Localized Delivery of Dexamethasone from Electrospun Fibers Reduces the Foreign Body Response

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Ⓢ Supporting Information

ABSTRACT: Synthetic scaffolds are crucial to applications in regenerative medicine; however, the foreign body response can impede regeneration and may lead to failure of the implant. Herein we report the development of a tissue engineering scaffold that allows attachment and proliferation of regenerating cells while reducing the foreign body response by localized delivery of an anti-inflammatory agent. Electrospun fibers composed of poly(L-lactic) acid (PLLA) and poly(ε-caprolactone) (PCL) were prepared with and without the steroid anti-inflammatory drug, dexamethasone. Analysis of subcutaneous implants demonstrated that the PLLA fibers encapsulating dexamethasone evoked a less severe inflammatory response than the other fibers examined. They also displayed a controlled release of dexamethasone over a period of time conducive to tissue regeneration and allowed human mesenchymal stem cells to adhere to and proliferate on them in vitro. These observations demonstrate their potential as a building block for tissue engineering scaffolds.
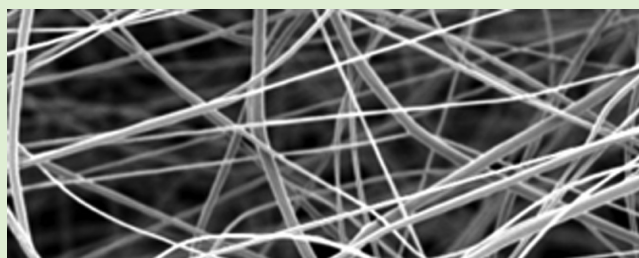
■ INTRODUCTION

Tissue engineering has been described as an interdisciplinary field where the principles of biology and engineering are applied to develop biological substitutes that restore, maintain, or improve tissue function.[1] This restored or improved function is often accomplished by the growth of new tissue from cells seeded onto an implanted synthetic scaffold.[1] In recent years, there has been a growing interest in the use of electrospun fibers as scaffolding materials in regenerative medicine.[2−4] Electrospun scaffolds have a high porosity and surface area to volume ratio, which is ideal for cell adhesion,[5] infiltration,[6,7] and angiogenesis.[8,9] Furthermore, the submicrometer fibrous structures mimic the arrangement of extracellular matrix, and their configurations have been shown, in vitro, to influence cell migration and proliferation.[10]

In addition to providing structural support, electrospun fibers can be designed to encapsulate, and release in a controlled fashion, therapeutic molecules such as antibiotics,[11] DNA,[12] growth factors,[13] and anti-inflammatory drugs.[14,15] Encapsulating therapeutic molecules in a tissue engineering scaffold allows local delivery of these molecules to regenerating tissues, possibly leading to a more potent effect and eliminating the side effects associated with systemic delivery.

A major problem arising from the use of synthetic tissue engineering scaffolds is the host's foreign body response. The goal of this study was to produce a tissue engineering scaffold that allows regenerating cells to attach and proliferate but evades the foreign body response and improves the biocompatibility of the synthetic material by locally releasing an anti-inflammatory agent.

Others have fabricated and examined electrospun scaffolds that release anti-inflammatory agents,[15−18] but, to our knowledge, in vivo evidence of a reduced foreign body response over a period of time conducive to tissue regeneration, that is, longer than 2 weeks, has not been reported. Electrospun fibers that release dexamethasone as an osteogenic differentiation agent have also been fabricated,[19−21] but their anti-inflammatory properties were not characterized.

Scaffolds composed of electrospun hyperbranched polyglycerol nanofibers that contain *Calendula officinalis* extract as a wound healing and anti-inflammatory agent have been examined in vivo for efficacy at reducing inflammation and promoting wound healing.[16] However conclusive in vivo

