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Improved Cellular Infiltration in Electrospun Fiber via Engineered Porosity

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Abstract

Small pore sizes inherent to electrospun matrices can hinder efficient cellular ingrowth. To facilitate infiltration while retaining its extracellular matrix-like character, electrospinning was combined with salt leaching to produce a scaffold having deliberate, engineered delaminations. We made elegant use of a specific randomizing component of the electrospinning process, the Taylor Cone and the falling fiber beneath it, to produce a uniform, well-spread distribution of salt particles. After 3 weeks of culture, up to 4 mm of cellular infiltration was observed, along with cellular coverage of up to 70% within the delaminations. To our knowledge, this represents the first observation of extensive cellular infiltration of electrospun matrices. Infiltration appears to be driven primarily by localized proliferation rather than coordinated cellular locomotion. Cells also moved from the salt-generated porosity into the surrounding electrospun fiber matrix. Given that the details of salt deposition (amount, size, and number density) are far from optimized, the result provides a convincing illustration of the ability of mammalian cells to interact with appropriately tailored electrospun matrices. These layered structures can be precisely fabricated by varying the deposition interval and particle size conceivably to produce *in vivo*-like gradients in porosity such that the resulting scaffolds better resemble the desired final structure.

INTRODUCTION

Tissue engineering has emerged as a promising means of replacing damaged organs. Cells are seeded *in vitro* in/on a scaffold, supplied with adequate nutrients and benefit from active removal of waste products. Such scaffolds should promote appropriate cellular adhesion, proliferation, and specific levels of function. Materials used are typically biodegradable and are resorbed either after or during successful tissue regeneration. In addition, a high level of porosity is desired to enable both the efficient influx of anabolic nutrients and the outflow of catabolic wastes.

Methods such as solvent casting/particulate leaching,[1] gas forming,[2] emulsion lyophilization,[3] and phase separation[4] have been used to generate 3-dimensional scaffolds from natural and synthetic biodegradable polymers including collagen, poly(lactic acid)

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(PLA), poly(glycolic acid) (PGA), poly(lactic-*co*-glycolic acid) (PLGA), and poly(ε-caprolactone) (PCL). These scaffolds show consistent promise in promoting overall levels of cellular proliferation.

Another method producing such highly interconnected porous structures is electrospinning. First patented by Formhals in 1934,[5] this technique yields a nonwoven fiber mesh that greatly resembles extracellular matrix (ECM). Typical fiber diameters range from 10 nm to 10 μm,[6] providing a substantial surface area per unit volume, a property promoting cellular adhesion. Electrospinning allows limited control of the pore sizes found between interfiber contacts by selecting an average diameter[7] via control of spinning parameters such as solution viscosity, distance, and voltage.

PCL is a good candidate material when scaffolds require short-term load-bearing ability owing to its relatively slow degradation rate *in vivo*.[8] Scaffolds made of PLA, PGA, or PLGA exhibit shrinkage and substantial chemical degradation shortly after biological exposures involving hydrolysis.[9] PCL, in contrast, is relatively inert; phagocytosis of PCL by macrophages and giant cells occurs only once the molecular weight of the polymer is reduced to 3000 or less by nonenzymatic bulk hydrolysis of the ester linkages.[10,11] Electrospun PCL has, logically, been selected as a scaffolding for bone[12–15] and cartilage[16–18] to support cell proliferation and ECM deposition.

Paradoxically, however, the small fiber size intrinsic to electrospinning can hinder efficient cellular infiltration. Eichhorn *et al.*[7] have shown that the mean pore radius of electrospun matrices varies with fiber diameter. For example, a 100-nm fiber diameter yields a mean pore radius less than 10 nm at a relative density of 80%. The comparative size of a rounded cell—ranging from 5 to 20 μm—shows that such small pore sizes will obstruct cellular migration. For a scaffold that requires minimal cellular infiltration (e.g., a vascular graft) proliferation limited to the surface may be acceptable or even desirable. However, the thickness of human articular cartilage in the knee has been observed to range from 0.5 to 7.1 mm.[19] To achieve uniform cellular proliferation throughout such thick scaffolds, both relatively large pore sizes and extended culture periods are necessary. Further, articular cartilage can be naturally anisotropic, being composed of superficial, middle, deep, and calcified zones,[20] which will require scaffolds possessing variable porosity and architecture.

In this study, we designed and fabricated an electrospun scaffold providing localized, controllable macroscopic porosity by combining electrospinning with the well-established technique of salt leaching.[1,21,22] Specific characteristics of the two processes form a useful synergy, producing a more uniform scaffold than would normally be expected from a salt-based technique. The result facilitates anisotropic cellular infiltration while retaining the highly porous, ECM-like nature of electrospun scaffolds.

MATERIALS AND METHODS

Electrospinning

A 12 wt.% solution of PCL (M_w 65,000, Sigma-Aldrich, St. Louis, MO) in acetone (Mallinckroff Chemicals, Phillipsburg, NJ) was prepared and electrospun at 23 kV using a high voltage DC power supply (Model FC50R2, Glassman high voltage, High Bridge, NJ) with a 20-cm tip-to-substrate distance and a flow rate of 18 mL/h onto aluminum foil wrapped on a 4- ×4-in. steel plate. Salt crystals previously sieved to sizes between 90 and 106 μm were introduced into the Taylor Cone and the falling fiber beneath it as shown in Fig. 1. The amount of NaCl in each layer was approximately 0.75 g and covered approximately 10% of the surface area of the aluminum foil. Deposition of each allotment of NaCl required 30–60 s during electrospinning; the interval between each allotment was either 5 or 10 min. Electrospinning under these conditions required approximately 90 min to make approximately 5 mm thick sheets. For specific comparisons of cellular penetration, approximately 4 mm thick sheets were spun without salt incorporation utilizing otherwise identical conditions.

In specific cases, fluorescein isothiocyanate (FITC) (Sigma-Aldrich) was added (0.1 mg/mL) to the polymer–acetone solution before electrospinning to fluorescently label electrospun PCL fiber. This enabled us to observe both the electrospun fiber and the infiltrating cells in sectioned samples via fluorescent microscopy.

The deposited sheet was carefully punched with a dermal biopsy punch (Miltex, York, PA) to generate cylindrical "plugs" 3 or 6 mm in diameter and approximately 5 mm in height. The plugs were then inserted into a sealed plastic bag and this bag placed into a 45°C water bath for 10 min to achieve the partial sintering needed to prevent the extensive delamination otherwise observed after exposure to aqueous solution. The embedded salt crystals were then leached out by exposure to DI water at 37°C for 3 days; the DI water was replaced every 24 h.

Cell culture

The CFK2 cell line (obtained from Dr. Henderson's lab, Department of Medicine, McGill University, Montreal, Canada) having the phenotypic characteristics of chondrocytes derived from fetal rat calvariae[23] was used. Approximately 650,000 cells per sample placed in a 12-well tissue culture plate (Falcon, Franklin Lakes, NJ) were gravitationally seeded onto salt-leached plugs lying on their sides in each well. The seeded cells were cultured in Ham's F-12 medium (Mediatech, Herndon, VA) containing 10% fetal bovine serum (FBS; Mediatech, Herndon, VA), 1% penicillin–streptomycin (Fisher Scientific, Fair Lawn, NJ), and 1% L-glutamine (Mediatech, Herndon, VA). The culture medium was changed every other day. The samples were harvested at day 3 and week 3 before subsequent characterization.

SEM

Samples were coated with an 8 nm thick layer of osmium (OPC-80T, SPI Supplies, West Chester, PA). Samples emerging from cell culture were fixed with 10% formalin (Richard-Allen Scientific, Kalamazoo, MI) and exposed to a graded ethanol series in DI water (50%, 70%, 85%, 90%, and 100% ethanol) to achieve dehydration that was then finalized using a graded ethanol–hexamethyldisilazane (HMDS, Electron Microscopy Sciences, Hatfield, PA) series (25%, 50%, 75%, and 100% HMDS) followed by drying under a hood overnight. The dried sample was either coated directly with osmium or gently parted along the salt-generated gaps to observe cell migration before osmium coating.

Cryosection

Samples cultured for 3 weeks were fixed with 10% formalin followed by three 10-min rinses in phosphate-buffered saline (PBS, Mediatech, Herndon, VA). The fixed samples were embedded in OCT compound (Sakura Finetek, Torrance, CA) and then frozen at −80°C. The frozen samples were cut to 12-μm sections via a cryostat (CM3050S, Leica Microsystems, Bannockburn, IL) and placed onto Super Frost Plus glass slides (Fisher Scientific). The samples were stored at –80°C until staining.

Nuclear staining

The cryosectioned samples were rinsed 4 times with PBS to remove residual OCT. The cells were then permeablized with 0.2% TritonX-100 (Sigma-Aldrich) for 30 min followed by 3 rinses with PBS. The samples were stained with 0.01% 4′,6-diamidino-2-phenyl-indole dihydrochloride (DAPI, Sigma-Aldrich) in PBS containing 0.5% bovine serum albumin (BSA) for 5 min in the dark. The stained samples were observed via an Axioplan2 microscope (Carl Zeiss, Thornwood, NY).

Histochemical staining

The cryosectioned samples were rinsed 4 times with PBS. The samples were then stained using a 1.5% Safranin O (Sigma-Aldrich) reagent for 40 min. The Safranin O solution was filtered through a 0.45-μm pore size nitrocellulose membrane (Bio-Rad, Hercules, CA) before use. The stained samples were quickly rinsed several times in DI water and observed under a microscope.

RESULTS

The interaction between the dispensed salt crystals and the fibers during electrospinning is shown in Fig. 2a. Under normal circumstances the falling salt would deposit directly below the exit of the sheath in Fig. 1. Interaction with the Taylor Cone and the solidifying fiber beneath it carried falling salt out beyond the circumference of the sheath via spirally moving fibers, resulting in a uniform distribution within the deposited layer. Uniformity was dependent on the weight, and hence the size, of the crystals. Previous use of salt particles larger than 150 μm produced visibly nonuniform salt distributions concentrated directly below the sheath. Figure 2b shows electrospun fibers wrapped around an incorporated NaCl crystal. A normal electrospun morphology (rounded fibers) is observed in spite of the presence of the salt crystal.

A low-magnification scanning electron microscopy (SEM) image of a cylindrical plug containing incorporated salt crystals is shown in Fig. 3a. A partially delaminated layered structure is evident owing to the volume of incorporated particles. Gap sizes appear to range from 100 to 200 μm. Salt distribution within each delamination is relatively uniform. The side of the plug was slightly smeared because of shear forces applied during punching. Much more shallow (less than 10 μm in depth) delaminations were also produced within each layer, and these are likely a characteristic of the electrospinning process itself because no salt was being deposited at those points during the spinning. A salt crystal entrapped by fibers marked by an arrow is shown (Fig. 3b). Each delaminated layer is held together by fibers deposited during salt incorporation. These fiber meshes were strong enough, thanks in part to the limited sintering (45°C for 10 min) that caused the formation of simple “necks” between fibers, to maintain an overall monolithic structure during salt leaching and subsequent cell culture. The structure of PCL fibrous plug after salt leaching is shown in Fig. 4a. As expected, leaching did not visibly degrade the fibers but some slight swelling of the overall structure was observed. This expansion seemed to be largely due to springback resulting from the release of compressive forces applied during punching. Figure 4b shows partially delaminated layers joined by “vertically” (in this image) oriented fibers. Salt incorporation followed by leaching increased relative as-spun porosity from 79 (salt-free) to 83.2%.

The distance between each delamination can be controlled by the intervals between additions of salt crystals. A 10-min interval resulted in approximately 450 μm thick layers while 5-min intervals resulted in approximately 230 μm thick layers. The shorter (5-min) time intervals also increased the overall porosity, to 86.5%.

Figure 5 documents the various morphologies adopted by CFK2 cells seeded on the salt-leached plug at day 3. Some cells are rounded (Fig. 5a) while others display a slightly elongated morphology (Fig. 5b). ECM accumulation on the plasma membrane is apparent in Fig. 5a and b.

Figure 6 provides images taken from a stained plug cryosection after 3 weeks of culture. Cellular nuclei are blue and the FITC-labeled PCL fibers are green. The presence of cells along the deposition plane shows that they clearly infiltrated along the pores formed by the salt crystals. A few cells infiltrate “vertically” into the electrospun mesh (see the circle in Fig. 6a) but the majority appears to be enclosed within the cavities formed by salt dissolution. Figure 6b shows a magnified image of the cells (marked with a circle in Fig. 6a) infiltrating both horizontally and vertically. Compared to the salt-leached samples, the cellular infiltration into electrospun structure that did not contain salt generated pores was minimal over the same culture duration (Fig. 6c and d). The maximum infiltration we were able to observe was approximately 160 μm (see Fig. 6d).

Horizontal cross-sections of the cell-seeded plug cultured for 3 weeks were observed (Fig. 7). Even though the samples were 6 mm in diameter these cross-sections were infiltrated in the range from 35% to 70% (Fig. 7a and b). The average (from 18 layers) and standard deviation of the cell coverage was determined to be 59.5 ± 9.2% (see Table 1). Lighter colored regions (marked as regions 1 and 3 in the picture) contain cells while no cells were present in the darker colored region (marked as region 2). High-resolution images of regions 1 and 3 reveal infiltrating cells along with the presence of accumulated ECM. Cellular penetration began at the edge of the salt-generated porosity and progressed inwards (in some cases substantially).

Finally, Fig. 8 shows glycosaminoglycan (GAG) distribution in the cryosectioned sample after 3 weeks of culture using a Safranin O (red) stain. The majority of the GAG content is observed in the gaps generated by salt leaching consistent with the cell distributions shown in Figs. 6 and 7. In Fig. 8, the vertically aligned fibers that hold the delaminated layers together, and the associated adherent cells, can be clearly observed.

