling and crosslinking time from 1 to 2 h. Once crosslinking time exceeded two hours, small differences in degree of swelling were observed. Scaffolds crosslinked for 60, 90, 120 min, and 24 h had degrees of swelling of 11.3 ± 0.7 , 6.2 ± 0.3 , 3.9 ± 0.7 , and 2.3 ± 0.5 respectively (Fig. 6A).

The degradation profile of the scaffolds fabricated under B₁₅ conditions was also assessed as a function of GA vapor crosslinking. The scaffolds were placed in a 0.1 mg/mL collagenase solution and weight loss was recorded at specified time points. As expected, scaffold degradation was inversely proportional to crosslinking times. During the initial 12 h of incubation, scaffold weight loss ranged from $88 \pm 6\%$ for scaffolds crosslinked for 1 h, to $74 \pm 3\%$ for 1.5 h, $53 \pm 7\%$ for 2 h, and $10 \pm 5\%$ for 24 h of crosslinking. An additional hour of crosslinking (from 1 to 2 h) led to a 35% decrease in the rate of degradation during the initial 12 h of incubation (Fig. 6B). Over the total incubation period (72 h), the scaffolds that were crosslinked for 1 h were completely dissolved in the collagenase medium while scaffolds that were crosslinked for 24 h had a weight loss of about 20% (Fig. 6B).

3.2. Cell studies

3.2.1. Cell proliferation and morphology as a function of scaffold architecture

—HUVES adhered successfully onto 3-D electrospun gelatin B scaffolds and cell proliferation was bFGF dependent at loading concentrations greater than 25 ng/ml for both, 2-D and 3-D culture conditions (Fig. 7). At 100 ng/ml of bFGF loading concentrations, cell density on the surface of the gelatin scaffolds was determined to be 128 ± 47 cells/mm² (by epifluorescence microscopy) while at the control groups (2-D culture plates) was determined to be 294 ± 124 cells/mm². The difference on the cell density measurements was attributed to cell migration within the porous scaffold. The gelatin structure was conducive to cell adhesion and had minimum effect of cell viability.

Topographical features of tissue engineering constructs can significantly influence cellular behavior [42]. Scaffold architecture with respect to nanofiber alignment (random vs. aligned) had a pronounced effect on individual cell morphology (Fig. 8). Cells seeded onto aligned gelatin B scaffolds had spindle shape, were elongated and parallel to the direction of the nanofibers indicating the ability of the fiber surface to guide cell orientation (i.e. contact guidance)(Fig. 8B and E). Conversely, cells seeded onto randomly deposited gelatin scaffolds maintained a rounded pellet-like phenotype, lacked tubular formation and had no specific orientation within the nanofibrous mesh (Fig. 8C and F). The differences in cellular behavior may be explained by the differences in pore size of the nanofibrous scaffolds. Parallel fiber deposition led to reduction in pore size and cell infiltration while directed cell adhesion and proliferation along the fiber axis. On the other hand, randomly deposited nanofibrous scaffolds allowed the cells to penetrate and organize themselves within the porous scaffold. This cell infiltration was evident from fluorescent microscopic images at various scaffold depths in the random orientation groups, while minimum infiltration was observed in the aligned nanofibrous scaffolds. Similar findings on the effect of fiber orientation of electrospun scaffolds on pore size and cellular infiltration have been reported by others [29,43].