Table 1. Electrospinning Parameters

PCL composition of fibers (mass fraction)[a]	PLLA composition of fibers (mass fraction)[a]	mass percent of dexamethasone relative to dexamethasone and polymer	PCL composition of electrospinning mixture (mass fraction)[a]	PLLA composition of electrospinning mixture (mass fraction)[a]	$CHCl_3$ or DCM to DMF ratio (v/v)	flow rate (mL/h)	voltage (kV)
1.00	0.00	5.7%	0.100	0.000	$CHCl_3$:DMF 75/25	3.20	20
0.00	1.00	5.7%	0.000	0.040	DCM:DMF 62.5/37.5	4.00	22
1.00	0.00	0.0%	0.100	0.000	$CHCl_3$:DMF 75/25	3.20	20
0.00	1.00	0.0%	0.000	0.040	DCM:DMF 62.5/37.5	4.75	20

[a]Excluding dexamethasone.

evidence of a reduced inflammatory response to these fibers was not reported. Cyclosporine A (CsA)-loaded PLLA electrospun fibers were shown to reduce the production of the proinflammatory cytokines IL-2 and IFN-γ by concanavalin-A-stimulated mouse spleen cells and to reduce T-cell proliferation in vitro. Explanted mouse skin allografts also displayed reduced expression of these cytokines when covered with CsA-loaded fibers for 7 days in vivo.[18] Poly(lactic-co-glycolic acid) (PLGA) electrospun fibers that release the anti-inflammatory drug ibuprofen were shown to reduce NF-κB nuclear translocation in human fibroblasts in response to a bacterial lipopolysaccharide,[15] and mouse monocyte-macrophages seeded on curcumin-loaded poly(ε-caprolactone) fibers displayed a reduction in the release of interleukin-6 following stimulation by *Escherichia coli* derived lipopolysaccharide;[17] however, neither of the latter two mentioned studies examined the inflammatory response in vivo. The inflammatory response to blended PLGA and dextran electrospun fibers has been shown, in vivo, to be less severe than that to a commercially available PLGA suture.[22] However in vivo results were not reported beyond 1 week post implantation, and this effect was not accomplished through controlled release of therapeutic molecules.

Dexamethasone, a steroid anti-inflammatory drug, has been shown to reduce the severity of the inflammatory response when delivered locally.[23−26] In this study, electrospun fibers composed of poly(L-lactic acid) (PLLA) and poly(ε-caprolactone) (PCL) were prepared with and without dexamethasone encapsulated (PLLA, PCL, PLLA/dex, and PCL/dex fibers, respectively). The surface morphologies and compositions of these fibers were examined with scanning electron microscopy (SEM) and X-ray photoelectron spectroscopy (XPS), respectively. Their thermal properties were studied using differential scanning calorimetry (DSC), the release rates of dexamethasone from the PCL/dex and PLLA/dex fibers were examined in vitro by ultraviolet-visual spectroscopy, and the in vivo inflammatory responses to all of the fibers were examined by histological analysis of subcutaneous implants. Finally, to assess further the ability of the fibers to serve as a tissue engineering scaffolding material, the alomarBlue viability assay was performed on human mesenchymal stem cells cultured on the fibers.

■ MATERIALS AND METHODS

Fabrication of Electrospun Fibers. To fabricate PLLA fibers, PLLA (MW 300 000 g/mol, Polysciences) was dissolved in dichloromethane (DCM) (Sigma) prior to the addition of *N,N*-dimethylformamide (DMF) (Sigma), similar to an established experimental procedure.[3] The DMF was added immediately prior to electro-

spinning. The 4.0 mass percent mixture of PLLA in 62.5/37.5 (v/v) DCM/DMF was loaded into a glass syringe and pumped (Harvard PHD 2000 syringe pump) through a 0.10 cm inner diameter (ID) metallic needle at a flow rate of 4.75 mL/h. A voltage of 20 kV was applied (Gamma high-voltage power supply ES5OP-5W/DAM), and the collection plate was held 35 cm from the tip of the needle.

To generate PLLA/dex fibers, dexamethasone (Sigma) was added to the PLLA-containing DCM/DMF mixture at 5.7% of the total drug and polymer mass. The DMF was added immediately prior to electrospinning. The flow rate was set to 4.00 mL/h, and a voltage of 22 kV was applied. The metallic collection plate was held 35 cm from the tip of the needle.

