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Electrospun fiber membranes enable proliferation of genetically modified cells

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Abstract: Polycaprolactone (PCL) and its blended composites (chitosan, gelatin, and lecithin) are well-established biomaterials that can enrich cell growth and enable tissue engineering. However, their application in the recovery and proliferation of genetically modified cells has not been studied. In the study reported here, we fabricated PCL-biomaterial blended fiber membranes, characterized them using physicochemical techniques, and used them as templates for the growth of genetically modified HCT116-19 colon cancer cells. Our data show that the blended polymers are highly miscible and form homogenous electrospun fiber membranes of uniform texture. The aligned PCL nanofibers support robust cell growth, yielding a 2.5-fold higher proliferation rate than cells plated on standard plastic plate surfaces. PCL-lecithin fiber membranes yielded a 2.7-fold higher rate of proliferation, while PCL-chitosan supported a more modest growth rate (1.5-fold higher). Surprisingly, PCL-gelatin did not enhance cell proliferation when compared to the rate of cell growth on plastic surfaces.

Keywords: nanofibers, PCL-biomaterial blends, miscibility, gene editing, cell proliferation

Introduction

It is becoming increasingly apparent that biomaterials have an important role to play in the development and evolution of regenerative medicine. The fabrication of simple or complex matrices with secondary and tertiary structures is rapidly becoming key in nanotechnology approaches to human cell therapy.[1,2] Perhaps not surprisingly, human cells appear to proliferate robustly on nanostructures, and this phenomenon can afford the opportunity for detailed evaluation of cellular growth mechanisms.[3–5]

The central objective of fabricating nanostructures for biological applications is the creation of compatible and biodegradable three-dimensional scaffolds upon which human cells can either differentiate, divide, or both. Polycaprolactone (PCL), a hydrophobic polymer, is a well-known biocompatible polymer on which to grow human cells and PCL nanofiber scaffolds can be produced by electrospinning.[6–9] The nanofiber scaffolds are particularly useful because of their high surface area and porosity, which stimulates cell adhesion. Modifications and blending of PCL with other chemicals or proteins can create environments that can become even more conducive to cell growth. Among the more prominent sources of natural biomaterials are gelatin, chitosan, and lecithin, all of which can enhance the hydrophilicity and biocompatibility of PCL.[10–15] Gelatin is composed of glycine, proline, and hydroxyproline, which act together to increase cell attachment. Nanofibers fabricated from a PCL-gelatin blend enhance cell adhesion and neurite outgrowth.[16,17] Chitosan, derived from the deacetylation of chitin and composed of linear polysaccharides linked by β(1-4)-linked D-glucosamine,

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is well known for its capacity to support cell growth and expansion.[13,14] Again, scaffolds prepared from a PCL-chitosan blend support excellent cell growth.[18,19] Lecithin is composed of a complex mixture of phospholipids that contain two long carbon chains derived from soybean oils.[20] Due to its amphiphilic chemical features, which are similar to the phospholipid components of the cell membrane, lecithin can serve as a superior biocompatible support material for cell attachment and growth. Lecithin has been successfully electrospun to fabricate fiber membranes[21–23] and the fiber made from a PCL-lecithin blend has been used to construct ureteral[15] and vascular[24] grafts in murine animal models. In addition, a three-dimensional material with a porous structure fabricated from a lecithin-poly (L-lactic acid) blend was used in a rat bone-graft study,[25] demonstrating improved hydrophilicity and biocompatibility. Hence, blending these biomaterials with PCL seems to provide an environment conducive to growth for cell differentiation and tissue engineering.

Investigations are underway with the goal of utilizing nanofiber templates that mimic biological structures to enable proliferation of stem cells in vitro.[5,7,12] It is believed that, as nanofibers resemble an extracellular matrix, at least structurally, they can provide multilayered physical support for stem cell adhesion.[26,27] While this approach has provided dynamic and exciting results for hematopoietic stem cells,[5] neuronal stem cells,[28] and mesenchymal stem calls,[7,29] relatively little is known about how such nanofiber scaffolds enable the recovery and re-ignition of the proliferation of genetically modified human cells. These cells are particularly relevant to nanomedicine because they are the product of ex vivo gene therapy approaches.

The emergence of several techniques that can direct the correction of point mutations in human genes through the process of gene editing has provided real hope for ex vivo therapy of inherited diseases such as sickle cell disease. The active components of these techniques, oligonucleotides, zinc finger nucleases, and transcription activator-like effector nucleases,[30–32] must be delivered into the target cell at levels that can have unintended effects – for example, zinc finger nuclease-offsite targeting.[33,34] One documented side effect is the phenomenon known as "reduced proliferation phenotype,"[35] in which cells that have undergone genetic modification have inherently reduced rates of replication and proliferation.[36] This barrier needs to be removed before clinical applications of nanomedicine are designed and realized.

Recently, Borjigin et al[37] showed that a single type of electrospun nanofiber can be used as a scaffold upon which genetically modified (gene-edited) cells can reignite the process of DNA replication and enable cell growth. These data suggest for the first time that electrospun PCL fibers have the capacity to enable the growth of human cells that bear a genetic change.