3.2.2. Image processing to estimate cell distribution and orientation—To

quantitate the cell distribution/orientation on the image plane, we used image processing methods, exploiting the color information to segment the nuclei (blue) and cytoplasm (green) and extract shape and topographical features (Fig. 9 and Fig. 1-S in Supplemental information). For estimating the approximate positions of the cells, we segmented the nuclei, by applying Harmonic K-Harmonic Means (KHM) clustering to the values of the blue chromatic channel of the input images (Fig. 1-S, top row—blue, Supplemental information) [44,45]. For the KHM clustering, we used a varying number of pre-determined output clusters: for each KHM repetition, we obtained the probability of each pixel belonging to a nucleus region, converting cluster memberships into probabilities [46]. The final probability field was thresholded, the nuclei regions were extracted, and ellipses were fitted (using least squares) at the segmented nuclei positions. The features of the ellipses, namely orientation, length ratio major/minor axis, and size, determined the spatial coordination of the nuclei. For the segmentation of cytoplasm regions, we used the green chromatic channel of the input color image in a similar way by estimating the probability of every pixel belonging to a cytoplasm region (Fig. 1-S, bottom row—green, Supplemental information). The boundaries of the cytoplasm regions can provide the desired alignment information using tensor voting [47]. Essentially, the results of second-order tensor voting provided a field that corresponds to saliency and orientation of the estimated region boundaries. We compared the tensor voting fields of cells seeded on aligned and randomly oriented fibrous scaffolds, after estimating their probability maps and thresholding them successively with four values in [0.80, . . . 0.95] (Fig. 9A). Low feature saliency is illustrated in the random (“r”) cell orientation, and high saliency regions are observed in the aligned (“a”) cell image, over all

probability thresholds. To show this result quantitatively, we plotted the normalized (in [0,1]) average saliency voting values (over the entire images), for different thresholds for the cytoplasm probability maps (Fig. 9B). There are distinctly higher saliency values for the aligned cell coordination in the image case, as opposed to the much lower orientation saliency in the randomly coordinated cells. Finally, using the procedure above, we examined 20 cell images (10 for random and 10 for aligned scaffolds) and we calculated the average saliency over the entire images, for different probability thresholds in [0.7,1.0] (Fig. 9C). The average orientation saliency which was normalized in [0,1] corresponds to the average saliency value for a cell image over all thresholds in the examined range. There was a clear distinction between the cell coordination produced by aligned versus the random fiber orientations.

3.2.3. Capillary formation as a function of growth factor concentration and scaffold architecture—The effect of scaffold architecture and growth factor delivery on capillary formation was assessed in a 3-D in vitro angiogenesis assay. The scaffolds were loaded with two different bFGF concentrations (50 or 100 ng per scaffold) and the number of sprouts and their lengths were determined over six days. The overall length of sprouts ranged from $230\ \mu\text{m} \pm 44$ (i.e. aligned fiber scaffolds loaded with 100 ng bFGF) to $125\ \mu\text{m} \pm 58$ (i.e. non-loaded random fiber scaffolds). Capillary formation (i.e. sprouts length and number of sprouts per bead) was a function of bFGF loading concentration for both types of electrospun scaffolds (Figs. 10 and 11). At days 3 and 6, there was a significant increase in sprout length for both aligned and random nanofibrous, bFGF-releasing scaffolds ($p < 0.05$) in comparison to control groups (i.e. non-loaded scaffolds). Fiber orientation (i.e. scaffolds with aligned or random fiber orientation) had a statistically significant effect on sprout length especially on day 3 (Fig. 10A). Specifically, scaffolds with aligned fiber configuration (loaded with 100 ng of bFGF) had a 28% increase in sprout length in comparison to scaffolds with random fiber configuration (loaded with 100 ng of bFGF) ($p = 0.0012$). This could be attributed to the differences on the swelling profiles between the aligned and random scaffolds, which in turn could influence cytokine release profiles [48–51]. In fact when we determined the swelling profiles of aligned and random scaffolds, there was a 13% increase in the rate of swelling for aligned versus random (Fig. 12A). Furthermore, based on ELISA measurements there was an 11% increase on the amount of bFGF being released from the scaffolds with aligned fiber orientation in comparison to the amount of bFGF being released from scaffolds with random fiber orientation over a 24 h period (Fig. 12B).

Sprout number per bead was also a function of bFGF concentration with a peak average number of 11 ± 1 sprouts per bead at day 3, a 50% increase in sprout number for both (aligned or random) bFGF releasing scaffolds ($p < 0.05$) compared to the control groups (Fig. 10B). Conversely, at day 6 there was a 3.6-fold reduction for all groups in the number of sprouts per bead in comparison to day 3. The decrease in sprout number was attributed to bFGF exhaustion resulting in capillary regression as well as the interconnection/fusion of adjacent sprouts [52,53].