To fabricate PCL fibers, a 10.0% by mass solution of PCL (MW 70 000 to 90 000 g/mol, Sigma) in 75/25 (v/v) chloroform/DMF (Sigma) was pumped through a 0.10 cm ID needle at a rate of 3.20 mL/h. The other parameters were maintained identical to those as in the fabrication of the PLLA fibers.

To generate PCL/dex fibers, dexamethasone was dissolved in the PCL-containing chloroform/DMF solution at 5.7% of the total drug and polymer mass. All other parameters were set identical to those used in the fabrication of the PCL fibers. Electrospinning conditions are summarized in Table 1.

Membranes composed of all four fibers were fabricated using a flat, aluminum collection plate during the electrospinning process. Fibers were allowed to deposit until a film, on the order of one hundred to several hundred micrometers in thickness, had formed. The film was placed under vacuum at 20 mTorr for 2 days before being peeled off of the collection plate.

Scanning Electron Microscopy. The sizes and morphologies of the fibers were examined with a scanning electron microscope (JEOL JSM 6060). All images were captured using an acceleration voltage of 5 kV. The software, ImageJ (National Institute of Health), was used to measure the distributions of fiber diameters from the SEM micrographs.

X-ray Photoelectron Spectroscopy. The X-ray photoelectron spectra of the PCL, PCL/dex, PLLA, and PLLA/dex fibrous membranes were measured with a Physical Electronics Multiprobe. XPS provided a means to quantify the elemental surface compositions of the fibers. The X-ray source was an aluminum anode, powered by 250 W and producing X-rays with a photon energy of 1486.6 eV. The takeoff angle with respect to the sample was 45°, and a pass energy of 100 eV was set to obtain survey spectra between 0 and 1200 eV. Spectral analytical software (CasaXPS) was used to subtract a baseline from each spectrum, integrate the peaks, and quantify the relative atomic surface compositions.

Differential Scanning Calorimetry. DSC measurements were obtained with a PerkinElmerDSC 8000 differential scanning calorimeter. Samples were loaded into an aluminum capsule and heated at a rate of 10 °C/min from 0 to 300 °C concurrently with an empty reference aluminum capsule in an atmosphere of nitrogen gas. DSC thermograms were obtained for each fibrous membrane (6−9 mg samples) as well as for pure dexamethasone (2.4 mg) (Figure 3).

In Vitro Release Measurements. To obtain in vitro release profiles of dexamethasone from the fibers, we submerged samples of
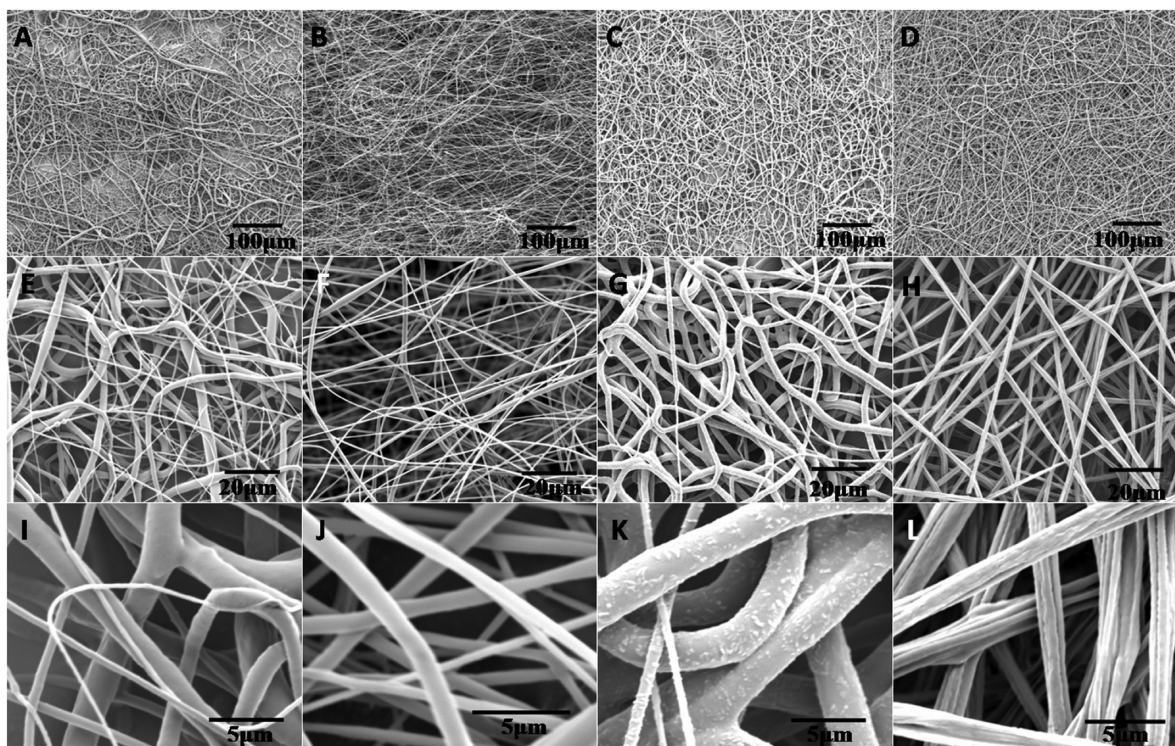
Figure 1. SEM micrographs of electrospun fibers. (A) PCL, (B) PLLA, (C) PCL/dex, (D) PLLA/dex, (E) PCL, (F) PLLA, (G) PCL/dex, (H) PLLA/dex, (I) PCL, (J) PLLA, (K) PCL/dex, and (L) PLLA/dex.