In the work reported here, we fabricated fiber membranes from PCL–biomaterial blends, examined their composition and miscibility, and evaluated their capacity to potentiate recovery and proliferation of genetically modified cells.

Materials and methods

Materials

PCL (MW 80,000), chitosan (MW mid-size), and gelatin (type A) were purchased from Sigma-Aldrich (St Louis, MO, USA). Lecithin was purchased from TCI America (Portland, OR, USA). Dichloromethane (DCM), chloroform, and trifluoroethanol were purchased from Acros Organics (Monroeville, NJ, USA). N,N-Dimethylformamide and trifluoroacetic acid were purchased from Thermo Fisher Scientific (Waltham, MA, USA). and were used without further purification. HyClone culture medium was purchased from Fisher Scientific. The HCT116-19 cell line was constructed in one author's (EBK) laboratory by incorporating the enhanced green fluorescent protein (*eGFP*) mutant gene into the wild-type HCT116 (American Type Culture Collection, Manassas, VA, USA) genome.

Electrospinning

PCL solutions (10%–20%) were made by dissolving PCL pellets in DCM and N,N-Dimethylformamide (8:2) according to the published procedures.[38] The PCL fibers were fabricated using an electrospinning apparatus (described in Borjigin et al)[37] setting the voltage at 12.5 kV, flow rate of the solution at 0.25 mL/h, and the distance between the spinneret and the collector at 10 cm.

The PCL-chitosan solution was prepared by mixing 1.5 mL 20% PCL solution and 0.5 mL 7% chitosan solution (made by dissolving chitosan powder in trifluoroacetic acid and DCM at a ratio 7:3, as described by Dhandayuthapani et al)[13] under sonication for 3 hours. Following this, PCL-chitosan fibers were fabricated by electrospinning the blended polymers with the voltage set at 12.5 kV, a flow rate of 0.35 mL/h, and a distance between spinneret and gap collector of 10 cm.

The PCL-gelatin solution was prepared by blending equal volumes of 15% PCL and 10% gelatin. The gelatin solution was made by dissolving the powder in trifluoroethanol as described by Gupta et al.[17] The electrospinning parameters

for the fabrication of PCL-gelatin fibers were a voltage of 12.5 kV, flow rate of 0.20 mL/h, and distance between the spinneret and the collector of 10 cm.

Lastly, the PCL-lecithin solution was prepared by mixing an equal volume of 10% PCL and 80% lecithin and vortexing. The 80% lecithin was made by dissolving lecithin in chloroform and DCM (7:3) as described by McKee et al.[21] The electrospinning parameters were a voltage of 15 kV, flow rate of 0.30 mL/h, and distance between spinneret and collector of 10 cm.

Characterization of electrospun fibers

Scanning electron microscopy (SEM) was used to visualize the texture of the fiber membranes and to measure the diameter of the electrospun fibers. The fiber membranes were placed on an aluminum mount-M4 (cat # 75610) (Electron Microscopy Sciences, Hatfield, PA, USA) and coated with Au/Pt using a Denton Bench Top Turbo III carbon evaporator (Denton Vacuum, Moorestown, NJ, USA) prior to imaging under a Hitachi S4700 Field Emission Scanning Electron Microscope (Hitachi High Technologies, Tokyo, Japan) with magnification between 2500 and 70,000×. The fiber diameters were measured using ImageJ software (v 1.4; National Institute of Health, Bethesda, MD, USA). Eight fibers from each sample were measured, and the average and standard deviation were calculated.

A Nicolet 6700 FT-IR Spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) was used to perform Fourier transform infrared spectroscopy (FTIR) using air as the (blank) control to determine the composition of the each fiber membrane by detecting the characteristic absorption band patterns associated with the rotational, rocking, twisting, and scissoring of the specific bonds within the fibers. The FTIR analysis was carried out over a wave number range between 4000 and 400 cm^{-1} at a resolution of 4 cm^{-1}.

The miscibility of the blended polymers in the electrospun fiber membranes was investigated using X-ray diffraction (XRD) and differential scanning calorimetry (DSC). XRD was used to determine the crystalline structure and amorphousness of the electrospun fibers. The fiber samples were analyzed using an X-ray diffractometer (X-pert PW3040, PANalytical, Lelyweg, The Netherlands), operating at 40 kV and 20 mA with a Cu-Kα source. The diffraction intensity was measured in the range of 2θ angles between 10° and 30° with a scanning rate of 5°/min. DSC analysis was performed on fiber samples (~5 mg) using a Pyris Diamond TGA/DGA High Temperature 115 thermogravimetric/differential analyzer (PerkinElmer, Waltham, MA, USA). The temperature was increased from ambient to 500°C in an argon atmosphere at a constant heating rate of 5°C/min.