4. Conclusions

In this study, we described a novel electrospinning approach to fabricate bFGF-loaded nanofibrous scaffolds with patterned fiber architecture and assessed their ability to direct cellular behavior and morphology. Electrospun gelatin scaffolds with variable fiber orientations were developed by dynamically controlling the electrostatic field during the electrospinning process. This was achieved by utilizing a series of electrodes whose polarity were controlled with a high voltage relay switch. Fiber dimensions and scaffold degradation were tailored by controlling the fabrication parameters (i.e. needle-to-collector distance,

electric field potential, feed rate, glutaraldehyde crosslinking time, etc.). HUVEC adhesion and orientation was highly dependent on scaffold fiber orientation and morphology. Development of long extended filopodia was observed in cells seeded onto aligned scaffolds. This is particularly important since the formation of new vessels in response to pro-angiogenic growth factor stimulation or matrix rearrangement is associated with the activation of quiescent endothelial cells which in part involves changes in cell morphology (i.e. elongation) and the formation of new contacts with the underlying substrate [54]. In a 3-D angiogenic assay, capillary formation (i.e. sprouts length and number of sprouts per bead) was a function of bFGF loading concentration for both types of electrospun scaffolds (i.e. aligned or random fiber orientation). We are currently investigating the chemical immobilization of bFGF onto the electrospun scaffolds and the effect of gradient growth factor distribution on vessel direction.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Appendix A. Figures with essential colour discrimination

Certain figures in this article, particularly Figure 9, are difficult to interpret in black and white. The full colour images can be found in the on-line version, at doi:10.1016/j.actbio.2011.12.008.

Appendix B. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.actbio.2011.12.008.

References

1. Zisch AH, Lutolf MP, Hubbell JA. Biopolymeric delivery matrices for angiogenic growth factors. *Cardiovasc Pathol.* 2003; 12:295–310. [PubMed: 14630296]
2. Biondi M, Ungaro F, Quaglia F, Netti PA. Controlled drug delivery in tissue engineering. *Adv Drug Delivery Rev.* 2008; 60:229–42.
3. Freedman SB, Isner JM. Therapeutic angiogenesis for ischemic cardiovascular disease. *J Mol Cell Cardiol.* 2001; 33:379–93. [PubMed: 11181008]
4. Hockel M, Schlenger K, Doctrow S, Kissel T, Vaupel PP. Therapeutic angiogenesis. *Arch Surg.* 1993; 128:423–9. [PubMed: 7681277]
5. Phelps EA, Garcia AJ. Update on therapeutic vascularization strategies. *Regen Med.* 2009; 4:65–80. [PubMed: 19105617]
6. Spadaccio C, Chello M, Trombetta M, Rainer A, Toyoda Y, Genovese JA. Drug releasing systems in cardiovascular tissue engineering. *J Cell Mol Med.* 2009; 13:422–39. [PubMed: 19379142]
7. Eble JA, Niland S. The extracellular matrix of blood vessels. *Curr Pharm Des.* 2009; 15:1385–400. [PubMed: 19355976]
8. Chien KR, Domian IJ, Parker KK. Cardiogenesis and the complex biology of regenerative cardiovascular medicine. *Science.* 2008; 322:1494–7. [PubMed: 19056974]