the fibrous membranes in 10 mL of phosphate-buffered saline (PBS), pH 7.4 (GIBCO). The 15 mL vials containing the PBS and fibrous membranes were fixed in a shaker rotisserie (Barnstead Thermolyne) and placed in an incubator (VWR) at 37 °C. At designated time intervals, the PBS medium was replaced, and an ultraviolet-visual spectrophotometer (Cary 100) was used to measure the former medium's absorbance around 241 nm. The concentration of dexamethasone was read off of the calibration curve created for this experiment (not shown), allowing the cumulative amount of dexamethasone released to be calculated.

Subcutaneous Implant Study. To assess the inflammatory response to the fibers, we performed a subcutaneous implant study. Membranes composed of PCL, PLLA, PCL/dex, and PLLA/dex fibers were prepared (as previously described). We anesthetized 250 g female Lewis rats with isoflurane(2%)/oxygen inhalation immediately prior to surgery. The back of each rat was shaved with electrical clippers and then cleansed with three cycles of a wash and rinse with betadine and alcohol, respectively. A longitudinal incision was made along the right side of the posterior portion of each rat's back, and a subcutaneous pocket was created on the medial side of the incision by blunt dissection. A square sample of a fibrous membrane, about 0.5 cm by 0.5 cm with a mass on the order of 2 mg, was placed in the pocket, and the incision was closed with wound clips. All surgeries were carried out in an aseptic field using aseptic technique. Buprenorphine (0.03-mg/kg Reckitt & Colman) was administered preemptively and every 8−12 h thereafter for 24 h. Nine samples of each of the four fibrous membranes were implanted, one sample of one fibrous membrane per rat. After 3 days, 2 weeks, and 4 weeks, three animals from each material group were euthanized and the implants were harvested. The harvested implants were fixed in a 10% neutral-buffered formalin solution (Richard-Allan Scientific) overnight and then stored in a 70/30 (v/v) ethanol/water mixture. Explants were paraffin-embedded and sectioned (5 μm) on a Thermo Shandon Microtome. Sections were stained with hematoxylin and eosin (H&E) and mounted on 3 in. microscope slides.

To evaluate the biocompatibility of samples 3 days post implantation, prior to the formation of fibrous capsules,[27] we quantified cell infiltration by counting the cells present per unit area

within the circumference of each sample cross section. Higher infiltrating cell densities were taken as an indication of poorer biocompatibility.[28] Measurements were made, using the ImageJ software, over two separate areas on each sample.

The biocompatibility of each sample was quantitatively assessed after 2 and 4 weeks by measuring the thickness of the inflammatory capsule formed around a cross section of the implant. The formation of thicker capsules was taken as an indication of poorer biocompatibility.[29−33] Measurements were made at eight points along the circumference of each harvested implant cross section using the ImageJ software.