DISCUSSION

Electrospinning is a promising technique allowing efficient, economical production of tissue engineering scaffolds. The process produces a unique nonwoven nano- and/ or microfibrous structure that resembles natural ECM. The influence of nano- or micro-structures on cellular migration, orientation, and cytoskeletal organization has been demonstrated,[24–27] and electrospun topographies significantly enhance cellular behavior.[28]

However, as has been pointed out, pore size exponentially decreases with fiber size.[7] Zhang *et al.* reported cellular infiltration of only 48 μm using bone marrow stromal cells.[29] Van Lieshout *et al.* also showed poor penetration of human myofibroblasts into electrospun PCL compared to a knitted equivalent.[30] Li *et al.* postulate that cells on an electrospun "surface" could penetrate by enzymatic degradation of individual fibers[31] but this mechanism is improbable for relatively resistant synthetics such as PCL. Faster "degraders" such as PGA, PLA, and gelatin can be used but at the cost of poorer initial chemical and mechanical stability.

Within this framework, it seems clear that efficient initial seeding is critical for tailored cellular ingrowth into electrospun scaffolds to produce *in vivo*-like cellular distributions. Dynamic depth filtration has achieved effective seeding in other fibrous scaffolds[32,33] but would not be as successful in standard electrospun matrices owing to the small pore sizes. Stankus *et al.*[34] developed a method that simultaneously introduces cells into a scaffold simultaneous with electrospinning providing concurrent deposition of cells and fibers throughout the scaffold.

The inherent appeal of marrying salt deposition with electrospinning is that it utilizes the inherent randomness of the Taylor Cone and the falling fiber beneath it to achieve uniform results that guide subsequent cell proliferation. Simple vertical additions of salt to the surface of the collector plate would be biased based on their method of introduction. The result could not be uniform unless considerable effort went into altering the normal gravity-driven trajectory of the introduced salt particles. With the current technique, a much simpler approach succeeds in providing a uniform distribution because it interacts with the elongating, whipping fiber formed by the balance between electrostatic repulsion and solution viscosity to result in a relatively random, uniform arrangement of salt particles in the as-deposited mass. Neither solvent evaporation nor the formation of nanometer-scale fiber diameters appears to be affected by the presence of the adhering salt particles, as indicated by the insignificant changes in average fiber diameter in each delaminated layer (Table 1). In other contexts involving the use of salt as a porogen a lack of control of salt placement can result in distinct gradients in porosity and pore size. An additional consequence, poor diffusion between largely disconnected porosity, is avoided here because electrospinning is characterized by highly interconnected (albeit cell-impermeable; see Fig. 6) pores.

Figure 5 shows cells in various shapes in which they exhibit different levels of mobility.[35–37] Most surprising, given the relative inefficiency of the simple gravity-based seeding employed here, was that the nearly complete (in some cases) penetration of the salt-generated gap observed (Figs. 6 and 7). Figure 8 validates the observations of both horizontal and vertical penetration while showing that the cells have apparently retained an appropriate phenotype capable of producing glycosaminoglycan. Given that the details of salt deposition (amount size, number density) are far from optimized, the result provides a clear illustration of the ability of mammalian cells to infiltrate appropriately engineered electrospun matrices.

To our knowledge, this represents the first observation of extensive cellular infiltration of electrospun matrices. Thus it is important to examine and understand the nature of infiltration. Figure 9 shows that cell clustering can take several forms. Relatively "thick" cell populations are observed at points of initial cell seeding (Fig. 9a) at the edge of the cylinders. These represent the initial gravity-driven cellular deposition into the edge of the gap (Fig. 4b) followed by proliferation leading to the observed mass of cells. Internal to the plug we see evidence for both large numbers of cells (Fig. 9b) as well as more scattered, isolated cell populations (Fig. 9c). Infiltration was highly variable; Fig. 7a shows nearly complete penetration of a delaminated cross-section and Fig. 7b shows cell populations scattered in between areas in which fiber density apparently remains high enough such that no cells are present. The origin of these disparate populations merits discussion. Distances of 3000–6000 μm were clearly spanned by these cells. Given the apparent good adhesion of the cells to the electrospun fiber, it seems likely that infiltration is primarily driven by localized proliferation rather than cellular locomotion. The latter, at roughly 100 μm/day,[38] would not be sufficient to allow for the cross-sectional coverage observed after only 3 weeks of culture. It would be interesting to determine how much culture time or initial porosity would be needed for proliferation to lead to physical delamination of these structures. The given method of salt incorporation used here leads to variations in porosity substantial enough to allow easy access of cells in some cases (Fig. 6a and b) while minimizing access in others (Table 1). Areas in between salt deposition appear to be no more cell permeable than the as-electrospun fiber (Fig. 6c and d).

The method developed in this study provides large pores into which cells can infiltrate without first requiring fiber degradation. We showed that cells can migrate up to approximately 4 mm and can cover approximately 70% of the cross-sectional area of these 6 mm diameter plugs using simple gravitational seeding. If a dynamic seeding—like the depth-infiltration seeding developed by Li *et al.*[33]—is applied to this scaffold infiltration would likely be even more effective. This technique clearly provides considerable flexibility in scaffold design and in promoting cellular ingrowth along specific directions. Both the amount of salt and the particle size will doubtless be important. Further, the layered structure can be precisely tailored by varying the deposition interval to produce controlled gradients in pore distribution. This gradient can lead to cellular density gradient in a scaffold that better resembles the *in vivo* equivalent, for example, the zonal anisotropic structure of cartilage.

CONCLUSIONS

By combining salt leaching with electrospinning, we demonstrate a method that produces a delaminated structure in an electrospun scaffold. With this method, not only can cellular infiltration into a thick, 3-dimensional electrospun scaffold be facilitated, but scaffolds having designed, anisotropic structures can also be produced to guide cellular proliferation to better approximate targeted tissues.

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References

1. Liao CJ, Chen CF, Chen JH, Chiang SF, Lin YJ, Chang KY. Fabrication of porous biodegradable polymer scaffolds using a solvent merging/particulate leaching method. J Biomed Mater Res. 2002; 59:676. [PubMed: 11774329]
2. Kim SS, Park MS, Jeon O, Choi CY, Kim BS. Poly(lactide-co-glycolide)/hydroxyapatite composite scaffolds for bone tissue engineering. Biomaterials. 2006; 27:1399. [PubMed: 16169074]
3. Hou QP, Grijpma DW, Feijen J. Preparation of interconnected highly porous polymeric structures by a replication and freeze-drying process. J Biomed Mater Res B Appl Biomater. 2003; 67B:732. [PubMed: 14598400]
4. Tu CF, Cai Q, Yang J, Wan YQ, Bei JZ, Wang S. The fabrication and characterization of poly(lactic acid) scaffolds for tissue engineering by improved solid-liquid phase separation. Polym Adv Technol. 2003; 14:565.
5. Formhals, A. Apparatus for Producing Artificial Filaments from Material Such as Cellulose Acetate. Schreiber-Gastell, Richard; 1934.
6. Fridrikh SV, Yu JH, Brenner MP, Rutledge GC. Controlling the fiber diameter during electrospinning. Phys Rev Lett. 2003; 90:144502. [PubMed: 12731920]
7. Eichhorn SJ, Sampson WW. Statistical geometry of pores and statistics of porous nanofibrous assemblies. J Roy Soc Interface. 2005; 2:309. [PubMed: 16849188]
8. Woodward SC, Brewer PS, Moatamed F. The intracellular degradation of poly(e-caprolactone). J Biomed Mater Res. 1985; 19:437. [PubMed: 4055826]
9. Shin HJ, Lee CH, Cho IH, Kim YJ, Lee YJ, Kim IA, Park KD, Yui N, Shin JW. Electrospun PLGA nanofiber scaffolds for articular cartilage reconstruction: mechanical stability, degradation and cellular responses under mechanical stimulation *in vitro*. J Biomater Sci Polym Ed. 2006; 17:103. [PubMed: 16411602]
10. Pitt CG, Chasalow FI, Hibionada YM, Klimas DM, Schindler A. Aliphatic polyesters. I. The degradation of poly(e-caprolactone) *in vivo*. J Appl Polym Sci. 1981; 26:3779.
11. Pitt CG, Gratzl MM, Kimmel GL, Surles J, Schindler A. Aliphatic polyesters II. The degradation of poly (DL-lactide), poly (epsilon-caprolactone), and their copolymers *in vivo*. Biomaterials. 1981; 2:215. [PubMed: 7326315]
12. Fujihara K, Kotaki M, Ramakrishna S. Guided bone regeneration membrane made of polycaprolactone/calcium carbonate composite nanofibers. Biomaterials. 2005; 26:4139. [PubMed: 15664641]
13. Shin M, Yoshimoto H, Vacanti Joseph P. *In vivo* bone tissue engineering using mesenchymal stem cells on a novel electrospun nanofibrous scaffold. Tissue Eng. 2004; 10:33. [PubMed: 15009928]
14. Yoshimoto H, Shin YM, Terai H, Vacanti JP. A biodegradable nanofiber scaffold by electrospinning and its potential for bone tissue engineering. Biomaterials. 2003; 24:2077. [PubMed: 12628828]

15. Wutticharoenmongkol P, Sanchavanakit N, Pavasant P, Supaphol P. Novel bone scaffolds of electrospun polycaprolactone fibers filled with nanoparticles. J Nanosci Nanotechnol. 2006; 6:514. [PubMed: 16573054]

16. Li, W-j; Danielson, KG.; Alexander, PG.; Tuan, RS. Biological response of chondrocytes cultured in three-dimensional nanofibrous poly(e-caprolactone) scaffolds. J Biomed Mater Res A. 2003; 67A:1105. [PubMed: 14624495]

17. Li W-J, Tuli R, Huang X, Laquerriere P, Tuan RS. Multilineage differentiation of human mesenchymal stem cells in a three-dimensional nanofibrous scaffold. Biomaterials. 2005; 26:5158. [PubMed: 15792543]

18. Li WJW-J, Tuli R, Okafor C, Derfoul A, Danielson KGKG, Hall DJDJ, Tuan RSRS. A three-dimensional nanofibrous scaffold for cartilage tissue engineering using human mesenchymal stem cells. Biomaterials. 2005; 26:599. [PubMed: 15282138]

19. Kladny B, Martus P, Schiwy-Bochat KH, Weseloh G, Swoboda B. Measurement of cartilage thickness in the human knee-joint by magnetic resonance imaging using a three-dimensional gradient-echo sequence. Int Orthop. 1999; 23:264. [PubMed: 10653290]

20. Woodfield TBF, Bezemer JM, Pieper JS, van Blitterswijk CA, Riesle J. Scaffolds for tissue engineering of cartilage. Crit Rev Eukaryot Gene Express. 2002; 12:209.

21. Katoh K, Tanabe T, Yamauchi K. Novel approach to fabricate keratin sponge scaffolds with controlled pore size and porosity. Biomaterials. 2004; 25:4255. [PubMed: 15046915]

22. Lu L, Peter SJ, Lyman MD, Lai HL, Leite SM, Tamada JA, Uyama S, Vacanti JP, Langer R, Mikos AG. *In vitro* and *in vivo* degradation of porous poly(DL-lactic-co-glycolic acid) foams. Biomaterials. 2000; 21:1837. [PubMed: 10919687]

23. Bernier SM, Goltzman D. Regulation of expression of the chondrocytic phenotype in a skeletal cell line (CFK2) *in vitro*. J Bone Miner Res. 1993; 8:475. [PubMed: 8475797]

24. Flemming RG, Murphy CJ, Abrams GA, Goodman SL, Nealey PF. Effects of synthetic micro- and nano-structured surfaces on cell behavior. Biomaterials. 1999; 20:573. [PubMed: 10213360]

25. Powell HM, Kniss DA, Lannutti JJ. Nanotopographic control of cytoskeletal organization. Langmuir. 2006; 22:5087. [PubMed: 16700598]

26. Powell HM, Lannutti JJ. Nanofibrillar surfaces via reactive ion etching. Langmuir. 2003; 19:9071.

27. Von Recum AF, Van Kooten TG. The influence of microtopography on cellular response and the implications for silicone implants. J Biomater Sci Polym Ed. 1995; 7:181. [PubMed: 7654632]

28. Nam J, Huang Y, Agarwal S, Lannutti JJ. Enhanced chondrocytic cell response to an electrospun topography. Submitted.

29. Zhang Y, Ouyang H, Lim Chwee T, Ramakrishna S, Huang Z-M. Electrospinning of gelatin fibers and gelatin/ PCL composite fibrous scaffolds. J Biomed Mater Res Part B, Applied Biomat. 2005; 72:156.