9. Krenning G, Moonen JR, van Luyn MJ, Harmsen MC. Generating new blood flow: integrating developmental biology and tissue engineering. *Trends Cardiovasc Med*. 2008; 18:312–23. [PubMed: 19345319]
10. LeBleu VS, Macdonald B, Kalluri R. Structure and function of basement membranes. *Exp Biol Med* (Maywood). 2007; 232:1121–9. [PubMed: 17895520]
11. de Mel A, Jell G, Stevens MM, Seifalian AM. Biofunctionalization of biomaterials for accelerated in situ endothelialization: a review. *Biomacromolecules*. 2008; 9:2969–79. [PubMed: 18831592]
12. Spinale FG. Myocardial matrix remodeling and the matrix metalloproteinases: influence on cardiac form and function. *Physiol Rev*. 2007; 87:1285–342. [PubMed: 17928585]
13. Vogel V, Sheetz MP. Cell fate regulation by coupling mechanical cycles to biochemical signaling pathways. *Curr Opin Cell Biol*. 2009; 21:38–46. [PubMed: 19217273]
14. Friedland JC, Lee MH, Boettiger D. Mechanically activated integrin switch controls alpha5 beta1 function. *Science*. 2009; 323:642–4. [PubMed: 19179533]
15. Layman H, Sacasa M, Murphy AE, Murphy AM, Pham SM, Andreopoulos FM. Co-delivery of FGF-2 and G-CSF from gelatin-based hydrogels as angiogenic therapy in a murine critical limb ischemic model. *Acta Biomater*. 2009; 5:230–9. [PubMed: 18713669]
16. Layman H, Rahnama-Azar AA, Pham SM, Tsechenakis G, Andreopoulos FM. Synergistic angiogenic effect of codelivering fibroblast growth factor 2 and granulocyte-colony stimulating factor from fibrin scaffolds and bone marrow transplantation in critical limb ischemia. *Tissue Eng Part A*. 2011; 17:243–54. [PubMed: 20712534]
17. Williams DF. To engineer is to create: the link between engineering and regeneration. *Trends Biotechnol*. 2006; 24:4–8. [PubMed: 16289395]
18. Ghanaati S, Webber MJ, Unger RE, Orth C, Hulvat JF, Kiehna SE, et al. Dynamic in vivo biocompatibility of angiogenic peptide amphiphile nanofibers. *Biomaterials*. 2009; 30:6202–12. [PubMed: 19683342]
19. Rajangam K, Behanna HA, Hui MJ, Han X, Hulvat JF, Lomasney JW, et al. Heparin binding nanostructures to promote growth of blood vessels. *Nano Lett*. 2006; 6:2086–90. [PubMed: 16968030]
20. Rieux AD, Ucakar B, Mupendwa BP, Colau D, Feron O, Carmeliet P, et al. 3D systems delivering VEGF to promote angiogenesis for tissue engineering. *J Control Release*. 2010; 150:272–8. [PubMed: 21130820]
21. Mironov V, Kasyanov V, Markwald RR. Nanotechnology in vascular tissue engineering: from nanoscaffolding towards rapid vessel biofabrication. *Trends Biotechnol*. 2008; 26:338–44. [PubMed: 18423666]
22. Hosseinkhani H, Hosseinkhani M, Khademhosseini A, Kobayashi H, Tabata Y. Enhanced angiogenesis through controlled release of basic fibroblast growth factor from peptide amphiphile for tissue regeneration. *Biomaterials*. 2006; 27:5836–44. [PubMed: 16930687]
23. Vasita R, Katti DS. Growth factor-delivery systems for tissue engineering: a materials perspective. *Expert Rev Med Devices*. 2006; 3:29–47. [PubMed: 16359251]
24. Bouten CV, Dankers PY, Driessen-Mol A, Pedron S, Brizard AM, Baaijens FP. Substrates for cardiovascular tissue engineering. *Adv Drug Delivery Rev*. 2011; 63:221–41.
25. Xie J, Li X, Xia Y. Putting electrospun nanofibers to work for biomedical research. *Macromol Rapid Commun*. 2008; 29:1775–92. [PubMed: 20011452]
26. Rutledge GC, Fridrikh SV. Formation of fibers by electrospinning. *Adv Drug Delivery Rev*. 2007; 59:1384–91.
27. Heath DE, Lannutti JJ, Cooper SL. Electrospun scaffold topography affects endothelial cell proliferation, metabolic activity, and morphology. *J Biomed Mater Res Part A*. 2010; 94:1195–204.
28. Liu L, Dzenis YA. Analysis of the effects of the residual charge and gap size on electrospun nanofiber alignment in a gap method. *Nanotechnology*. 2008; 19:355307. [PubMed: 21828847]
29. Ma Z, He W, Yong T, Ramakrishna S. Grafting of gelatin on electrospun poly(caprolactone) nanofibers to improve endothelial cell spreading and proliferation and to control cell orientation. *Tissue Eng*. 2005; 11:1149–58. [PubMed: 16144451]