Cell recovery and proliferation on fiber membranes

The integrated mutant *eGFP* gene in HCT116-19 cells was corrected by means of a standard gene-editing protocol.[35–37] Briefly, cells were synchronized with 6 μM aphidicolin followed by 4 hours of release prior to introduction of the 3′ PTO (phosphorothioate)-modified 72-nucleotide (NT) single-stranded oligodeoxynucleotide (ssODN) by electroporation. One million cells in 100 μL of Hyclone McCoy's 5A serum free medium (Thermo Scientific, Logan, UT, USA) were mixed with the ssODN (4 μM) in an electroporation cuvette with a 4 mm gap (Fisher Scientific, Hampton, NH, USA). The cells were electropermealized using a Gene Pulser Xcell™ electroporation apparatus (Bio-Rad, Hercules, CA, USA) with settings of 250 V, 13 ms, 2 pulses, and a 1-second interval for delivery of ssODN into the cells. Immediately after the electroporation, the cells were transferred onto nanofibers for recovery and growth. Triplicate samples of cells recovered on fiber membranes were harvested at Day 4 (96 hours) and Day 7 (168 hours) for analysis of gene editing using fluorescence-activated cell-sorting (FACS) analysis (Guava EasyCyte HT, Millipore, Billerica, MA, USA). The corrected cells on the fibers at Day 4 (96 hours) were visualized and images were taken using an EVOS FL microscope (AMG Micro, Bothell, WA, USA).[37]

Results

Electrospun PCL-biomaterial blended fibers

Four different fibers were electrospun to fabricate parallel-aligned fiber membranes using a standard electrospining apparatus setup, as described in "Materials and methods" (see Borjigin et al).[37] A 21-gauge flat-tip syringe needle was used as a spinneret and a gap collector was used to electrospin the fibers. The distance between the spinneret – from where the polymer solution spins out – and the collector was set 10 cm for the fabrication of all four fiber membranes. The flow rate of polymer solutions from the spinneret and the voltage gap applied between the spinneret and the collector were optimized for the specific polymer fiber fabrication. A flow rate of 0.25 mL/h and voltage of 12.5 kV were used in PCL nanofiber electrospinning; 0.35 mL/h and 12.5 kV, 0.20 mL/h and 15 kV, and 0.30 mL/h and 15 kV were applied in electrospinning PCL-chitosan, PCL-gelatin, and PCL-lecithin, respectively. The morphology and texture of

fiber membranes fabricated from pure PCL polymer were more uniform and the fibers were aligned more neatly than in the blended polymers. The diameter of a PCL nanofiber was determined to be 428 ± 40 nm (Figure 1A), while that of a PCL-chitosan nanofiber was thinner (113 ± 31 nm) and exhibited a rougher surface (Figure 1B). The two other electrospun blend fibers, PCL-gelatin and PCL-lecithin, had a diameter of 3.20 ± 1.65 μm and 1.80 ± 0.90 μm, respectively (Figure 1C and D).

Composition of blended fibers

FTIR analysis was conducted using a Nicolet 6700 FT-IR spectrometer to examine the composition of fiber membranes and to determine the presence of each component in the miscible blended fibers. FTIR was performed using air as a blank control over the range of 4000–400 wavenumber/cm at a resolution of 4 cm^{-1}. PCL nanofibers exhibited a characteristic band at 1160/cm in the fingerprint range of the FTIR absorption spectrum; this is attributed to C–O–C bond stretching within the PCL polymer. In addition, a very strong carbonyl group bond stretching at 1720/cm and C–H stretching at 2860 and 2940/cm were detected in the PCL nanofibers (Figure 2). This spectrum pattern was also present in all three blended fibers, albeit at weaker intensity, signifying the presence of PCL in the blended polymers. The PCL-chitosan blend (10% PCL, 3.5% chitosan in polymer solution, comprising 10:3.5 ratio in the dry fibers) nanofibers exhibited a unique band at 1540/cm, indicating N–H stretching from the primary amine of chitosan. PCL-gelatin

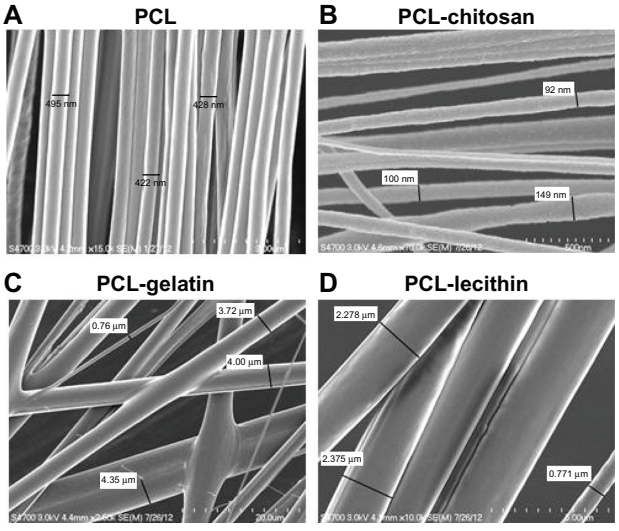

Figure 1 Scanning electron microscope (SEM) images of parallel-aligned electrospun fibers: (**A**) polycaprolactone (PCL), (**B**) PCL-chitosan, (**C**) PCL-gelatin, and (**D**) PCL-lecithin.
Note: Eight fibers observed in each sample were measured and the average and standard deviation were calculated.