30. Van Lieshout MI, Vaz CM, Rutten MCM, Peters GWM, Baaijens FPT. Electrospinning versus knitting: Two scaffolds for tissue engineering of the aortic valve. J Biomater Sci Polym Ed. 2006; 17:77. [PubMed: 16411600]

31. Li WJ, Laurencin CT, Caterson EJ, Tuan RS, Ko FK. Electrospun nanofibrous structure: A novel scaffold for tissue engineering. J Biomed Mater Res. 2002; 60:613. [PubMed: 11948520]

32. Feng Z, Teng M. Perfusion bioreactor system for human mesenchymal stem cell tissue engineering: Dynamic cell seeding and construct development. Biotechnol Bioeng. 2005; 91:482. [PubMed: 15895382]

33. Li Y, Ma T, Kniss DA, Lasky LC, Yang ST. Effects of filtration seeding on cell density, spatial distribution, and proliferation in nonwoven fibrous matrices. Biotechnol Prog. 2001; 17:935. [PubMed: 11587587]

34. Stankus JJ, Guan JJ, Fujimoto K, Wagner WR. Microintegrating smooth muscle cells into a biodegradable, elastomeric fiber matrix. Biomaterials. 2006; 27:735. [PubMed: 16095685]

35. Aszodi A, Hunziker EB, Brakebusch C, Fassler R. beta 1 integrins regulate chondrocyte rotation, G1 progression, and cytokinesis. Genes Dev. 2003; 17:2465. [PubMed: 14522949]

36. Grashoff C, Aszodi A, Sakai T, Hunziker EB, Faessler R. Integrin-linked kinase regulates chondrocyte shape and proliferation. EMBO Rep. 2003; 4:432. [PubMed: 12671688]

37. Panda DK, Miao DS, Lefebvre V, Hendy GN, Goltzman D. The transcription factor SOX9 regulates cell cycle and differentiation genes in chondrocytic CFK2 cells. J Biol Chem. 2001; 276:41229. [PubMed: 11514554]

38. Chao PH, Roy R, Mauck RL, Liu W, Valhmu WB, Hung CT. Chondrocyte translocation response to direct current electric fields. J Biomech Eng. 2000; 122:261. [PubMed: 10923294]

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1. Lee, Jung Min; Chae, Taesik; Sheikh, Faheem A.; Ju, Hyung Woo; Moon, Bo Mi; Park, Hyun Jung; Park, Ye Ri; Park, Chan Hum. Three dimensional poly(ε-caprolactone) and silk fibroin nanocomposite fibrous matrix for artificial dermis. Materials Science and Engineering: C. 2016; 68:758–767.

2. Pauly, Hannah M.; Kelly, Daniel J.; Popat, Ketul C.; Trujillo, Nathan A.; Dunne, Nicholas J.; McCarthy, Helen O.; Haut Donahue, Tammy L. Mechanical properties and cellular response of novel electrospun nanofibers for ligament tissue engineering: Effects of orientation and geometry. Journal of the Mechanical Behavior of Biomedical Materials. 2016; 61:258–270. [PubMed: 27082129]

3. Li, Dawei; Chen, Weiming; Sun, Binbin; Li, Haoxuan; Wu, Tong; Ke, Qinfei; Huang, Chen; EI-Hamshary, Hany; Al-Deyab, Salem S.; Mo, Xiumei. A Comparison of Nanoscale and Multiscale PCL/Gelatin Scaffolds Prepared by Disc-electrospinning. Colloids and Surfaces B: Biointerfaces. 2016

4. Taskin, Mehmet Berat; Xu, Ruodan; Gregersen, Hans; Nygaard, Jens Vinge; Besenbacher, Flemming; Chen, Menglin. Three-Dimensional Polydopamine Functionalized Coiled Microfibrous Scaffolds Enhance Human Mesenchymal Stem Cells Colonization and Mild Myofibroblastic Differentiation. ACS Applied Materials & Interfaces. 2016; 8(25):15864–15873. [PubMed: 27265317]

5. Mahjour, Seyed Babak; Sefat, Farshid; Polunin, Yevgeniy; Wang, Lichen; Wang, Hongjun. Improved cell infiltration of electrospun nanofiber mats for layered tissue constructs. Journal of Biomedical Materials Research Part A. 2016; 104(6):1479–1488. [PubMed: 26845076]

6. Cho, Mira; Kim, Seung-Hyun; Jin, Gyuhyung; Park, Kook In; Jang, Jae-Hyung. Salt-Induced Electrospun Patterned Bundled Fibers for Spatially Regulating Cellular Responses. ACS Applied Materials & Interfaces. 2016; 8(21):13320–13331. [PubMed: 27167566]

7. Hwang, Patrick TJ.; Lim, Dong-Jin; Fee, Timothy; Alexander, Grant C.; Tambralli, Ajay; Andukuri, Adinarayana; Tian, Liqun; Cui, Wanxing; Berry, Joel; Gilbert, Shawn R.; Jun, Ho-Wook. A bio-inspired hybrid nanosack for graft vascularization at the omentum. Acta Biomaterialia. 2016

8. Maldonado, Maricela; Ico, Gerardo; Low, Karen; Luu, Rebeccah J.; Nam, Jin. Enhanced Lineage-Specific Differentiation Efficiency of Human Induced Pluripotent Stem Cells by Engineering Colony Dimensionality Using Electrospun Scaffolds. Advanced Healthcare Materials. 2016; 5(12): 1408–1412. [PubMed: 27187808]

9. Hwang, Patrick TJ.; Murdock, Kyle; Alexander, Grant C.; Salaam, Amanee D.; Ng, Joshua I.; Lim, Dong-Jin; Dean, Derrick; Jun, Ho-Wook. Poly(ε-caprolactone)/gelatin composite electrospun scaffolds with porous crater-like structures for tissue engineering. Journal of Biomedical Materials Research Part A. 2016; 104:1017–1029.doi: 10.1002/jbma.v104.4 [PubMed: 26567028]

10. Chen, Huizhi; Peng, Yan; Wu, Shucheng; Tan, Lay. Electrospun 3D Fibrous Scaffolds for Chronic Wound Repair. Materials. 2016; 9(4):272.

11. Narayanan, Naagarajan; Jiang, Chunhui; Uzunalli, Gozde; Thankappan, Shalumon Kottappally; Laurencin, Cato T.; Deng, Meng. Polymeric Electrospinning for Musculoskeletal Regenerative Engineering. Regenerative Engineering and Translational Medicine. 2016

12. Jun, Indong; Chung, Yong-Woo; Heo, Yun-Hoe; Han, Hyung-Seop; Park, Jimin; Jeong, Hongsoo; Lee, Hyunjung; Lee, Yu Bin; Kim, Yu-Chan; Seok, Hyun-Kwang; Shin, Heungsoo; Jeon, Hojeong. Creating Hierarchical Topographies on Fibrous Platforms Using Femtosecond Laser Ablation for Directing Myoblasts Behavior. ACS Applied Materials & Interfaces. 2016; 8:3407–3417. [PubMed: 26771693]

13. Lee, Boram; Shafiq, Muhammad; Jung, Youngmee; Park, Jong-Chul; Kim, Soo Hyun. Characterization and preparation of bio-tubular scaffolds for fabricating artificial vascular grafts by combining electrospinning and a co-culture system. Macromolecular Research. 2016; 24:131–142.
14. Capulli AK, MacQueen LA, Sheehy Sean P, Parker KK. Fibrous scaffolds for building hearts and heart parts. Advanced Drug Delivery Reviews. 2016; 96:83–102. [PubMed: 26656602]
15. Horner CB, Low K, Nam J. Electrospun scaffolds for cartilage regeneration. :213–240.
16. Hong Y. Electrospun fibrous polyurethane scaffolds in tissue engineering. :543–559.
17. Clark, Elizabeth S.; Best, Cameron; Onwuka, Ekene; Sugiura, Tadahisa; Mahler, Nathan; Bolon, Brad; Niehaus, Andrew; James, Iyore; Hibino, Narutoshi; Shinoka, Toshiharu; Johnson, Jed; Breuer, Christopher K. Effect of cell seeding on neotissue formation in a tissue engineered trachea. Journal of Pediatric Surgery. 2016; 51:49–55. [PubMed: 26552897]
18. Cortez Tornello, Pablo R.; Ballarin, Florencia Montini; Caracciolo, Pablo C.; Gustavo, A. AbrahamMicro/nanofiber-based scaffolds for soft tissue engineering applications. :201–229.
19. Mao N. Nonwoven fabric filters. :273–310.
20. Yuan, Huihua; Zhou, Qihui; Li, Biyun; Bao, Min; Lou, Xiangxin; Zhang, Yanzhong. Direct printing of patterned three-dimensional ultrafine fibrous scaffolds by stable jet electrospinning for cellular ingrowth. Biofabrication. 2015; 7:045004. [PubMed: 26538110]
21. Efimov AE, Agapova OI, Parfenov VA, Pereira FDAS, Bulanova EA, Mironov VA, Agapova II. Investigating the micro- and nanostructure of microfibrous biocompatible polyurethane scaffold by scanning probe nanotomography. Nanotechnologies in Russia. 2015; 10:925–929.
22. Orr, Steven B.; Chainani, Abby; Hippensteel, Kirk J.; Kishan, Alysha; Gilchrist, Christopher; William Garrigues, N.; Ruch, David S.; Guilak, Farshid; Little, Dianne. Aligned multilayered electrospun scaffolds for rotator cuff tendon tissue engineering. Acta Biomaterialia. 2015; 24:117–126. [PubMed: 26079676]
23. Qian, Yuna; Li, Linhao; Jiang, Chao; Xu, Wei; Lv, Yonggang; Zhong, Li; Cai, Kaiyong; Yang, Li. The effect of hyaluronan on the motility of skin dermal fibroblasts in nanofibrous scaffolds. International Journal of Biological Macromolecules. 2015; 79:133–143. [PubMed: 25940528]
24. Brennan, Meadhbh Á.; Renaud, Audrey; Gamblin, Annelaure; D'Arros, Cyril; Nedellec, Steven; Trichet, Valerie; Layrolle, Pierre. 3D cell culture and osteogenic differentiation of human bone marrow stromal cells plated onto jet-sprayed or electrospun microfiber scaffolds. Biomedical Materials. 2015; 10:045019. [PubMed: 26238732]
25. Jones, Desiree; Park, DoYoung; Anghelina, Mirela; Pécot, Thierry; Machiraju, Raghu; Xue, Ruipeng; Lannutti, John J.; Thomas, Jessica; Cole, Sara L.; Moldovan, Leni; Moldovan, Nicanor I. Actin grips: Circular actin-rich cytoskeletal structures that mediate the wrapping of polymeric microfibers by endothelial cells. Biomaterials. 2015; 52:395–406. [PubMed: 25818446]
26. Maldonado, Maricela; Wong, Lauren Y.; Echeverria, Cristina; Ico, Gerardo; Low, Karen; Fujimoto, Taylor; Johnson, Jed K.; Nam, Jin. The effects of electrospun substrate-mediated cell colony morphology on the self-renewal of human induced pluripotent stem cells. Biomaterials. 2015; 50:10–19. [PubMed: 25736491]
27. Sheikh, Faheem A.; Ju, Hyung Woo; Lee, Jung Min; Moon, Bo Mi; Park, Hyun Jung; Lee, Ok Joo; Kim, Jung-Ho; Kim, Dong-Kyu; Park, Chan Hum. 3D electrospun silk fibroin nanofibers for fabrication of artificial skin. Nanomedicine: Nanotechnology, Biology and Medicine. 2015; 11:681–691.
28. Choi, Ji Suk; Kim, Hye Sung; Yoo, Hyuk Sang. Electrospinning strategies of drug-incorporated nanofibrous mats for wound recovery. Drug Delivery and Translational Research. 2015; 5:137–145. [PubMed: 25787739]
29. Kathleen, Hoffman; Drago, Skrtic; Jirun, Sun; Wojtek, Tutak. Airbrushed Composite Polymer Zr-ACP Nanofiber Scaffolds with Improved Cell Penetration for Bone Tissue Regeneration. Tissue Engineering Part C: Methods. 2015; 21(3):284–291. [PubMed: 25128269]
30. Frohbergh Michael E, Anya Katsman, Mondrinos Mark J, Stabler Collin T, Hankenson Kurt D, Oristaglio Jeffrey T, Lelkes Peter I. Osseointegrative Properties of Electrospun Hydroxyapatite-Containing Nanofibrous Chitosan Scaffolds. Tissue Engineering Part A. 2015; 21(5–6):970–981. [PubMed: 25336062]

31. Pu, Juan; Yuan, Falei; Li, Song; Komvopoulos, Kyriakos. Electrospun bilayer fibrous scaffolds for enhanced cell infiltration and vascularization in vivo. Acta Biomaterialia. 2015; 13:131–141. [PubMed: 25463495]

32. Zhao, Wen; Liu, Wenlong; Li, Jiaojiao; Lin, Xiao; Wang, Ying. Preparation of animal polysaccharides nanofibers by electrospinning and their potential biomedical applications. Journal of Biomedical Materials Research Part A. 2015; 103:807–818.doi: 10.1002/jbm.v103.2 [PubMed: 24733749]

33. Khorshidi, Sajedeh; Solouk, Atefeh; Mirzadeh, Hamid; Mazinani, Saeedeh; Lagaron, Jose M.; Sharifi, Shahriar; Ramakrishna, Seeram. A review of key challenges of electrospun scaffolds for tissue-engineering applications. Journal of Tissue Engineering and Regenerative Medicine. 2015 n/a-n/a.

34. Mohan, Neethu; Michael, S. DetamoreBiomimetic Nanofibers for Musculoskeletal Tissue Engineering. :57–75.

35. Durham, ER.; Tronci, G.; Yang, X.; Wood, DJ.; Russell, SJ. Nonwoven scaffolds for bone regeneration. p. 45-65.

36. Vellayappan MV, Jaganathan SK, Supriyanto E. Review: unraveling the less explored flocking technology for tissue engineering scaffolds. RSC Adv. 2015; 5:73225–73240.

37. Rivet, Christopher J.; Zhou, Kun; Gilbert, Ryan J.; Finkelstein, David I.; Forsythe, John S. Cell infiltration into a 3D electrospun fiber and hydrogel hybrid scaffold implanted in the brain. Biomatter. 2015; 5:e1005527. [PubMed: 25996265]

38. Goonoo N, Bhaw-Luximon A, Rodriguez IA, Wesner D, Schönherr H, Bowlin GL, Jhurry D. Poly(ester-ether)s: III. assessment of cell behaviour on nanofibrous scaffolds of PCL, PLLA and PDX blended with amorphous PMeDX. J Mater Chem B. 2015; 3:673–687.