All animal procedures were conducted according to protocols approved by the Committee on Animal Care at MIT in compliance with NIH guidelines. Research was conducted in compliance with the Animal Welfare Act Regulations and other Federal statutes relating to animals and experiments involving animals and adheres to the principles set forth in the Guide for Care and Use of Laboratory Animals, National Research Council, 1996.

In Vitro Cell Viability Assay. Sections of each fibrous membrane (PCL, PCL/dex, PLLA, PLLA/dex) were adhered to 21 mm glass slides (VWR) using a medical silicon adhesive (Factor II). Membranes were positioned to cover the entire slide, and these slides were placed in poly(2-hydroxyethyl methacrylate)-treated (Sigma-Aldrich) six-well plates (BD-Falcon) (one slide per well) to prevent cell attachment to anything other than the fibrous membranes. The six-well plates containing the scaffolds were sterilized under UV light overnight and washed once with PBS before use.

Human mesenchymal stem cells (hMSCs) (Lonza) were expanded in MesenPRO culture medium (Gibco-Invitrogen) at 37 °C and 5% CO_2. Once expanded, cells were resuspended in hMSC medium (DMEM (Invitrogen) supplemented with 10% (by volume) fetal bovine serum (Invitrogen) and penicillin/streptomycin (Invitrogen) at final concentrations of 100 U/mL (penicillin) and 100 mg/L (streptomycin)).

Cells were plated on the fibrous membranes at a density of 5000 cells/cm^2. 500 μL of the cell solution was placed in the center of each fibrous membrane and incubated for 1 h at 37 °C and 5% CO_2 prior to addition of the remaining culture medium. As a positive control, cells

were plated in the same manner on gelatin-coated glass slides. Four samples were prepared for each of the four fibrous membranes and the positive control.

Cell growth on the fibrous membranes was monitored with the alamarBlue cell viability assay according to the manufacturer's instructions (Invitrogen). In brief, at 1, 4, and 7 days post cell seeding, culture medium was replaced with the working alamarBlue solution (1:10, by volume, solution of alamarBlue to hMSC medium) in each well and allowed to incubate for 2 h. Four 100 μL samples of the supernatant from each well were collected and read on a fluorescence plate reader (Perkin-Elmer Victor). The working alamarBlue solution was then replaced with hMSC medium in each well.

Cell Staining. Cells were fixed by incubation in a 4% (m/v) solution of formaldehyde in PBS for 15 min. The cell membranes were then permeabilized by incubation in a 0.1% (by volume) solution of TritonX-100 (Sigma Aldrich) in PBS. After the membranes were permeabilized, cells were incubated in a 0.2 μg/mL solution of phalloidin-tetramethylrhodamine B isothiocynate (TRITC) (Sigma Aldrich) in PBS for 45 min. Finally, the cells were incubated in a 1.5 μg/mL solution of 4′,6-diamidino-2-phenylindole (DAPI) (Sigma Aldrich) in PBS for 5 min and then washed with PBS. Before each step, the supernatant was removed from the culture wells, and the cells were washed with PBS. TRITC and DAPI fluorescently labeled the cytoskeleton (actin filaments) and nuclei, respectively. The stained cells were analyzed with a wide-field Zeiss Axiovert 200 M microscope (Carl Zeiss) equipped with DAPI, rhodamine, and fluorescein isothiocyanate (FITC)/green fluorescent protein (GFP) filters. Electrospun fibers exhibited fluorescence signals under the GFP filter without additional staining. In the images (Figure 8), actin filaments, nuclei, and scaffold fibers are shown in red, blue, and green, respectively.