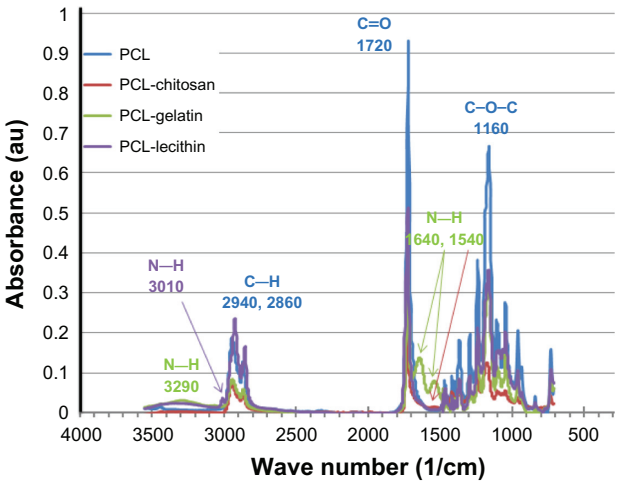

Figure 2 Fourier transform infrared spectroscopy spectra of the fiber membranes.
Abbreviation: PCL, polycaprolactone.

(7.5% PCL and 5% gelatin, ratio of dry weight 7.5:5) fibers showed characteristic bands at 1540, 1640, and 3290/cm, signaling N–H stretching from the primary and secondary amines from peptides and proteins inherent in the gelatin mix. Finally, PCL-lecithin (5% PCL, 40% lecithin, weight ratio of 5:40) fibers exhibited a weak band at 3010/cm, indicating N–H stretching in the quaternary amine group of lecithin phosphatidylcholine (Figure 2). Our FTIR absorption spectra of PCL, PCL/chitosan, and PCL/gelatin are consistent with previously published data.[13,16,17] Our data confirm the presence of the indicated natural materials in the blended membranes at the predicted composition.

XRD and DSC

XRD of the fibers was carried out using the X-pert PW3040 diffractometer (PANalytical) operating at 40 kV and 20 mA with a Cu-Kα source. The PCL nanofibers exhibited two strong diffraction peaks at Bragg angles 2θ = 21.3° and 23.6°, which represent the (110) and (200) reflections respectively of a polyethylene-like crystal structure with orthorhombic unit cell parameters[8,39,40] (see Figure 3). The PCL-chitosan nanofiber displayed a much weaker diffraction at 2θ = 20.2° and 22.4°, shifting one degree left of the PCL nanofiber peaks, indicating a different type of crystal form with different D spacing within the crystal lattice. PCL-gelatin fiber diffracted at 2θ = 22.8° and 24.7° (right shift) with a much weaker intensity than that of the pure PCL nanofibers, indicating a different crystal form and a less ordered structure or a lesser degree of crystallinity in the blend material. Lastly, PCL-lecithin showed moderate intensity diffractions at 2θ = 20.8°, 21.7°, 23.1°, and 24.1°, indicating that different forms of crystals were present in this blended material