30. Qian T, Wang Y. Micro/nano-fabrication technologies for cell biology. *Med Biol Eng Comput.* 2010; 48:1023–32. [PubMed: 20490938]
31. Xu C. Aligned biodegradable nanofibrous structure: a potential scaffold for blood vessel engineering. *Biomaterials.* 2004; 25:877–86. [PubMed: 14609676]
32. Sahoo S, Ang LT, Goh JC, Toh S. Growth factor delivery through electrospun nanofibers in scaffolds for tissue engineering applications. *J Biomed Mater Res Part A.* 2009; 93:1539–50.
33. Zhang X, Baughman CB, Kaplan DL. In vitro evaluation of electrospun silk fibroin scaffolds for vascular cell growth. *Biomaterials.* 2008; 29:2217–27. [PubMed: 18279952]
34. Dersch R, Liu T, Schaper AK, Greiner A, Wendorff JH. Electrospun nanofibers: Internal structure and intrinsic orientation. *J Polym Sci. Part A. Polym Chem.* 2003; 41:545–53.
35. Khil M, Bhattarai SR, Kim H, Kim S, Lee K. Novel fabricated matrix via electrospinning for tissue engineering. *J Biomed Mater Res. Part B. Appl Biomater.* 2005; 72B:117–24. [PubMed: 15389495]
36. Theron A, Zussman E, Yarin AL. Electrostatic field-assisted alignment of electrospun nanofibres. *Nanotechnology.* 2001; 12:384–90.
37. Katta P, Alessandro M, Ramsier RD, Chase CG. Continuous electrospinning of aligned polymer nanofibers onto a wire drum collector. *Nano Lett.* 2004; 4:2215–8.
38. Li D, Wang Y, Xia Y. Electrospinning of polymeric and ceramic nanofibers as uniaxially aligned arrays. *Nano Lett.* 2003; 3:1167–71.
39. Sundaray B, Subramanian V, Natarajan TS, Xiang R, Chang C, Fann W. Electrospinning of continuous aligned polymer fibers. *Appl Phys Lett.* 2004; 84:1222–5.
40. Laham RJ, Garcia L, Baim DS, Post M, Simons M. Therapeutic angiogenesis using basic fibroblast growth factor and vascular endothelial growth factor using various delivery strategies. *Curr Interv Cardiol Rep.* 1999; 1:228–33. [PubMed: 11096629]
41. Chen RR, Silva EA, Yuen WW, Mooney DJ. Spatio-temporal VEGF and PDGF delivery patterns blood vessel formation and maturation. *Pharm Res.* 2007; 24:258–64. [PubMed: 17191092]
42. Gentile F, Tirinato L, Battista E, Causa F, Liberale C, di Fabrizio EM, et al. Cells preferentially grow on rough substrates. *Biomaterials.* 2010; 31:7205–512. [PubMed: 20637503]
43. Baker BM, Gee AO, Metter RB, Nathan AS, Marklein RA, Burdick JA, et al. The potential to improve cell infiltration in composite fiber-aligned electrospun scaffolds by the selective removal of sacrificial fibers. *Biomaterials.* 2008; 29:2348–58. [PubMed: 18313138]
44. Hamerly G, Elkan C. Alternatives to the k-Means Algorithm That Find Better Clusterings. *Proceedings int'l Conference on information and Knowledge Management.* 2002:600–607.
45. Zhang, B. Generalized K-harmonic means: boosting in unsupervised learning (HP laboratories technical report): Hewlett-Packard laboratories. 2000. Available from: <http://www.hpl.hp.com/techreports/2000/HPL-2000-137.html>
46. Platt, JC.; Smola, A.; Bartlett, P.; Scholkopf, B.; Schuurmans, D. *Advances in Large Margin Classifiers.* MIT Press; 2000. Probabilities for SV machines in advances in large margin classifiers.; p. 61-74.
47. Medioni, G.; Mordohai, P.; Nicolescu, M. The tensor voting framework.. In: Corrochano, Eduardo Bayro, editor. *Handbook of Geometric Computing: Applications in Pattern Recognition, Computer Vision, Neural Computing, and Robotics.* Springer-Verlag Berlin Heidelberg; 2005. p. 535-68.
48. Farra R, Caliceti P, Guarnieri G, Salmaso S, Carenza M, Grassi M. Temperature-sensitive hydrogels: potential therapeutic applications. *Am J Drug Delivery.* 2005; 3:239–51.
49. Young S, Wong M, Tabata Y, Mikos AG. Gelatin as a delivery vehicle for the controlled release of bioactive molecules. *J Control Release.* 2005; 109:256–74. [PubMed: 16266768]
50. Li WJ, Jiang YJ, Tuan RS. Chondrocyte phenotype in engineered fibrous matrix is regulated by fiber size. *Tissue Eng.* 2006; 12:1775–85. [PubMed: 16889508]
51. Wang, L.; Carrier, R. Biomimetic topography: bioinspired cell culture substrates and scaffolds.. In: George, A., editor. *Advances in biomimetics.* InTech; 2011. p. 453
52. Chen Z, Htay A, Dos Santos W, Gillies GT, Fillmore HL, Sholley MM, et al. In vitro angiogenesis by human umbilical vein endothelial cells (HUVEC) induced by three-dimensional co-culture with glioblastoma cells. *J Neurooncol.* 2009; 92:121–8. [PubMed: 19039523]