■ RESULTS AND DISCUSSION

Fiber Size, Morphology, and Drug Loading. Fiber sizes and morphologies were examined using a scanning electron microscope. SEM micrographs of the PCL, PLLA, PCL/dex, and PLLA/dex fibers can be seen in Figure 1. The fibers appear defect free and fairly uniform in morphology within each sample. The average diameters of the PLLA and PCL fibers are ~1 μm, and those of the dexamethasone-encapsulating fibers are ~2 μm (Table 2). The encapsulation of dexamethasone by

Table 2. Molar Surface Compositions and Fiber Diameters[a]

	C 1s (%)	O 1s (%)	F 1s (%)	$d \pm \sigma$ (μm)
PCL	76.00	24.00	0.00	1.2 ± 0.9
PCL/dex	74.15	25.65	0.20	2.2 ± 0.7
PLLA	61.73	38.27	0.00	0.7 ± 0.3
PLLA/dex	63.48	36.52	0.00	1.8 ± 0.4

[a]Symbols d and σ represent the averaged measured diameters and sample standard deviations, respectively.

PCL and PLLA during the electrospinning process does appear to have affected the fiber surface morphologies. High-resolution SEM micrographs (Figure 1I−L) show rough, jagged surfaces of the PCL/dex fibers and smooth, ruffled surfaces of the PLLA/dex fibers; whereas the PCL and PLLA fiber surfaces appear smooth and free of bumps or ruffles. Fibers containing dexamethasone were fabricated to have a 5.7% composition (by mass) of the drug so that the total quantity implanted would be comparable to doses given as intraperitoneal injections to rodents.[34]

Determination of Surface Compositions. The jagged surfaces of the PCL/dex fibers were possibly a result of phase separation of dexamethasone and PCL, indicating that the

PCL/dex fibers may have a different surface composition or distribution of dexamethasone than the PLLA/dex fibers. The surface compositions were measured using XPS. The X-ray photoelectron spectra of the fibrous membranes gave a quantitative measure of the elemental surface compositions. A very small peak corresponding to elemental fluorine[35] is seen on the spectrum of the PCL/dex fibers around 689 eV (Figure 2B). Fluorine is unique to dexamethasone in this system;
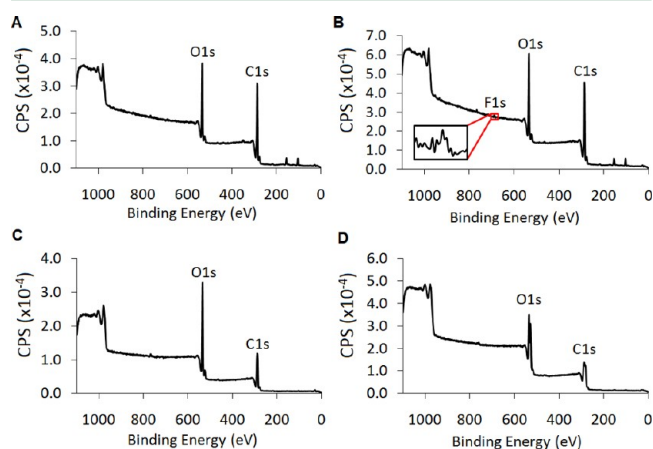

Figure 2. XPS spectra of electrospun fibers. (A) PCL, (B) PCL/dex, (C) PLLA, and (D) PLLA/dex. CPS stands for counts per second.

therefore, the presence of fluorine on the surface indicates the presence of dexamethasone. Integration of the X-ray photoelectron spectrum suggests that the surfaces of the PCL/dex fibers consist of 0.2%, by mole (excluding hydrogen), atomic fluorine (Table 2). The absence of a fluorine peak in the X-ray photoelectron spectrum of the PLLA/dex fibrous membrane (Figure 2D) indicates that the surface concentration of fluorine is below the detectable limit of the spectrometer and thus below that of the PCL/dex fibrous membrane.

The X-ray photoelectron spectrum of the PLLA/dex fibrous membrane (Figure 2D) contains wide/multiple carbon and oxygen peaks as opposed to the single, narrow peaks present in the spectrum of the PLLA fibrous membrane (Figure 2C). This is indicative of intermolecular interactions[36] and suggests that the encapsulated dexamethasone formed intermolecular interactions, such as hydrogen bonds, with the poly(L-lactic acid).