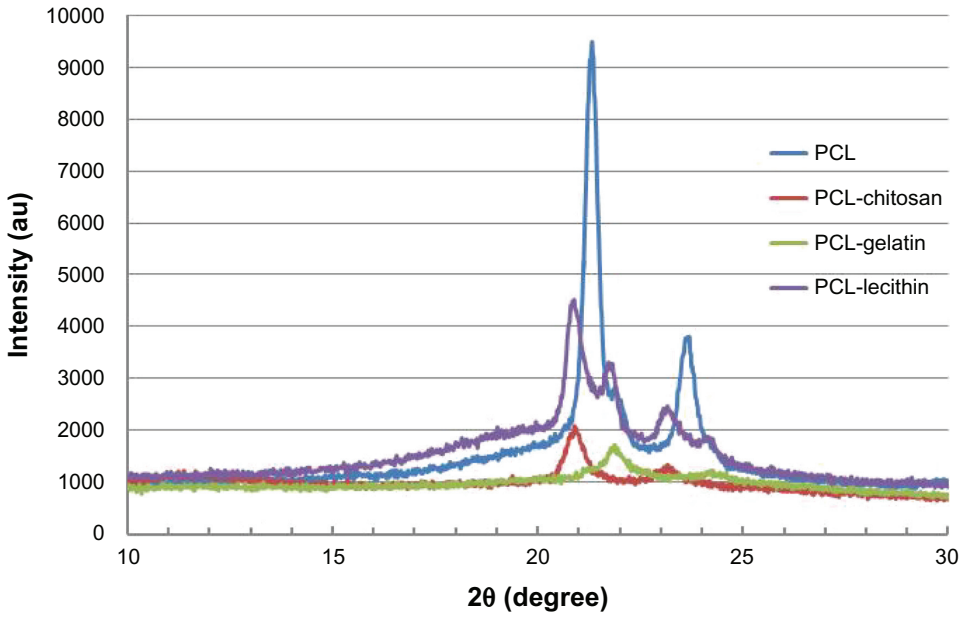

Figure 3 X-ray diffraction patterns of the fiber membranes.
Notes: Polycaprolactone (PCL) nanofibers exhibit two strong diffraction peaks at Bragg angles 2θ = 21.3° and 23.6°. PCL-chitosan shows much weaker diffraction at 2θ = 22.2° and 22.4°, while PCL-gelatin is weaker again at 2θ = 22.8° and 24.7°. PCL-lecithin shows moderate intensity diffraction at 2θ = 20.8°, 21.7°, 23.1°, and 21.7°.

(Figure 3). These data demonstrate that crystal forms and the orientation in the blended polymer fibers are different from those found in pure PCL nanofiber membranes. These results further indicate that the transparent and visually miscible polymer solutions of blended PCL-biomaterials formed amorphous composite fibers with different types of crystalline structure.

To further examine whether the homogeneity and miscibility of the blended fibers were affected by the lesser degree of crystallinity or the different forms of crystals in the fibers, DSC was conducted using the Pyris Diamond thermogravimetric/differential analyzer. Theoretically, a completely miscible blend should result in the formation of a single phase-transition temperature rather than the separate phase transition of individual parent polymers. Fiber samples (5 mg) were sealed in aluminum pans of the instrument and the pan was heated from ambient temperature to 500°C at 5°C/min increments under argon purge at a 20 mL/min rate. The analysis showed that the PCL nanofiber has an onset temperature of 52.6°C, at which point the PCL nanofiber starts transitioning to a liquid state, with a melting point of approximately 61°C. Noticeably, the three natural polymers (chitosan, gelatin, and lecithin) blended into PCL led to an increase in the melting temperature. The onset temperature of the PCL-chitosan composite nanofibers was 57.2°C, and its melting point was 65.3°C; the PCL-gelatin composite fibers showed an onset temperature of 58.4°C and melting point at 64.7°C; and the PCL-lecithin fibers displayed an onset temperature of 55.5°C and a melting point of 63.2°C.

In contrast, the depolymerization temperature of pure PCL nanofibers was higher than that of the three blended PCL-biomaterial fibers. The depolymerization of PCL nanofibers occurred at a temperature of 389°C, whereas the depolymerization of PCL-chitosan nanofibers occurred at 368°C, that of PCL-gelatin fibers at 379°C, and that of PCL-lecithin fibers at 382°C (Figure 4). The single peak each for phase-transition temperature and decomposition temperature of pure PCL and blended natural polymers confirms that PCL blended into the three natural polymers forms highly miscible polymer fibers, albeit with different levels and types of crystallinity present.

Recovery and proliferation of genetically modified cancer cells

Gene editing in human cells consists of the molecular exchange of a nucleotide in the coding region of a gene, as directed by a ssODN.[30,41–46] Largely, mechanistic studies have utilized a mutated *eGFP* gene as the target and a functional eGFP is produced if the gene is repaired; then, the corrected cells express *eGFP* and display green fluorescence. In the standard model system, a single copy of the *eGFP* gene with a single base mutation – TAC → TAG – generating a stop codon has been stably integrated in the sequence of HCT116 cells. To correct the mutation, the appropriate oligonucleotide is introduced into the cell population by