39. Kirchmajer DM, Gorkin R III, in het Panhuis M. An overview of the suitability of hydrogel-forming polymers for extrusion-based 3D-printing. J Mater Chem B. 2015; 3:4105–4117.

40. Sun, Bin; Jiang, Xue-Jun; Zhang, Shuchao; Zhang, Jun-Cheng; Li, Yi-Feng; You, Qin-Zhong; Long, Yun-Ze. Electrospun anisotropic architectures and porous structures for tissue engineering. J Mater Chem B. 2015; 3:5389–5410.

41. Li, Yan-Fang; Gregersen, Hans; Nygaard, Jens Vinge; Cheng, Weilu; Yu, Ying; Huang, Yudong; Dong, Mingdong; Besenbacher, Flemming; Chen, Menglin. Ultraporous nanofeatured PCL PEO microfibrous scaffolds enhance cell infiltration, colonization and myofibroblastic differentiation. Nanoscale. 2015; 7:14989–14995. [PubMed: 26308365]

42. Miller, Kristin S.; Khosravi, Ramak; Breuer, Christopher K.; Humphrey, Jay D. A hypothesis-driven parametric study of effects of polymeric scaffold properties on tissue engineered neovessel formation. Acta Biomaterialia. 2015; 11:283–294. [PubMed: 25288519]

43. Kim, Hye Sung; Yoo, Hyuk Sang. Surface-polymerized biomimetic nanofibrils for the cell-directed association of 3-D scaffolds. Chem Commun. 2015; 51:306–309.

44. Hung, Ben P.; Huri, Pinar Yilgor; Temple, Joshua P.; Dorafshar, Amir; Grayson, Warren L. Craniofacial Bone. :215–230.

45. Rocco Kevin A, Maxfield Mark W, Best Cameron A, Dean Ethan W, Breuer Christopher K. In Vivo Applications of Electrospun Tissue-Engineered Vascular Grafts: A Review. Tissue Engineering Part B: Reviews. 2014; 20(6):628–640. [PubMed: 24786567]

46. Wright LD, McKeon-Fischer KD, Cui Z, Nair LS, Freeman JW. PDLA/PLLA and PDLA/PCL nanofibers with a chitosan-based hydrogel in composite scaffolds for tissue engineered cartilage. Journal of Tissue Engineering and Regenerative Medicine. 2014; 8:946–954.doi: 10.1002/term.v8.12 [PubMed: 23109502]

47. Jin, Gyuhyung; Lee, Slgirim; Kim, Seung-Hyun; Kim, Minhee; Jang, Jae-Hyung. Bicomponent electrospinning to fabricate three-dimensional hydrogel-hybrid nanofibrous scaffolds with spatial fiber tortuosity. Biomedical Microdevices. 2014; 16:793–804. [PubMed: 24972552]

48. Wade, Ryan J.; Burdick, Jason A. Advances in nanofibrous scaffolds for biomedical applications: From electrospinning to self-assembly. Nano Today. 2014; 9:722–742.

49. Nukavarapu, Syam; Kumbar, Sangamesh; Merrell, Jonathan; Laurencin, Cato. Electrospun Polymeric Nanofiber Scaffolds for Tissue Regeneration. :229–254.

50. William Garrigues N, Little Dianne, Sanchez-Adams Johannah, Ruch David S, Guilak Farshid. Electrospun cartilage-derived matrix scaffolds for cartilage tissue engineering. Journal of Biomedical Materials Research Part A. 2014; 102:3998–4008.doi: 10.1002/jbm.a.v102.11 [PubMed: 24375991]
51. Liu, Shuqiong; He, Zhihang; Xu, Guojie; Xiao, Xiufeng. Fabrication of polycaprolactone nanofibrous scaffolds by facile phase separation approach. Materials Science and Engineering: C. 2014; 44:201–208. [PubMed: 25280697]
52. Bonvallet Paul P, Culpepper Bonnie K, Bain Jennifer L, Schultz Matthew J, Thomas Steven J, Bellis Susan L. Microporous Dermal-Like Electrospun Scaffolds Promote Accelerated Skin Regeneration. Tissue Engineering Part A. 2014; 20(17–18):2434–2445. [PubMed: 24568584]
53. Domingues, Rui MA.; Gomes, Manuela E.; Reis, Rui L. The Potential of Cellulose Nanocrystals in Tissue Engineering Strategies. Biomacromolecules. 2014; 15:2327–2346. [PubMed: 24914454]
54. Woods, Ian; Flanagan, Thomas C. Electrospinning of biomimetic scaffolds for tissue-engineered vascular grafts: threading the path. Expert Review of Cardiovascular Therapy. 2014; 12:815–832. [PubMed: 24903895]
55. Jia, Chao; Mahjour, Seyed; Chan, Lawrence; Hou, Da-wei; Wang, Hongjun. Biomimetic Design of Extracellular Matrix-Like Substrate for Tissue Regeneration. :199–230.
56. Castro, Nathan; O'Brien, Joseph; Zhang, Lijie. Engineering Functional Bone Grafts for Craniofacial Regeneration. :589–620.
57. Sun B, Long YZ, Zhang HD, Li MM, Duvail JL, Jiang XY, Yin HL. Advances in three-dimensional nanofibrous macrostructures via electrospinning. Progress in Polymer Science. 2014; 39:862–890.
58. Wang, Hongjun; Xu, Meng. A Biomimetic Approach toward the Fabrication of Epithelial-like Tissue. :175–194.
59. Ondeck, Matthew G.; Engler, Adam J. Dynamic Materials Mimic Developmental and Disease Changes in Tissues. :25–43.
60. Tyler Nelson M, Pattanaik Lagnajit, Allen Marcia, Gerbich Matthew, Hux Kelvin, Allen Matthew, Lannutti John J. Recrystallization improves the mechanical properties of sintered electrospun polycaprolactone. Journal of the Mechanical Behavior of Biomedical Materials. 2014; 30:150–158. [PubMed: 24295966]
61. Haghi A, Zaikov G. Polymeric Nanosystems. :105–145.
62. Shabani, Iman; Haddadi-Asl, Vahid; Soleimani, Masoud; Seyedjafari, Ehsan; Hashemi, Seyed Mahmoud. Ion-Exchange Polymer Nanofibers for Enhanced Osteogenic Differentiation of Stem Cells and Ectopic Bone Formation. ACS Applied Materials & Interfaces. 2014; 6:72–82. [PubMed: 24328219]
63. Xu, Helan; Yang, Yiqi. 3D Electrospun Fibrous Structures from Biopolymers. :103–126.
64. Rampichová, Michala; Buzgo, Matej; Chvojka, Jiří.; Prosecká, Eva; Kofroňová, Olga; Amler, Evžen. Cell penetration to nanofibrous scaffolds. Cell Adhesion & Migration. 2014; 8:36–41. [PubMed: 24429388]
65. Simonet, Marc; Stingelin, Natalie; Wismans, Joris GF.; Oomens, Cees WJ.; Driessen-Mol, Anita; Baaijens, Frank PT. Tailoring the void space and mechanical properties in electrospun scaffolds towards physiological ranges. J Mater Chem B. 2014; 2:305–313.
66. Li, Song; Sengupta, Debanti; Chien, Shu. Vascular tissue engineering: from in vitro to in situ. Wiley Interdisciplinary Reviews: Systems Biology and Medicine. 2014; 6:61–76.doi: 10.1002/wsbm.2014.6.issue-1 [PubMed: 24151038]
67. Pilehrood, Mohammad Kazemi; Dilamian, Mandana; Mirian, Mina; Sadeghi-Aliabadi, Hojjat; Maleknia, Laleh; Nousiainen, Pertti; Harlin, Ali. Nanofibrous Chitosan-Polyethylene Oxide Engineered Scaffolds: A Comparative Study between Simulated Structural Characteristics and Cells Viability. BioMed Research International. 2014; 2014:1–9.
68. Jeong, Sung In; Burns, Nancy A.; Bonino, Christopher A.; Kwon, Il Keun; Khan, Saad A.; Alsberg, Eben. Improved cell infiltration of highly porous 3D nanofibrous scaffolds formed by combined fiber fiber charge repulsions and ultrasonication. J Mater Chem B. 2014; 2:8116–8122.

69. Li, Longchao; Ge, Juan; Wang, Ling; Guo, Baolin; Ma, Peter X. Electroactive nanofibrous biomimetic scaffolds by thermally induced phase separation. Journal of Materials Chemistry B. 2014; 2:6119.

70. Lee, Hyeongjin; Jang, Chul Ho; Kim, Geun Hyung. A polycaprolactone/silk-fibroin nanofibrous composite combined with human umbilical cord serum for subacute tympanic membrane perforation; an in vitro and in vivo study. Journal of Materials Chemistry B. 2014; 2:2703.

71. Chainani, Abby; Hippensteel, Kirk J.; Kishan, Alysha; William Garrigues, N.; Ruch, David S.; Guilak, Farshid; Little, Dianne. Multilayered Electrospun Scaffolds for Tendon Tissue Engineering. Tissue Engineering Part A. 2013; 19(23–24):2594–2604. [PubMed: 23808760]

72. Wang, Kai; Xu, Meng; Zhu, Meifeng; Su, Hong; Wang, Hongjun; Kong, Deling; Wang, Lianyong. Creation of macropores in electrospun silk fibroin scaffolds using sacrificial PEO-microparticles to enhance cellular infiltration. Journal of Biomedical Materials Research Part A. 2013; 101:3474–3481.doi: 10.1002/jbm.a.v101.12 [PubMed: 23606405]

73. Yuan, Wenjie; Feng, Yakai; Wang, Heyun; Yang, Dazhi; An, Bo; Zhang, Wencheng; Khan, Musammir; Guo, Jintang. Hemocompatible surface of electrospun nanofibrous scaffolds by ATRP modification. Materials Science and Engineering: C. 2013; 33:3644–3651. [PubMed: 23910260]

74. Kane, Robert J.; Peter, X. MaBiomimetic Nanofibrous Scaffolds for Bone Tissue Engineering Applications. :69–89.

75. Liu, Wenying; Thomopoulos, Stavros; Xia, Younan. Electrospun Nanofibers for Regenerative Medicine. :265–295.

76. Nguyen, Luong TH.; Chen, Shilin; Elumalai, Naveen K.; Prabhakaran, Molamma P.; Zong, Yun; Vijila, Chellappan; Allakhverdiev, Suleyman I.; Ramakrishna, Seeram. Biological, Chemical, and Electronic Applications of Nanofibers. Macromolecular Materials and Engineering. 2013; 298:822–867.doi: 10.1002/mame.v298.8

77. Joshi, Vaidehi S.; Lei, Nan Ye; Walthers, Christopher M.; Wu, Benjamin; Dunn, James CY. Macroporosity enhances vascularization of electrospun scaffolds. Journal of Surgical Research. 2013; 183:18–26. [PubMed: 23769018]

78. Hu, Wei-Wen; Yu, Hsing-Ning. Coelectrospinning of chitosan/alginate fibers by dual-jet system for modulating material surfaces. Carbohydrate Polymers. 2013; 95:716–727. [PubMed: 23648033]

79. Farrugia, Brooke L.; Brown, Toby D.; Upton, Zee; Hutmacher, Dietmar W.; Dalton, Paul D.; Dargaville, Tim R. Dermal fibroblast infiltration of poly(ε-caprolactone) scaffolds fabricated by melt electrospinning in a direct writing mode. Biofabrication. 2013; 5:025001. [PubMed: 23443534]

80. Cheng, Qian; Lee, Benjamin L-P.; Komvopoulos, Kyriakos; Li, Song. Engineering the Microstructure of Electrospun Fibrous Scaffolds by Microtopography. Biomacromolecules. 2013; 14:1349–1360. [PubMed: 23534553]

81. Wang, Feng; Li, Zhenqing; Guan, Jianjun. Fabrication of mesenchymal stem cells-integrated vascular constructs mimicking multiple properties of the native blood vessels. Journal of Biomaterials Science, Polymer Edition. 2013; 24:769–783. [PubMed: 23594067]

82. Sreerekha, Perumcherry Raman; Menon, Deepthy; Nair, Shantikumar V.; Chennazhi, Krishna Prasad. Fabrication of Electrospun Poly (Lactide-co-Glycolide) Fibrin Multiscale Scaffold for Myocardial Regeneration In Vitro. Tissue Engineering Part A. 2013; 19(7–8):849–859. [PubMed: 23083104]

83. Kim, Hong Nam; Jiao, Alex; Hwang, Nathaniel S.; Kim, Min Sung; Kang, Do Hyun; Kim, Deok-Ho; Suh, Kahp-Yang. Nanotopography-guided tissue engineering and regenerative medicine. Advanced Drug Delivery Reviews. 2013; 65:536–558. [PubMed: 22921841]

84. Bosworth, Lucy A.; Turner, Lesley-Anne; Cartmell, Sarah H. State of the art composites comprising electrospun fibres coupled with hydrogels: a review. Nanomedicine: Nanotechnology, Biology and Medicine. 2013; 9:322–335.