53. Nakatsu M, Hughes C. Chapter 4. An optimized three-dimensional In vitro model for the analysis of angiogenesis. *Meth Enzymol.* 2008; 443:65–82. [PubMed: 18772011]
54. Bondar B, Fuchs S, Motta A, Migliaresi C, Kirkpatrick CJ. Functionality of endothelial cells on silk fibroin nets: comparative study of micro- and nanometric fibre size. *Biomaterials.* 2008; 29:561–72. [PubMed: 17942151]

Fig. 1.

(A) Diagrammatic representation of our custom-made electrospinning apparatus. The vacuum relay switch allows for controlling the polarity of the collecting electrodes to control fiber direction (SEM images C through E). (B) Graphical user interface (GUI) programmed in LabView 8.2 to control the fabrication parameters of the electrospun scaffolds. (C) A representative scanning electron microscopy image (SEM) of an electrospun scaffold (prepared from 10% gelatin B in HFIP) with random fiber orientation. (D) Scaffold with aligned fiber orientation, (E) with gridded fiber alignment, and (F) a tubular scaffold fabricated on a spinning mandrel.

Fig. 2.

(A) Histogram of fiber diameter distribution from 20 randomly selected fibers from an electrospun gelatin B scaffolds under sample B₁₅ parameters (15 kV, 10 cm N-t-C, 4 mL/hr). (B) Representative SEM image used to assess fiber diameters. Results yielded 5% of fibers diameters between 0.95–1.0 μm, 20% between 0.85–0.95 μm, 70% between 0.75–0.85 μm, and 5% between 0.65–0.75 μm in diameter. (C) Histogram of fiber diameter distribution from 20 randomly selected fibers from an electrospun gelatin B scaffolds under sample F₁₅ parameters (15 kV, 15 cm N-t-C, 8 mL/hr). (D) Representative SEM image used to assess fiber diameters. Results yielded 15% between 1.5–2 μm, 15% between 0.95–1.5 μm, 10% between 0.55–0.65 μm, 10% between 0.45–0.55 μm, 10% between 0.35–0.45 μm, 30% between 0.25–0.35 μm, 10% between 0.15–0.25 μm in diameter.