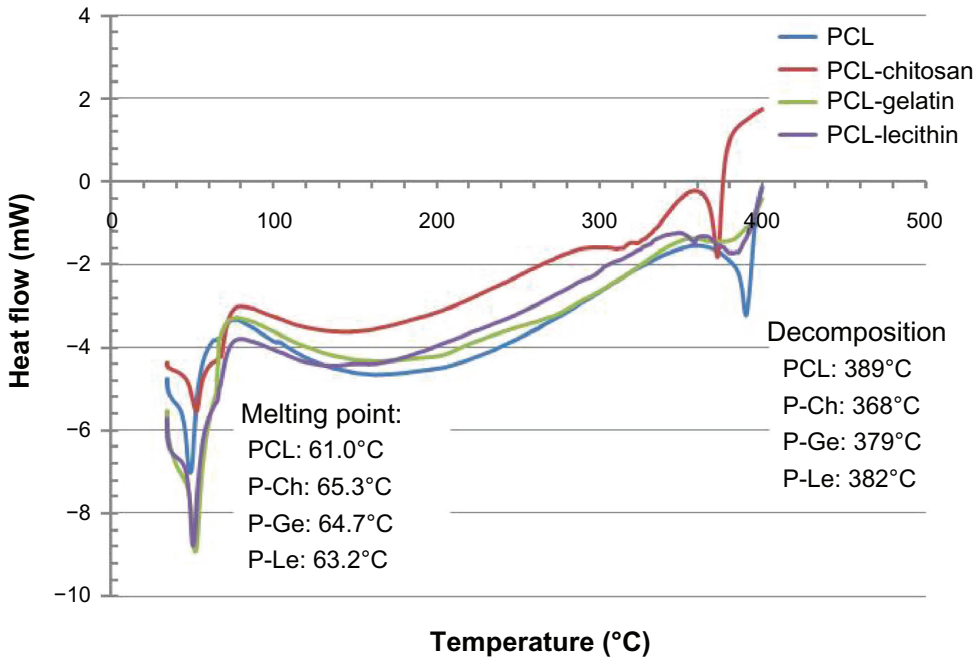

Figure 4 Differential scanning calorimetry thermograms of the fiber membranes.
Abbreviations: PCL, polycaprolactone; P-Ch, polycaprolactone-chitosan; P-Ge, polycaprolactone-gelatin; P-Le, polycaprolactone-lecithin.

electroporation. We have previously employed this type of system to evaluate gene editing in HCT116 cells.[30,35,36] The method involves synchronizing HCT116-19 cells using aphidicolin for 24 hours followed by a 4-hour release period prior to introduction of 72 NT DNA oligonucleotides by electroporation (Figure 5A). Immediately after electroporation, 0.5×10^6 cells are plated onto a polylysine-coated dish or an electrospun fiber membrane for recovery and expansion. Due to the large number of free ends delivered into the cell, which is part of the ODN-directed gene-editing process, the DNA damage response checkpoint proteins CK1 and CK2 are activated and DNA replication is slowed or halted. As a result, the targeted cell appears to enter a quiescent state of growth.[47,48] Such stress responses, along with the physical harm incurred by the process of electroporation itself, result in a long recovery phase, which we have termed the "reduced proliferation phenotype" (RPP).[30,35,47,48]

Since the three PCL-biomaterial-blended electrospun fiber membranes demonstrated excellent miscibility, we evaluated whether they were capable of supporting cell recovery and, essentially, can enable reversal of RPP. Our data show that corrected cells (green) grown on three of the electrospun fiber membranes, PCL, PCL-chitosan, and PCL-lecithin, divide at Day 4 (96 hours) in contrast to cells plated onto the standard plastic surface or onto PCL-gelatin fiber membranes (Figure 5B). Triplicate samples of cells from each fiber membrane and the plastic surface were harvested at Day 4 (96 hours), and the correction efficiency was quantified using FACS. The correction efficiency of cells on PCL, PCL-chitosan, and PCL-lecithin was comparable; approximately 1.8 and 1.9 times higher than that found for PCL-gelatin fiber membranes and plastic surfaces, respectively (see Figure 5C). These results suggest that a higher magnitude of cell recovery is enabled when cells are plated onto PCL, PCL-chitosan, and PCL-lecithin fiber membranes, and that RPP can be reversed.

Cell proliferation on the fiber membranes and plastic surfaces was also quantified at Day 7 (168 hours). PCL-lecithin fiber membranes were found to promote cell growth the best, with a rate 3.5 times higher than that seen with PCL-gelatin fiber membranes and 2.7 times higher than that seen with plastic surfaces. PCL and PCL-chitosan membranes enhanced the proliferation of the cells 3.2 and 1.9 times more than PCL-gelatin (Figure 5C). The correction efficiency of cells grown on PCL-gelatin fiber membranes declined precipitously at both time points, demonstrating that cell expansion on this fiber membrane was impeded.

Discussion

Electrospun nanofibers have attracted a great deal of attention for applications in tissue engineering due primarily to their topological similarity to the extracellular matrix, large surface to volume ratio, flexibility in surface functionality,