85. Zander, Nicole. Hierarchically Structured Electrospun Fibers. Polymers. 2013; 5:19–44.

86. Li, Huaqiong; Wong, Yee Shan; Wen, Feng; Ng, Kee Woei; Ng, Gary Ka Lai; Venkatraman, Subbu S.; Boey, Freddy Yin Chiang; Tan, Lay Poh. Human Mesenchymal Stem-Cell Behaviour On Direct Laser Micropatterned Electrospun Scaffolds with Hierarchical Structures. Macromolecular Bioscience. 2013; 13:299–310. [PubMed: 23233197]

87. Ionescu, Lara C.; Mauck, Robert L. Porosity and Cell Preseeding Influence Electrospun Scaffold Maturation and Meniscus Integration In Vitro. Tissue Engineering Part A. 2013; 19(3–4):538–547. [PubMed: 22994398]

88. Rampichová M, Chvojka J, Buzgo M, Prosecká E, Mikeš P, Vysloužilová L, Tvrdík D, Kochová P, Gregor T, Lukáš D, Amler E. Elastic three-dimensional poly (ε-caprolactone) nanofibre scaffold enhances migration, proliferation and osteogenic differentiation of mesenchymal stem cells. Cell Proliferation. 2013; 46:23–37. [PubMed: 23216517]

89. Rim, Nae Gyune; Shin, Choongsoo S.; Shin, Heungsoo. Current approaches to electrospun nanofibers for tissue engineering. Biomedical Materials. 2013; 8:014102. [PubMed: 23472258]

90. Vaquette, Cédryck; Cooper-White, Justin. A simple method for fabricating 3-D multilayered composite scaffolds. Acta Biomaterialia. 2013; 9:4599–4608. [PubMed: 22902817]

91. Zander, Nicole E.; Orlicki, Joshua A.; Rawlett, Adam M.; Beebe, Thomas P. Electrospun polycaprolactone scaffolds with tailored porosity using two approaches for enhanced cellular infiltration. Journal of Materials Science: Materials in Medicine. 2013; 24:179–187. [PubMed: 23053801]

92. Kai, Dan; Jin, Guorui; Prabhakaran, Molamma P.; Ramakrishna, Seeram. Electrospun synthetic and natural nanofibers for regenerative medicine and stem cells. Biotechnology Journal. 2013; 8:59–72. [PubMed: 23139231]

93. Walther, Anja; Hoyer, Birgit; Springer, Armin; Mrozik, Birgit; Hanke, Thomas; Cherif, Chokri; Pompe, Wolfgang; Gelinsky, Michael. Novel Textile Scaffolds Generated by Flock Technology for Tissue Engineering of Bone and Cartilage. Materials. 2012; 5:540–557.

94. Izadifar, Zohreh; Chen, Xiongbiao; Kulyk, William. Strategic Design and Fabrication of Engineered Scaffolds for Articular Cartilage Repair. Journal of Functional Biomaterials. 2012; 3:799–838. [PubMed: 24955748]

95. Khadka, Dhan B.; Haynie, Donald T. Protein- and peptide-based electrospun nanofibers in medical biomaterials. Nanomedicine: Nanotechnology, Biology and Medicine. 2012; 8:1242–1262.

96. Wade, Ryan J.; Burdick, Jason A. Engineering ECM signals into biomaterials. Materials Today. 2012; 15:454–459.

97. Lee, Jongman; Yoo, James J.; Atala, Anthony; Lee, Sang Jin. The effect of controlled release of PDGF-BB from heparin-conjugated electrospun PCL/gelatin scaffolds on cellular bioactivity and infiltration. Biomaterials. 2012; 33:6709–6720. [PubMed: 22770570]

98. Leung, Linus H.; Naguib, Hani. Novel fabrication technique for three-dimensional micropatterned electrospun poly(DL-lactide-co-glycolide) acid. Journal of Applied Polymer Science. 2012; 125:E61–E70.

99. Schofer, Markus D.; Tünnermann, Lisa; Kaiser, Hendric; Roessler, Philip P.; Theisen, Christina; Heverhagen, Johannes T.; Hering, Jacqueline; Voelker, Maximilian; Agarwal, Seema; Efe, Turgay; Fuchs-Winkelmann, Susanne; Paletta, Jürgen RJ. Functionalisation of PLLA nanofiber scaffolds using a possible cooperative effect between collagen type I and BMP-2: impact on colonization and bone formation in vivo. Journal of Materials Science: Materials in Medicine. 2012; 23:2227–2233. [PubMed: 22718044]

100. Zhang, Zhanpeng; Hu, Jiang; Ma, Peter X. Nanofiber-based delivery of bioactive agents and stem cells to bone sites. Advanced Drug Delivery Reviews. 2012; 64:1129–1141. [PubMed: 22579758]

101. Hu, Jin-Jia; Chao, Wei-Chih; Lee, Pei-Yuan; Huang, Chih-Hao. Construction and characterization of an electrospun tubular scaffold for small-diameter tissue-engineered vascular grafts: A scaffold membrane approach. Journal of the Mechanical Behavior of Biomedical Materials. 2012; 13:140–155. [PubMed: 22854316]

102. Kim, Mihye; Hong, Bohee; Lee, Jongman; Kim, Se Eun; Kang, Seong Soo; Kim, Young Ha; Tae, Giyoong. Composite System of PLCL Scaffold and Heparin-Based Hydrogel for Regeneration of Partial-Thickness Cartilage Defects. Biomacromolecules. 2012; 13:2287–2298. [PubMed: 22758918]

103. Kang, Jae Kyeong; Lee, Mi Hee; Kwon, Byeong Ju; Kim, Hak Hee; Shim, In Kyong; Jung, Mi Ra; Lee, Seung Jin; Park, Jong-Chul. Effective layer by layer cell seeding into non-woven 3D

electrospun scaffolds of poly-L-lactic acid microfibers for uniform tissue formation. Macromolecular Research. 2012; 20:795–799.

104. Lee, Benjamin Li-Ping; Jeon, Hojeong; Wang, Aijun; Yan, Zhiqiang; Yu, Jian; Grigoropoulos, Costas; Li, Song. Femtosecond laser ablation enhances cell infiltration into three-dimensional electrospun scaffolds. Acta Biomaterialia. 2012; 8:2648–2658. [PubMed: 22522128]
105. Lee, Bit Na; Kim, Da Yeon; Kang, Hwi Ju; Kwon, Jin Seon; Park, Young Hwan; Chun, Heung Jae; Kim, Jae Ho; Lee, Hai Bang; Min, Byoung Hyun; Kim, Moon Suk. In vivo biofunctionality comparison of different topographic PLLA scaffolds. Journal of Biomedical Materials Research Part A. 2012; 100A:1751–1760.doi: 10.1002/jbm.a.v100a.7 [PubMed: 22467280]
106. Bottino, Marco C.; Thomas, Vinoy; Schmidt, Gudrun; Vohra, Yogesh K.; Chu, Tien-Min Gabriel; Kowolik, Michael J.; Janowski, Gregg M. Recent advances in the development of GTR/GBR membranes for periodontal regeneration A materials perspective. Dental Materials. 2012; 28:703–721. [PubMed: 22592164]
107. Coburn JM, Gibson M, Monagle S, Patterson Z, Elisseeff JH. Bioinspired nanofibers support chondrogenesis for articular cartilage repair. Proceedings of the National Academy of Sciences. 2012; 109:10012–10017.
108. Wang, Heyun; Feng, Yakai; An, Bo; Zhang, Wencheng; Sun, Minglin; Fang, Zichen; Yuan, Wenjie; Khan, Massuri. Fabrication of PU/PEGMA crosslinked hybrid scaffolds by in situ UV photopolymerization favoring human endothelial cells growth for vascular tissue engineering. Journal of Materials Science: Materials in Medicine. 2012; 23:1499–1510. [PubMed: 22430593]
109. Shabani, Iman; Haddadi-Asl, Vahid; Seyedjafari, Ehsan; Soleimani, Masoud. Cellular infiltration on nanofibrous scaffolds using a modified electrospinning technique. Biochemical and Biophysical Research Communications. 2012; 423(1):50–54. [PubMed: 22618233]
110. Mickova, Andrea; Buzgo, Matej; Benada, Oldrich; Rampichova, Michala; Fisar, Zdenek; Filova, Eva; Tesarova, Martina; Lukas, David; Amler, Evzen. Core/Shell Nanofibers with Embedded Liposomes as a Drug Delivery System. Biomacromolecules. 2012; 13:952–962. [PubMed: 22401557]
111. Thomas, Vinoy; Vohra, Yogesh K. Functionally-Graded Biomimetic Vascular Grafts for Enhanced Tissue Regeneration and Bio-integration. :235–273.
112. Zhong, Shaoping; Zhang, Yanzhong; Lim, Chwee Teck. Fabrication of Large Pores in Electrospun Nanofibrous Scaffolds for Cellular Infiltration: A Review. Tissue Engineering Part B: Reviews. 2012; 18(2):77–87. [PubMed: 21902623]
113. Li, Linhao; Qian, Yuna; Jiang, Chao; Lv, Yonggang; Liu, Wanqian; Zhong, Li; Cai, Kaiyong; Li, Song; Yang, Li. The use of hyaluronan to regulate protein adsorption and cell infiltration in nanofibrous scaffolds. Biomaterials. 2012; 33:3428–3445. [PubMed: 22300743]
114. Lee, Sang; Yoo, James. Guidance of Cell Adhesion, Alignment, Infiltration and Differentiation on Electrospun Nanofibrous Scaffolds. :201–217.
115. Leung, Linus H.; Fan, Stephanie; Naguib, Hani E. Fabrication of 3D electrospun structures from poly(lactide-co-glycolide acid)-nano-hydroxyapatite composites. Journal of Polymer Science Part B: Polymer Physics. 2012; 50:242–249.
116. Liu, Wenying; Thomopoulos, Stavros; Xia, Younan. Electrospun Nanofibers for Regenerative Medicine. Advanced Healthcare Materials. 2012; 1:10–25. [PubMed: 23184683]
117. McClure, Michael J.; Wolfe, Patricia S.; Simpson, David G.; Sell, Scott A.; Bowlin, Gary L. The use of air-flow impedance to control fiber deposition patterns during electrospinning. Biomaterials. 2012; 33:771–779. [PubMed: 22054536]
118. Phipps, Matthew C.; Clem, William C.; Grunda, Jessica M.; Clines, Gregory A.; Bellis, Susan L. Increasing the pore sizes of bone-mimetic electrospun scaffolds comprised of polycaprolactone, collagen I and hydroxyapatite to enhance cell infiltration. Biomaterials. 2012; 33:524–534. [PubMed: 22014462]
119. Hakamada, Yoshihiro; Ohgushi, Nao; Fujimura-Kondo, Nozomi; Matsuda, Takehisa. Electrospun Poly(γ-Benzyl-L-Glutamate) and Its Alkali-Treated Meshes: Their Water Wettability and Cell-Adhesion Potential. Journal of Biomaterials Science, Polymer Edition. 2012; 23:1055–1067. [PubMed: 21619718]

120. Diban, Nazely; Stamatialis, Dimitrios F. Functional Polymer Scaffolds for Blood Vessel Tissue Engineering. Macromolecular Symposia. 2011; 309–310:93–99.

121. McCullen SD, Gittard SD, Miller PR, Pourdeyhimi Behnam, Narayan RJ, Loboa EG. Laser Ablation Imparts Controlled Micro-Scale Pores in Electrospun Scaffolds for Tissue Engineering Applications. Annals of Biomedical Engineering. 2011; 39:3021–3030. [PubMed: 21847685]

122. Lee, Carol H.; Lim, Yong C.; Farson, Dave F.; Powell, Heather M.; Lannutti, John J. Vascular Wall Engineering Via Femtosecond Laser Ablation: Scaffolds with Self-Containing Smooth Muscle Cell Populations. Annals of Biomedical Engineering. 2011; 39:3031–3041. [PubMed: 21971965]

123. Lee, Jung Bok; Jeong, Sung In; Bae, Min Soo; Yang, Dae Hyeok; Heo, Dong Nyoung; Kim, Chun Ho; Alsberg, Eben; Kwon, Il Keun. Highly Porous Electrospun Nanofibers Enhanced by Ultrasonication for Improved Cellular Infiltration. Tissue Engineering Part A. 2011; 17(21–22): 2695–2702. [PubMed: 21682540]

124. Rnjak-Kovacina, Jelena; Weiss, Anthony S. Increasing the Pore Size of Electrospun Scaffolds. Tissue Engineering Part B: Reviews. 2011; 17(5):365–372. [PubMed: 21815802]

125. Dahlin, Rebecca L.; Kurtis Kasper, F.; Mikos, Antonios G. Polymeric Nanofibers in Tissue Engineering. Tissue Engineering Part B: Reviews. 2011; 17(5):349–364. [PubMed: 21699434]

126. Rnjak-Kovacina, Jelena; Wise, Steven G.; Li, Zhe; Maitz, Peter KM.; Young, Cara J.; Wang, Yiwei; Weiss, Anthony S. Tailoring the porosity and pore size of electrospun synthetic human elastin scaffolds for dermal tissue engineering. Biomaterials. 2011; 32:6729–6736. [PubMed: 21683438]

127. Skotak, Maciej; Ragusa, Jorge; Gonzalez, Daniela; Subramanian, Anuradha. Improved cellular infiltration into nanofibrous electrospun cross-linked gelatin scaffolds templated with micrometer-sized polyethylene glycol fibers. Biomedical Materials. 2011; 6:055012. [PubMed: 21931195]

128. Kinikoglu, Beste; Rodríguez-Cabello, José Carlos; Damour, Odile; Hasirci, Vasif. The influence of elastin-like recombinant polymer on the self-renewing potential of a 3D tissue equivalent derived from human lamina propria fibroblasts and oral epithelial cells. Biomaterials. 2011; 32:5756–5764. [PubMed: 21592566]

129. Guan, Jianjun; Wang, Feng; Li, Zhenqing; Chen, Joseph; Guo, Xiaolei; Liao, Jun; Moldovan, Nicanor I. The stimulation of the cardiac differentiation of mesenchymal stem cells in tissue constructs that mimic myocardium structure and biomechanics. Biomaterials. 2011; 32:5568–5580. [PubMed: 21570113]

130. Sundararaghavan, Harini G.; Burdick, Jason A. Gradients with Depth in Electrospun Fibrous Scaffolds for Directed Cell Behavior. Biomacromolecules. 2011; 12:2344–2350. [PubMed: 21528921]

131. Lee, Wen-Yu; Cheng, Wei-Yuan; Yeh, Yi-Chun; Lai, Chih-Huang; Hwang, Shiaw-Min; Hsiao, Chun-Wen; Huang, Chia-Wen; Chen, Mei-Chin; Sung, Hsing-Wen. Magnetically Directed Self-Assembly of Electrospun Superparamagnetic Fibrous Bundles to Form Three-Dimensional Tissues with a Highly Ordered Architecture. Tissue Engineering Part C: Methods. 2011; 17(6): 651–661. [PubMed: 21375393]

132. Vaquette, Cedryck; Cooper-White, Justin John. Increasing electrospun scaffold pore size with tailored collectors for improved cell penetration. Acta Biomaterialia. 2011; 7:2544–2557. [PubMed: 21371575]

133. Shabani, Iman; Haddadi-Asl, Vahid; Soleimani, Masoud; Seyedjafari, Ehsan; Babaeijandaghi, Farshad; Ahmadbeigi, Naser. Enhanced Infiltration and Biomineralization of Stem Cells on Collagen-Grafted Three-Dimensional Nanofibers. Tissue Engineering Part A. 2011; 17:9–10. 1209–1218.