Fig. 3. Computational modeling and comparison of “model” scaffold fiber orientations. (I): a “model” example was constructed where the four edge maps (A1–D1, top) have been manually created, with increasing orientation randomness from left to right. The bottom row in (A1–D1) shows the Hough Transform accumulation matrices (position–orientation distributions) of the detected edges for each case. (II) Covariance matrix and Chamfer distances of the four orientation distributions. (III) The “collapsed accumulation matrices”, i.e. the fiber orientation distributions for the four cases; x -axis indicates orientation (0–180°) while the y -axis values result from the summation of all values in each column of the matrices (A1–D1, bottom).

Fig. 4. Computational modeling and comparison of electrospun scaffold fiber orientation. (I–II): Modeling and comparing the fiber orientations in four SEM images obtained for our electrospun scaffold with random and aligned fiber orientation, similarly to the “model” example, at two different magnifications (A2, B2 at 1500 and C2, D2 at 300× magnification respectively). (III) Represents the fiber orientation distributions for these four cases.

Fig. 5.

Randomly deposited nanofibrous scaffolds (A) prior to crosslinking and (B) after crosslinking. SEM images of scaffold prior to crosslinking (C) and after crosslinking (D) used for assessing fiber diameters and morphology are shown. Crosslinking of 10% gelatin B nanofibers was achieved via glutaraldehyde vapor from a 25% GA solution at 2 mL per 500 μL of electrospun gelatin for one hour. (E) Bar graph of fiber diameters from all conditions, i.e. aligned not crosslinked, random not crosslinked, aligned crosslinked, and random crosslinked. Measurements were taken from three SEM images per condition, and twenty fibers per image.

Fig. 6.

(A) Degree of swelling results as a function of GA vapor exposure time. Crosslinking of sample B15 electrospun scaffolds were crosslinked for 1, 1.5, 2, and 24 h degrees of swelling ranged from of 11.25 ± 0.69 to 2.32 ± 0.48 respectively. (B) Degradation kinetics of B₁₅ gelatin B electrospun scaffolds as a function of GA vapor exposure time. Samples were degraded in 0.1 mg/mL collagenase solution in PBS. (■) 1 h, (●) 1.5 h, (▲) 2 h, and (×) 24 h crosslinking time in GA vapor. Images C, D, and E represent scaffolds crosslinked for one hour and submerged in 0.1 mg/ml collagenase solution for 1 h (C), 12 h (D), and 24 h (E).

Fig. 7. HUVEC proliferation on culture plates (A) and electrospun gelatin scaffolds with random fiber orientation (B) as a function of bFGF loading concentration. Assessment was performed after 5 days in culture using a calcein AM stain and fluorescent microscopy. Representative fluorescent images (4× magnification) of cells growing on 2-D wells (A) and electrospun scaffolds (B) with random fiber orientation loaded with 100 ng/mL of bFGF. (*) indicates statistical significance at $p < 0.05$.

Fig. 8.

Cytoskeleton arrangement of HUVECs on electrospun gelatin scaffolds. (A & D) Bare well, (B & E) Scaffolds with aligned fiber orientation, and (C & F) Scaffolds with random fiber orientation. Images A, B, and C were taken at 20 $\times$ magnification while D, E, and F were taken at 40 $\times$. 10,000 cells were added to each well containing scaffolds (1 cm diameter, 24-well plates) and the cells were incubated at 37 $^{\circ}$ C/5%CO₂ for 5 days. Following incubation, the cells were stained with Alexa Fluor 488 Phalloidin and DAPI per manufacturer's protocol, and the cell-scaffolds were imaged with a fluorescent microscope to assess the effect of the scaffold's fiber orientation on cell morphology.

Fig. 9.