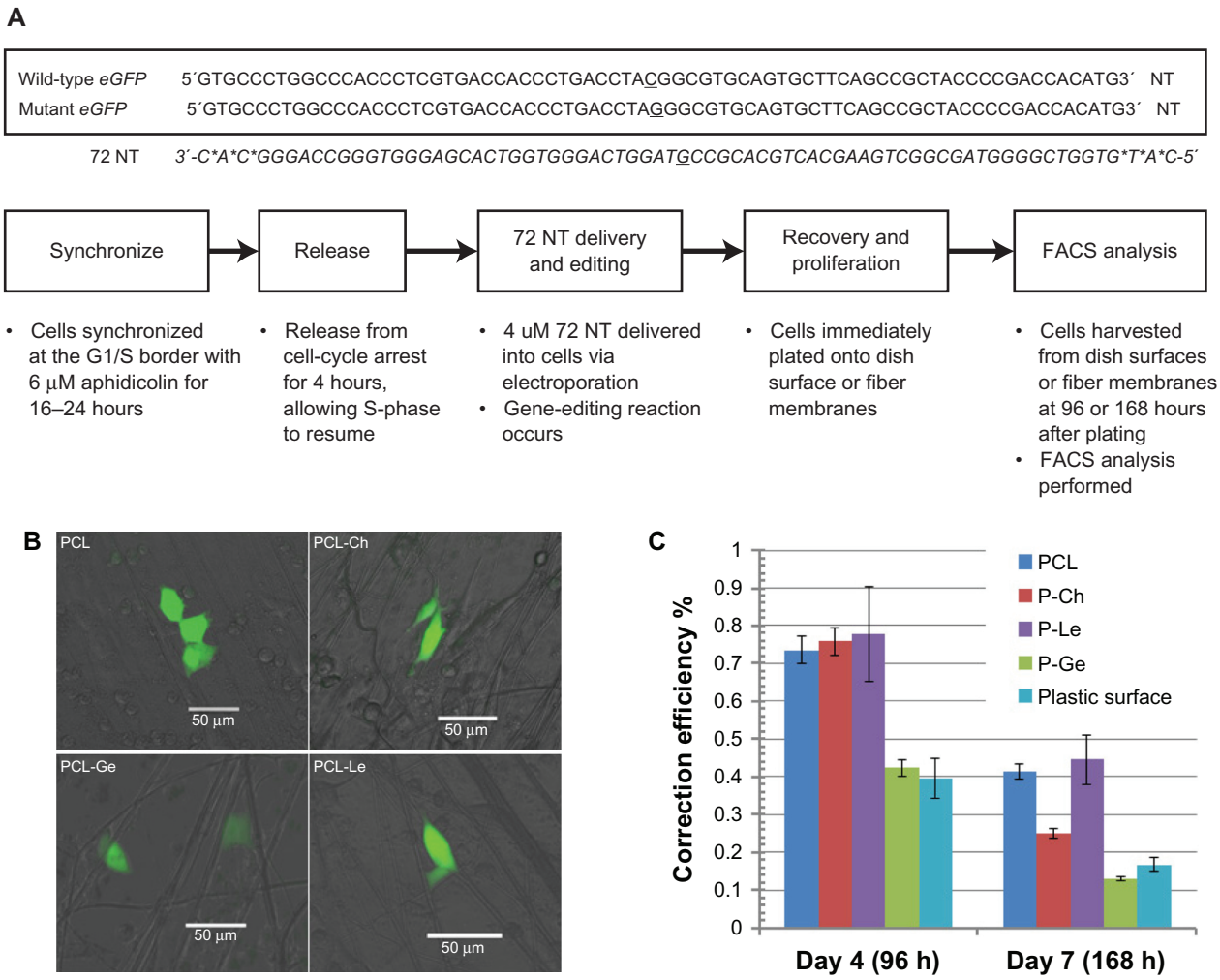

Figure 5 Recovery of the genetically modified cells on electrospun fiber membranes. (**A**) Model system and experimental protocol for gene editing. (**B**) Fluorescent microscope images of the genetically modified HCT116-19 cells on fiber membranes at Day 4 (96 hours) using an EVOS FL microscope (AMG Micro, Bothell, WA, USA) at 170× magnification. (**C**) Gene correction efficiency of the cells on fiber membranes at Day 4 (96 hours) and Day 7 (168 hours) was analyzed using a Guava fluorescence-activated cell-sorting (FACS) machine (Millipore, Billerica, MA, USA).

Abbreviations: eGFP, enhanced green fluorescent protein; NT, nucleotide; PCL, polycaprolactone; P-Ch, polycaprolactone-chitosan; P-Ge, polycaprolactone-gelatin; P-Le, polycaprolactone-lecithin.

and adjustable porosity.[4,49,50] Recently, homogenous PCL nanofibers have been used to enable proliferation of genetically modified cells.[37] Here, we extended that initial study and fabricated parallel-aligned PCL-chitosan, PCL-gelatin, and PCL-lecithin electrospun fiber membranes with visually well-blended PCL-biomaterial solutions, and confirmed the presence of the expected components in the fiber membranes by FTIR. We examined the fiber quality (amorphous, ordered structure, or crystallinity) of the fibers by XRD and determined the miscibility of the components by DSC analysis. Then, these membranes were used as recovery scaffolds to expand genetically modified cells that are known not to proliferate on standard plastic surfaces.

Certain features of electrospun fibers, such as morphology, diameter, and density, are important to the creation of a conducive nanofiber environment for cell growth.[50] The SEM data show that electrospun PCL-chitosan and PCL fibers were nanoscale, and PCL-gelatin fibers exhibit a larger diameter with higher deviation than other fibers (PCL, PCL-chitosan, and PCL-lecithin) (Figure 1). The fiber diameter variation was caused by ambient conditions (such as temperature and humidity), fluid properties, and electrospinning operating parameters. Fluid properties are viscosity, surface tension, boiling point, conductivity, and dielectric constant, and so forth.[51,52] In our study, we used published protocols to fabricate the fiber membranes, but did not explore modulating these variables. The operating parameters are flow rate, voltage, and distance between the tip and the collector; these parameters were adjusted to produce the well-distributed defect-free fibers.