134. Rivet CJ, Gilbert RJ. Fabrication and characterization of a hydrogel containing electrospun fibers. :1–2.

135. Nam, Jin; Johnson, Jed; Lannutti, John J.; Agarwal, Sudha. Modulation of embryonic mesenchymal progenitor cell differentiation via control over pure mechanical modulus in electrospun nanofibers. Acta Biomaterialia. 2011; 7:1516–1524. [PubMed: 21109030]

136. Szentivanyi, Andreas; Chakradeo, Tanmay; Zernetsch, Holger; Glasmacher, Birgit. Electrospun cellular microenvironments: Understanding controlled release and scaffold structure. Advanced Drug Delivery Reviews. 2011; 63:209–220. [PubMed: 21145932]
137. McCullen, Seth; Miller, Philip; Gittard, Shaun; Pourdeyhimi, Behnam; Gorga, Russell; Narayan, Roger; Loboa, Elizabeth. In situ collagen polymerization of layered cell-seeded electrospun scaffolds for bone tissue engineering applications. Tissue Engineering Part C: Methods. 2011; 26 110306233140095.
138. Whited, Bryce M.; Whitney, Jon R.; Hofmann, Matthias C.; Xu, Yong; Rylander, Marissa N. Pre-osteoblast infiltration and differentiation in highly porous apatite-coated PLLA electrospun scaffolds. Biomaterials. 2011; 32:2294–2304. [PubMed: 21195474]
139. Nerurkar, Nandan L.; Sen, Sounok; Baker, Brendon M.; Elliott, Dawn M.; Mauck, Robert L. Dynamic culture enhances stem cell infiltration and modulates extracellular matrix production on aligned electrospun nanofibrous scaffolds. Acta Biomaterialia. 2011; 7:485–491. [PubMed: 20728589]
140. Teo, Wee-Eong; Inai, Ryuji; Ramakrishna, Seeram. Technological advances in electrospinning of nanofibers. Science and Technology of Advanced Materials. 2011; 12:013002.
141. Rebollar, Esther; Cordero, Diego; Martins, Albino; Chiussi, Stefano; Reis, Rui L.; Neves, Nuno M.; León, Betty. Improvement of electrospun polymer fiber meshes pore size by femtosecond laser irradiation. Applied Surface Science. 2011; 257:4091–4095.
142. Szot, Christopher S.; Buchanan, Cara F.; Gatenholm, Paul; Rylander, Marissa Nichole; Freeman, Joseph W. Investigation of cancer cell behavior on nanofibrous scaffolds. Materials Science and Engineering: C. 2011; 31:37–42.
143. Meade K, Holley RJ, Merry CLR. Cell culture systems for stem cell research. :372–396.
144. Wright LD, Andric T, Freeman JW. Utilizing NaCl to increase the porosity of electrospun materials. Materials Science and Engineering: C. 2011; 31:30–36.
145. Simonet M, Driessen-Mol A, Baaijens FPT, Bouten CVC. Heart valve tissue regeneration. :202–224.
146. Mouthuy P-A, Ye H. Biomaterials. :23–36.
147. Hardingham T. Cartilage tissue regeneration. :111–126.
148. McCullen, Seth D.; Miller, Philip R.; Gittard, Shaun D.; Gorga, Russell E.; Pourdeyhimi, Behnam; Narayan, Roger J.; Loboa, Elizabeth G. In Situ Collagen Polymerization of Layered Cell-Seeded Electrospun Scaffolds for Bone Tissue Engineering Applications. Tissue Engineering Part C: Methods. 2010; 16(5):1095–1105. [PubMed: 20192901]
149. Singelyn, Jennifer M.; Christman, Karen L. Injectable Materials for the Treatment of Myocardial Infarction and Heart Failure: The Promise of Decellularized Matrices. Journal of Cardiovascular Translational Research. 2010; 3:478–486. [PubMed: 20632221]
150. Wright LD, Young RT, Andric T, Freeman JW. Fabrication and mechanical characterization of 3D electrospun scaffolds for tissue engineering. Biomedical Materials. 2010; 5:055006. [PubMed: 20844321]
151. Weber N, Lee Y-S, Shanmugasundaram S, Jaffe M, Arinzeh TL. Characterization and in vitro cytocompatibility of piezoelectric electrospun scaffolds. Acta Biomaterialia. 2010; 6:3550–3556. [PubMed: 20371302]
152. Ruckh, Timothy T.; Kumar, Kuldeep; Kipper, Matt J.; Popat, Ketul C. Osteogenic differentiation of bone marrow stromal cells on poly(ε-caprolactone) nanofiber scaffolds. Acta Biomaterialia. 2010; 6:2949–2959. [PubMed: 20144747]
153. Stella, John A.; D'Amore, Antonio; Wagner, William R.; Sacks, Michael S. On the biomechanical function of scaffolds for engineering load-bearing soft tissues. Acta Biomaterialia. 2010; 6:2365–2381. [PubMed: 20060509]
154. Seif-Naraghi, Sonya B.; Salvatore, Michael A.; Schup-Magoffin, Pam J.; Hu, Diane P.; Christman, Karen L. Design and Characterization of an Injectable Pericardial Matrix Gel: A Potentially Autologous Scaffold for Cardiac Tissue Engineering. Tissue Engineering Part A. 2010; 16(6):2017–2027. [PubMed: 20100033]

155. Guimarães, Ana; Martins, Albino; Pinho, Elisabete D.; Faria, Susana; Reis, Rui L.; Neves, Nuno M. Solving cell infiltration limitations of electrospun nanofiber meshes for tissue engineering applications. Nanomedicine. 2010; 5:539–554. [PubMed: 20528450]

156. Bhardwaj, Nandana; Kundu, Subhas C. Electrospinning: A fascinating fiber fabrication technique. Biotechnology Advances. 2010; 28:325–347. [PubMed: 20100560]

157. Kurpinski, Kyle T.; Stephenson, Jacob T.; Janairo, Randall Raphael R.; Lee, Hanmin; Li, Song. The effect of fiber alignment and heparin coating on cell infiltration into nanofibrous PLLA scaffolds. Biomaterials. 2010; 31:3536–3542. [PubMed: 20122725]

158. Ju, Young Min; Choi, Jin San; Atala, Anthony; Yoo, James J.; Lee, Sang Jin. Bilayered scaffold for engineering cellularized blood vessels. Biomaterials. 2010; 31:4313–4321. [PubMed: 20188414]

159. Sundararaghavan, Harini G.; Metter, Robert B.; Burdick, Jason A. Electrospun Fibrous Scaffolds with Multiscale and Photopatterned Porosity. Macromolecular Bioscience. 2010; 10:265–270.doi: 10.1002/mabi.v10:3 [PubMed: 20014198]

160. Kim, Sung Jin; Jang, Da Hyun; Park, Won Ho; Min, Byung-Moo. Fabrication and characterization of 3-dimensional PLGA nanofiber/microfiber composite scaffolds. Polymer. 2010; 51:1320–1327.

161. Gentsch, Rafael; Boysen, Bjoern; Lankenau, Andreas; Börner, Hans G. Single-Step Electrospinning of Bimodal Fiber Meshes for Ease of Cellular Infiltration. Macromolecular Rapid Communications. 2010; 31:59–64.doi: 10.1002/marc.v31:1 [PubMed: 21590837]

162. Wulkersdorfer B, Kao KK, Agopian VG, Ahn A, Dunn JC, Wu BM, Stelzner M. Bimodal Porous Scaffolds by Sequential Electrospinning of Poly(glycolic acid) with Sucrose Particles. International Journal of Polymer Science. 2010; 2010:1–9.

163. Sisson, Kristin; Zhang, Chu; Farach-Carson, Mary C.; Bruce Chase, D.; Rabolt, John F. Fiber diameters control osteoblastic cell migration and differentiation in electrospun gelatin. Journal of Biomedical Materials Research Part A. 2010 n/a-n/a.

164. Huang, Yi-You; Wang, De-Yao; Chang, Lee-Lee; Yang, Ying-Chi. Fabricating Microparticles/Nanofibers Composite and Nanofiber Scaffold with Controllable Pore Size by Rotating Multichannel Electrospinning. Journal of Biomaterials Science, Polymer Edition. 2010; 21:1503–1514. [PubMed: 20534198]

165. Patlolla A, Collins G, Livingston Arinzeh T. Solvent-dependent properties of electrospun fibrous composites for bone tissue regeneration. Acta Biomaterialia. 2010; 6:90–101. [PubMed: 19631769]

166. Veleva AN, Heath DE, Johnson JK, Nam J, Patterson C, Lannutti JJ, Cooper SL. Interactions between endothelial cells and electrospun methacrylic terpolymer fibers for engineered vascular replacements. Journal of Biomedical Materials Research Part A. 2009; 91A:1131–1139.doi: 10.1002/jbm.a.v91a:4 [PubMed: 19148926]

167. Jed Johnson M, Nowicki Oskar, Lee Carol H, Antonio Chiocca E, Viapiano Mariano S, Lawler Sean E, Lannutti John J. Quantitative Analysis of Complex Glioma Cell Migration on Electrospun Polycaprolactone Using Time-Lapse Microscopy. Tissue Engineering Part C: Methods. 2009; 15(4):531–540. [PubMed: 19199562]

168. Jang, Jun-Hyeog; Castano, Oscar; Kim, Hae-Won. Electrospun materials as potential platforms for bone tissue engineering. Advanced Drug Delivery Reviews. 2009; 61:1065–1083. [PubMed: 19646493]

169. Baker, Brendon M.; Handorf, Andrew M.; Ionescu, Lara C.; Li, Wan-Ju; Mauck, Robert L. New directions in nanofibrous scaffolds for soft tissue engineering and regeneration. Expert Review of Medical Devices. 2009; 6:515–532. [PubMed: 19751124]

170. Zucchelli A, Fabiani D, Gualandi C, Focarete ML. An innovative and versatile approach to design highly porous, patterned, nanofibrous polymeric materials. Journal of Materials Science. 2009; 44:4969–4975.

171. Mauck, Robert L.; Baker, Brendon M.; Nerurkar, Nandan L.; Burdick, Jason A.; Li, Wan-Ju; Tuan, Rocky S.; Elliott, Dawn M. Engineering on the Straight and Narrow: The Mechanics of Nanofibrous Assemblies for Fiber-Reinforced Tissue Regeneration. Tissue Engineering Part B: Reviews. 2009; 15(2):171–193. [PubMed: 19207040]

172. Zhang, Kai; Wang, Xuefen; Jing, Dazheng; Yang, Yin; Zhu, Meifang. Bionic electrospun ultrafine fibrous poly(L-lactic acid) scaffolds with a multi-scale structure. Biomedical Materials. 2009; 4:035004. [PubMed: 19439825]

173. Shabani, Iman; Haddadi-Asl, Vahid; Seyedjafari, Ehsan; Babaeijandaghi, Farshad; Soleimani, Masoud. Improved infiltration of stem cells on electrospun nanofibers. Biochemical and Biophysical Research Communications. 2009; 382:129–133. [PubMed: 19265673]

174. Drilling, Sarah; Gaumer, Jeremy; Lannutti, John. Fabrication of burst pressure competent vascular grafts via electrospinning: Effects of microstructure. Journal of Biomedical Materials Research Part A. 2009; 88A:923–934.doi: 10.1002/jbm.a.v88a:4 [PubMed: 18384169]

175. Nam, Jin; Rath, Bjoern; Knobloch, Thomas J.; Lannutti, John J.; Agarwal, Sudha. Novel Electrospun Scaffolds for the Molecular Analysis of Chondrocytes Under Dynamic Compression. Tissue Engineering Part A. 2009; 15(3):513–523. [PubMed: 18694324]

176. Baker BM, Nathan AS, Russell Huffman G, Mauck RL. Tissue engineering with meniscus cells derived from surgical debris. Osteoarthritis and Cartilage. 2009; 17:336–345. [PubMed: 18848784]

177. Yow SZ, Quek CH, Yim Evelyn KF, Lim CT, Leong KW. Collagen-based fibrous scaffold for spatial organization of encapsulated and seeded human mesenchymal stem cells. Biomaterials. 2009; 30:1133–1142. [PubMed: 19041132]

178. Tzezana, Roey; Zussman, Eyal; Levenberg, Shulamit. A Layered Ultra-Porous Scaffold for Tissue Engineering, Created via a Hydrospinning Method. Tissue Engineering Part C: Methods. 2008; 14(4):281–288. [PubMed: 18781888]