(A) Second order tensor voting for modeling cell alignment (blue = low, red = high). (B) Comparison of two cell images using the normalized tensor votes, after thresholding the cytoplasm probability maps with different thresholds. (C) Average normalized orientation votes for twenty images of cells seeded on random or aligned micro fibrous scaffolds indicating a clear distinction of cell orientation.

Fig. 10.

(A) Comparison of average length of HUVEC sprouting with respect to bFGF (50 ng and 100 ng/scaffold) release between scaffolds with aligned fiber orientation and scaffolds with random fiber orientation. (B) Comparison of average number of sprouts per dextran bead with respect to bFGF (50 ng and 100 ng/scaffold) releasing scaffolds with aligned fiber orientation and scaffolds with random fiber orientation. Statistical significance was determined at $p < 0.05$ ($n = 30$ beads per group at each time point). A solid line denotes statistical significance between the control (no bFGF) and scaffolds loaded with 50 ng bFGF. A dotted line denotes statistical significance between the control (no bFGF) and scaffolds loaded with 100 ng bFGF.

Fig. 11. Light microscopic images of cell sprouting in a fibrin gel matrix in response to bFGF release from electrospun scaffolds with variable fiber orientation. Scaffolds were loaded with 100 ng of bFGF per scaffold. All images were collected at 10× magnification.

Fig. 12.

(A) Evaluation of the degree of swelling as a function of scaffold architecture (i.e. scaffolds with aligned fiber orientation vs. scaffolds with random fiber orientation). (B) The release profile of bFGF from scaffolds with aligned and random fiber orientation. Aligned and random scaffolds were electrospun under parameters B₁₅ (Table 1) and crosslinked with glutaraldehyde vapor for one hour.

Table 1

Groups of electrospun 10% and 5% gelatin B in 1,1,1,3,3,3 hexafluoro-2-propanol nanofibrous scaffolds at different parameters. Subscripts on sample ID's refer to kV used.

Group	Voltage (kV)	N-t-C (cm)	Feed rate (mL/hr)
A ₁₅	15	10	1
B ₁₅			4
C ₁₅			8
D ₁₅	15	15	1
E ₁₅			4
F ₁₅			8

Table 2

Fiber diameter averages of all gelatin B scaffolds fabricated via electrospinning at different kV (15 or 20), needle-to-collector distances (10 or 15 cm), and flow rates (1,4, and 8ml/hr). All samples were electrospun from gelatin B in 1,1,1,3,3,3 hexafluoro-2-propanol either at 10% or 5% w/v.

Group	Fiber diameter (μm)	Group	Fiber diameter (μm)
<i>10% Gelatin B in HFIP</i>			
A ₁₅	0.41 $\pm$ 0.16	A ₂₀	0.46 $\pm$ 0.13
B ₁₅	0.82 $\pm$ 0.06	B ₂₀	0.46 $\pm$ 0.26
C ₁₅	0.58 $\pm$ 0.21	C ₂₀	0.57 $\pm$ 0.27
D ₁₅	0.59 $\pm$ 0.25	D ₂₀	0.45 $\pm$ 0.19
E ₁₅	0.53 $\pm$ 0.25	E ₂₀	0.43 $\pm$ 0.35
F ₁₅	0.67 $\pm$ 0.54	F ₂₀	0.34 $\pm$ 0.34
<i>5% Gelatin B in HFIP</i>			
A ₁₅	0.09 $\pm$ 0.03	A ₂₀	0.14 $\pm$ 0.05
B ₁₅	0.11 $\pm$ 0.03	B ₂₀	0.10 $\pm$ 0.03
C ₁₅	0.13 $\pm$ 0.04	C ₂₀	0.16 $\pm$ 0.11
D ₁₅	0.21 $\pm$ 0.10	D ₂₀	0.24 $\pm$ 0.06
E ₁₅	0.28 $\pm$ 0.08	E ₂₀	0.21 $\pm$ 0.09
F ₁₅	0.24 $\pm$ 0.10	F ₂₀	0.39 $\pm$ 0.26