The correction efficiency of HCT116-19 cells at Day 4 (96 hours) on PCL-gelatin was 1.8-fold lower than that on PCL, PCL-chitosan, or PCL-lecithin fiber membranes. Furthermore, the correction efficiency at Day 7 (168 hours) on PCL-gelatin fibers dropped 3.2- and 3.5-fold lower compared to PCL and PCL-lecithin, respectively (Figure 5C). After recovery on PCL-lecithin, PCL, and PCL-chitosan fiber membranes for 7 days (168 hours), the correction efficiency of HCT116-19 cells was 2.7-, 2.5-, and 1.5-fold higher, respectively, than that on standard plastic surfaces. PCL-lecithin membranes demonstrated the highest level of cell growth (along with pure PCL nanofiber membranes), while PCL-gelatin neither supported the recovery of cells up to Day 4 (96 hours) nor promoted the expansion of the cell growth until Day 7 (168 hours). Although the three blend biomaterials (chitosan, gelatin, and lecithin) provide conducive cell growth environments, as demonstrated in the literature,[10–15] the level of recovery and expansion of our genetically modified cells varied. One reason for this variation is the cell-specific interaction with the biomaterials. Such interaction is perhaps more important to our genetically modified cells, since the process of genetic modification in our model system can affect cell metabolism, including the delivery of a large amount of ssODN into cells via electroporation.

Studies on other types of cells grown on various fibers have revealed that the fiber diameter plays an important role in cell growth. Cells cultured on fibers of larger diameter (micron size) tend to adhere to a single fiber and are seen not to proliferate.[53] This observation is supported by the stronger growth of neural stem cells on nanofibers than on micro fibers.[54] In contrast, a recent study by Cardwell et al showed that human mesenchymal stem cells proliferate and differentiate better on microfibers than on nanofibers.[55] In our study, the correction efficiency on PCL-chitosan was comparably higher than those on PCL and PCL-lecithin during the recovery period (at Day 4), and it dropped significantly during the extended period. An explanation for this observation might be that a component released from the dissolved chitosan fiber interacts with cells to trigger a different pathway of physiology over the time. Therefore, the size of the fiber diameter might have a significant impact on cell attachment during the cell recovery period (4 days), while post-recovery cell physiology may be more dependent on the interaction between the cells and the biomaterials.

Not only is the miscibility of the blended polymers essential for formation of defect-free electrospun nanofibers, but it also provides uniform chemical properties that can enhance cell adhesion and proliferation.[18,39] In our study, FTIR analyses show that the PCL nanofibers exhibit strong carbonyl group bond stretching at 1720/cm and C–H stretching at 2860 and 2940/cm, which matches exactly with the characteristic absorption bands presented in the literature.[16,17] In addition, the blended fibers displayed unique peaks that can be attributed to the corresponding individual components. PCL-gelatin had several characteristic bands at 1540, 1640, and 3290/cm, attributed to N-H stretching from the primary and secondary amines in the peptide and protein components of the gelatin. PCL-chitosan nanofibers and PCL-lecithin fibers were distinguished from other blended fibers due to N-H stretching from the primary amine of chitosan at 1540/cm and a weak band at 3010/cm indicating N–H stretching in the quaternary amine group of lecithin phosphatidylcholine (Figure 2).

The physical nature of the components (ie, miscibility, amorphous or ordered structure, and crystallinity) in the blended fibers was examined using XRD and DSC analysis. The XRD data revealed that pure PCL fibers formed a more ordered structure or exhibited higher crystallinity, while the blended fibers were more amorphous (with less crystallinity) and the PCL nanofibers were found to have a higher diffraction intensity (Figure 3). Our data also indicate that the blended biomaterials contributed to the formation of different types of crystals and crystal-plane orientations, interpreted from the 2θ angle shift (Figure 3). This indicates that the blended polymers were highly miscible and formed uniform composite materials. DSC analysis demonstrated that all three blended fibers and PCL differ in terms of both melting point and decomposition temperature, which again confirms the component difference in the blends. There is only a single phase-transition temperature (melting point) for each blended fiber membrane, further verifying that each blended fiber membrane was highly miscible (Figure 4).

Although the electrospun nanofiber scaffolds made from PCL, PCL-chitosan, and PCL-gelatin have been examined in cell differentiation studies,[16,56,57] their application in the recovery and proliferation of genetically modified cells is, as far as the authors are aware, reported for the first time here. While PCL-lecithin is relatively less well-studied in cell growth and tissue engineering, it exhibits high potential for tissue grafts.[15,24] In this study, PCL-lecithin supported the recovery and proliferation of genetically modified cells in a robust fashion (Figure 5B). In contrast, PCL-gelatin displayed no enhancement over plastic surfaces for recovery and proliferation.

Conclusion

In our study, we fabricated electrospun fiber membranes using PCL, PCL-chitosan, PCL-gelatin, and PCL-lecithin, characterized them using chemical and physical methods, and applied them to the recovery and growth of genetically modified cells. Our data show that all the blended polymers formed miscible defect-free parallel-aligned fibers, and that PCL-lecithin and PCL were best at enhancing cell recovery and proliferation. In contrast, PCL-gelatin does not support the growth of genetically modified cells, and further study on this topic might reveal interesting mechanisms.

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Disclosure

The authors report no conflicts of interest in this work.

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