179. Yixiang, Dong; Yong, Thomas; Liao, Susan; Chan, Casey K.; Ramakrishna, S. Degradation of Electrospun Nanofiber Scaffold by Short Wave Length Ultraviolet Radiation Treatment and Its Potential Applications in Tissue Engineering. Tissue Engineering Part A. 2008; 14(8):1321–1329. [PubMed: 18466068]

180. Baker, Brendon M.; Gee, Albert O.; Metter, Robert B.; Nathan, Ashwin S.; Marklein, Ross A.; Burdick, Jason A.; Mauck, Robert L. The potential to improve cell infiltration in composite fiber-aligned electrospun scaffolds by the selective removal of sacrificial fibers. Biomaterials. 2008; 29:2348–2358. [PubMed: 18313138]

181. Rath, Bjoern; Nam, Jin; Knobloch, Thomas J.; Lannutti, John J.; Agarwal, Sudha. Compressive forces induce osteogenic gene expression in calvarial osteoblasts. Journal of Biomechanics. 2008; 41:1095–1103. [PubMed: 18191137]

182. Martins, Albino; Araújo, José V.; Reis, Rui L.; Neves, Nuno M. Electrospun nanostructured scaffolds for tissue engineering applications. Nanomedicine. 2007; 2:929–942. [PubMed: 18095855]

183. Allee, Tyler; Handorf, Andrew; Wan-Ju. LiElectrospinning. :48–78.

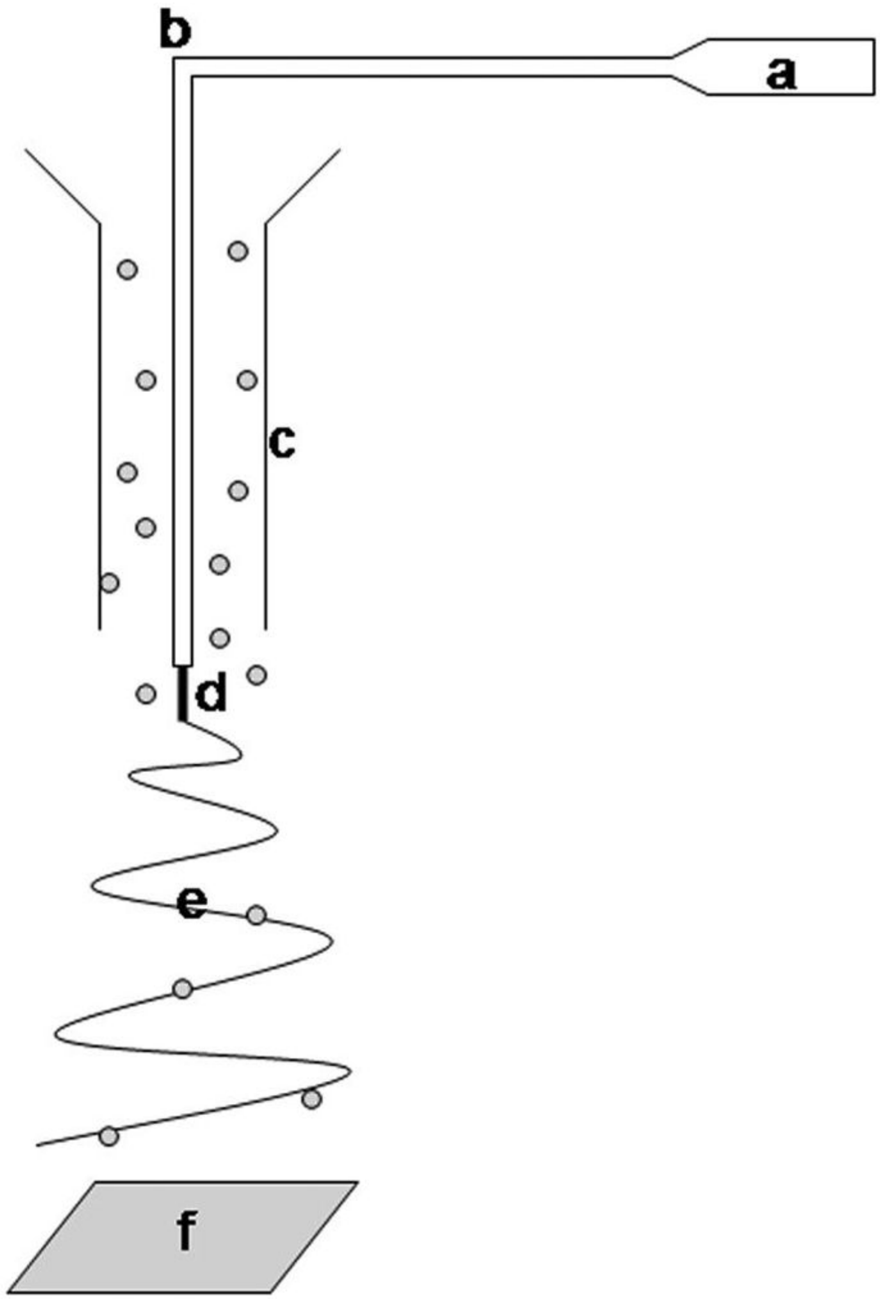

FIG. 1.

Schematic illustration of an electrospinning setup showing the mechanical introduction of salt crystals (gray particles): (**a**) syringe pump, (**b**) extension tubing, (**c**) sheath surrounding the needle into which the crystals are added, (**d**) needle through which voltage is applied, (**e**) electrospun fiber interacting with falling NaCl crystals, and (**f**) grounded collector.

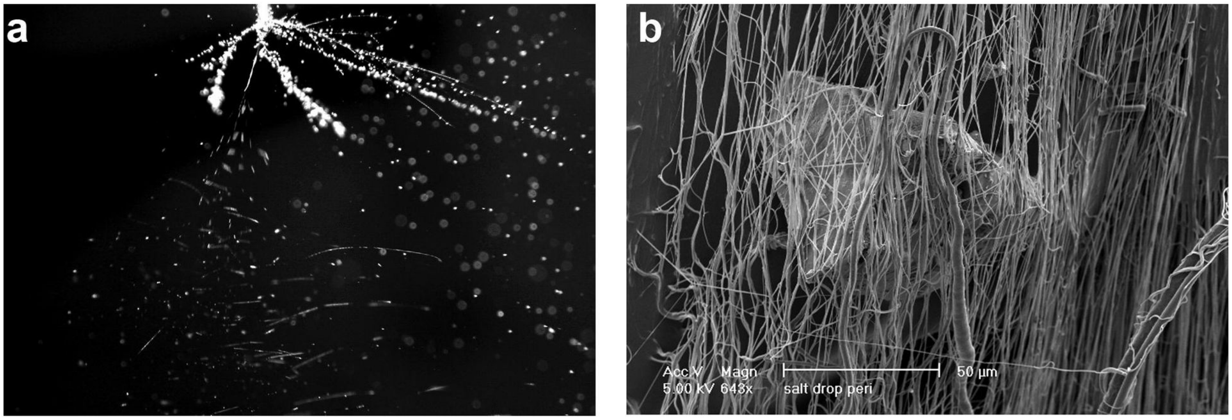

FIG. 2.

(**a**) Image of salt particles interacting with fibers during electrospinning. (**b**) SEM image of a salt particle entrapped by a large group of fibers.

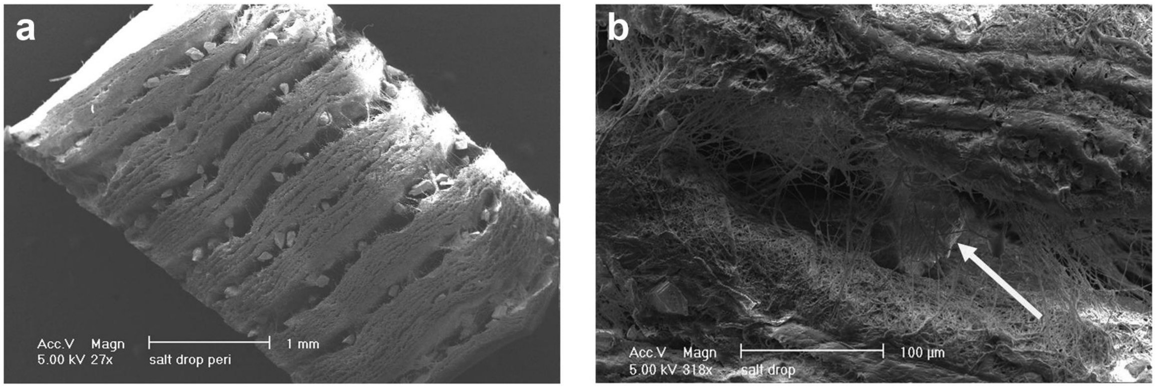

FIG. 3.

(**a**) Side view of an as-punched 37 mm diameter PCL plug containing salt crystals. (**b**) A salt particle (arrow) entrapped by large groups of fibers within a delamination.

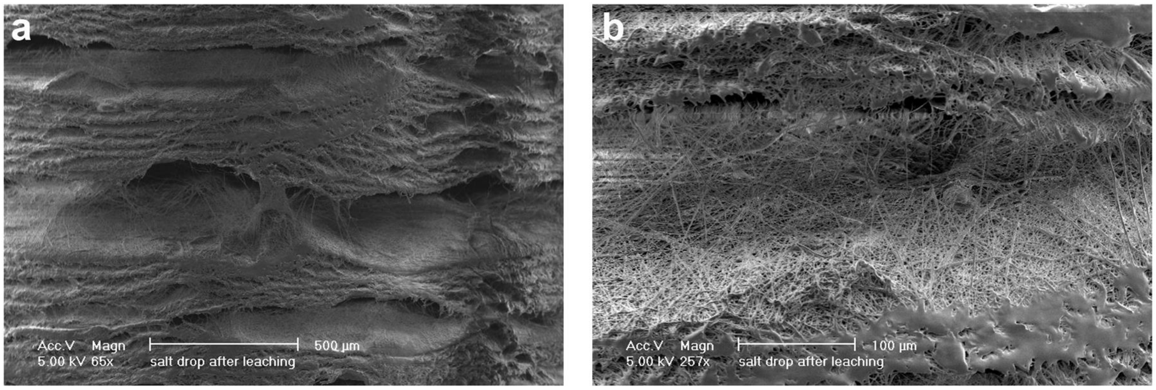

FIG. 4.

SEM images of (**a**) PCL plug after NaCl leaching showing approximately 200-µm pores and (**b**) fibers holding two partially delaminated layers together.

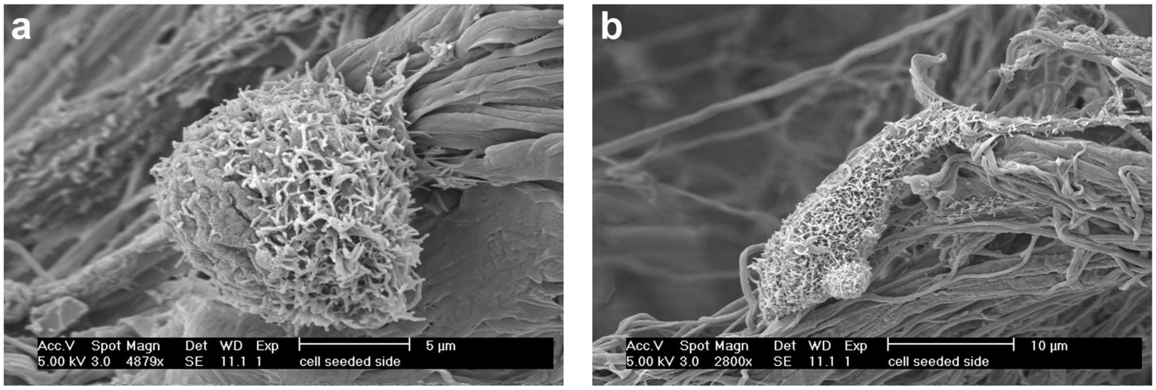

FIG. 5.
SEM images of the seeded cells showing either rounded (**a**) or slightly elongated (**b**) shapes on the salt leached PCL plug at day 3.

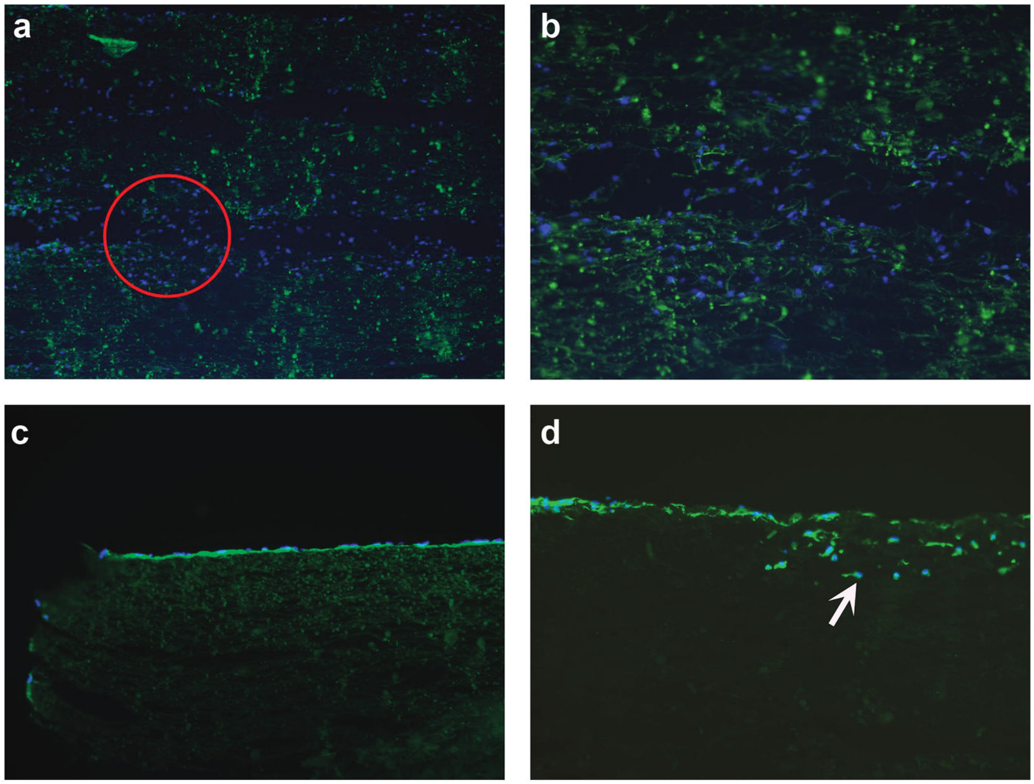

FIG. 6.

Nuclear stained (DAPI; blue stains) sample cryosectioned to 12 μm in thickness after 3 weeks of culture in the samples with (**a, b**) and without (**c, d**) prior salt incorporation. (**a**) Cellular infiltration through salt-generated pores (×100) and (**b**) cellular infiltration into the PCL fiber meshes in some area (magnified image of the circled area in Fig. 6a) (×200). (**c**) Outer surface cellular coverage without infiltration (×100) and (**d**) maximum cellular penetration (approximately 160 μm) observed in a sample spun without any incorporated salt. Color images available online at www.liebertpub.com/ten.

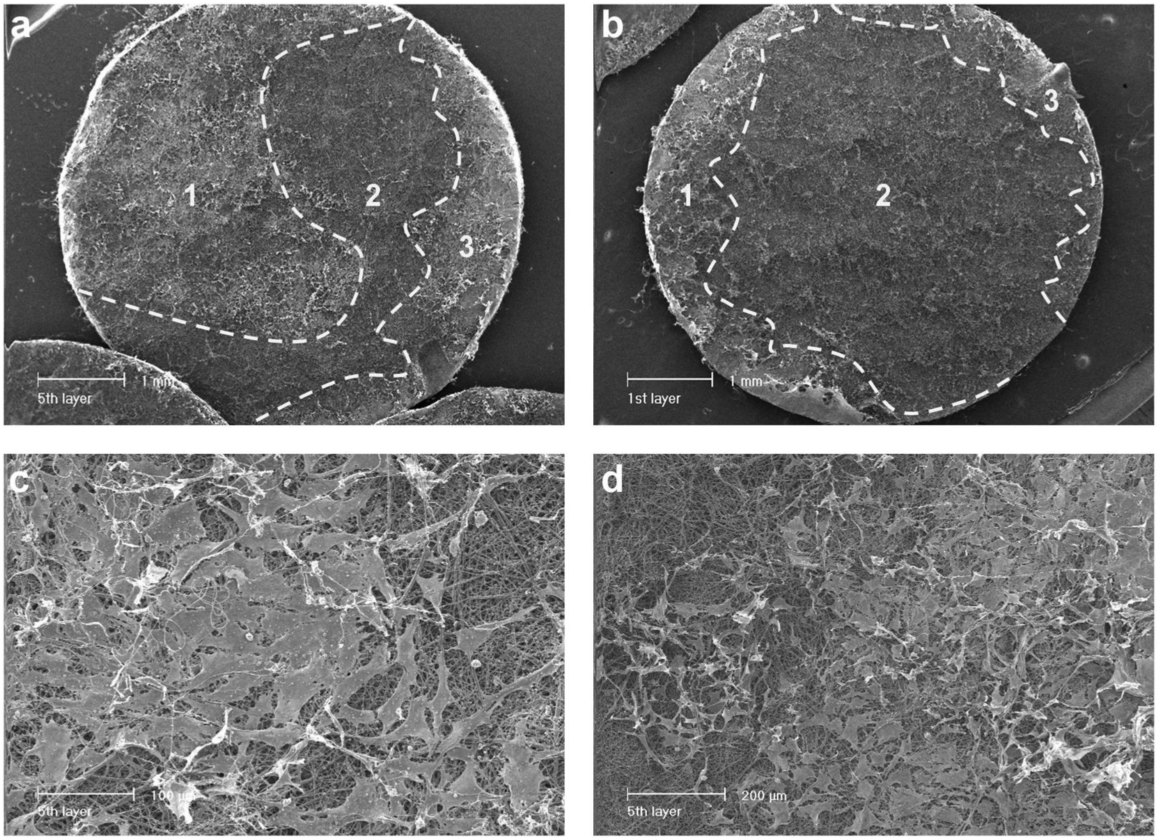

FIG. 7.
SEM images of the delaminated layers showing cellular infiltration after 3 weeks of culture; cells are shown covering (**a**) approximately 70% and (**b**) approximately 35% of the 6-mm cross sections in the same plug; regions 1 and 3 show cellular infiltrated areas; region 2, no cellular coverage. (**c**) High-resolution image of region 1 and (**d**) high-resolution image of region 3.

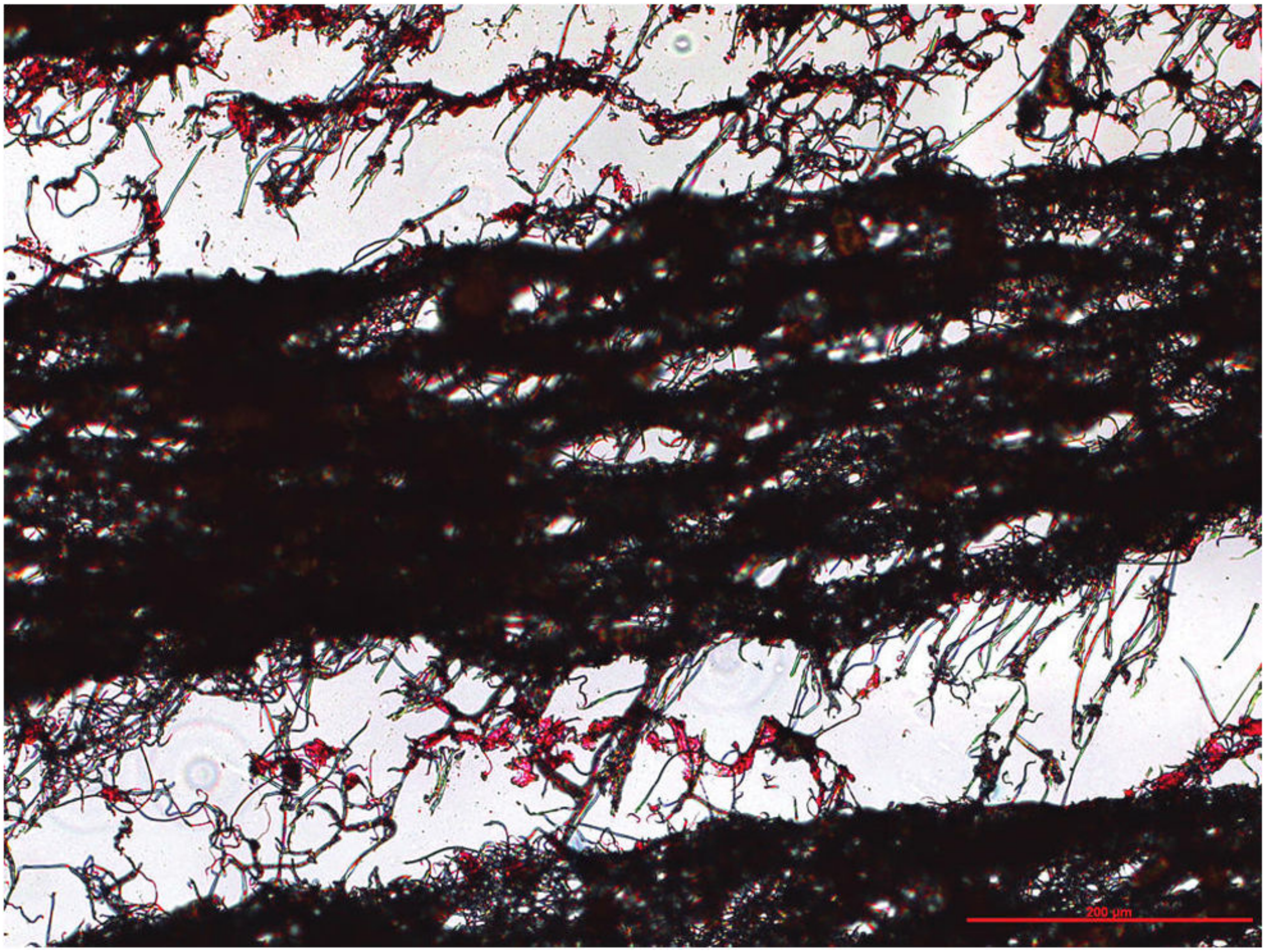

FIG. 8.

Safranin O–stained sample cryosectioned in 12 μm after 3-week culture shows GAG (red stains) accumulation in the NaCl crystal-generated pores. Vertical fibers that hold partially delaminated electrospun layers are also observed (×200). Color images available online at www.liebertpub.com/ten.

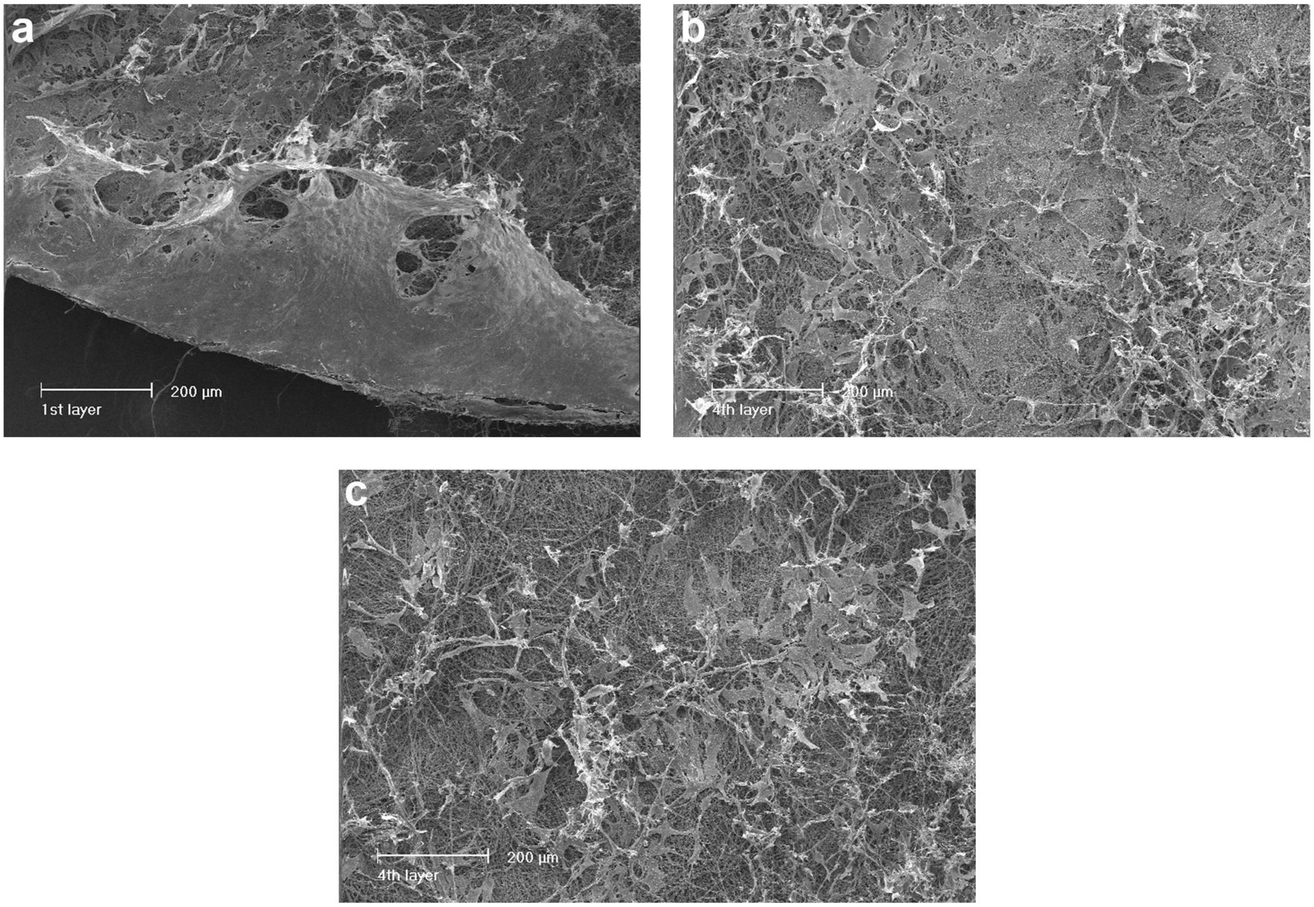

FIG. 9.
SEM images of (**a**) dense cellular population near the edge of the plug, (**b**) dense cellular population, and (**c**) sparse population in the middle of the plug.

Table 1

Variation in Fiber Diameter and Cellular Coverage for Delaminated Areas Taken from Two Specimens

	Sample 1		Sample 2	
Layer	**Average fiber diameter (μm)**	**Cellular coverage (%)**	**Average fiber diameter (μm)**	**Cellular coverage (%)**
1	0.71	35.2	0.72	50.7
2	0.73	50.8	0.72	64.4
3	0.73	55.3	0.73	59.3
4	0.74	69.8	0.75	68.2
5	0.76	68.8	0.74	67.4
6	0.75	64.5	0.74	70.5
7	0.74	67.7	0.74	54.1
8	0.75	58.2	0.74	58.9
9	0.75	50.7	0.75	56.6
Average	0.74	57.9 ± 11.3	0.74	61.1 ± 6.8

The average fiber diameters and cellular coverages are included along with the standard deviation of the latter.