The narrow carbon and oxygen peaks on the X-ray photoelectron spectrum of the PCL/dex fibrous membrane suggest that the intermolecular interactions that may have formed between PLLA and dexamethasone during the electrospinning process were weaker or absent between PCL and dexamethasone. This finding, along with the fluorine surface concentration measurements and the SEM images, suggests possible phase separation between dexamethasone and PCL.

Examination of Thermal Properties. To investigate further the interaction between dexamethasone and the scaffolding materials, we examined the thermal properties of the electrospun fibers using DSC. The crystalline melting peak of pure dexamethasone was detected to be ~260 °C (Figure 3A). DSC thermograms for the PCL and PCL/dex fibers both show endothermic peaks, indicative of the melting point (T_m), at 60 °C (Figure 3B). Similarly, encapsulation of dexamethasone did not appear to affect the glass-transition temperature (T_g) or the T_m of the fibers composed of PLLA, as the PLLA
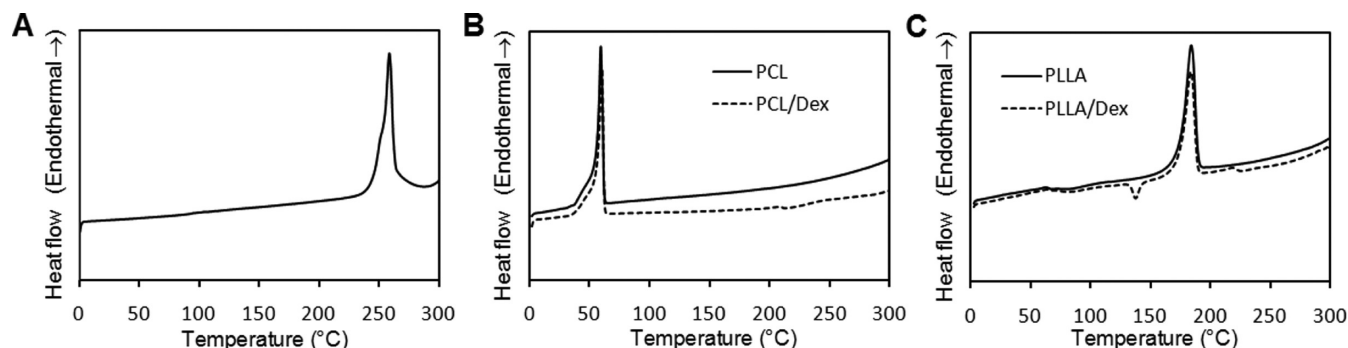
Figure 3. Differential scanning calorimetry thermograms. (A) Dexamethasone, (B) PCL and PCL/dex fibers, and (C) PLLA and PLLA/dex fibers.

and PLLA/dex fibers were both found to have a T_g and T_m of 67 and 183 °C, respectively (Figure 3C). However, a cold crystallization exothermic peak (T_c) appears at 138 °C in the DSC thermogram of the PLLA/dex fibers but not the PLLA fibers (Figure 3C). This may indicate that an interaction between dexamethasone and PLLA affected the crystallization of the PLLA/dex fibers.

The degree of crystallinity (X_c) was determined according to eq 1.

$$X_c = \frac{\Delta H_m - \Delta H_c}{\Delta H_m^0} \qquad (1)$$

where ΔH_m is the endothermic enthalpy of melting, ΔH_c is the exothermic enthalpy of cold crystallization, and ΔH_m^0 is the endothermic enthalpy of melting for a perfect PLLA crystal. The ΔH_m values of the PLLA and PLLA/dex fibers were determined to be 52.9 and 50.4 J/g, respectively, whereas the ΔH_c of the PLLA/dex fibers was determined to be 4.9 J/g. ΔH_m^0 has been found to be 91 J/g.[37] Therefore, by eq 1, X_c is 0.58 and 0.50 for the PLLA and PLLA/dex fibers, respectively. This 8% difference in crystallinity is not trivial considering that the overall concentration of dexamethasone in the fibrous scaffolds was only 5.7%. This suggests a significant PLLA-dexamethasone interaction at the molecular level.

The endothermic melting peaks corresponding to dexamethasone are seen at 209 and 218 °C in the DSC thermograms of the PCL/dex and PLLA/dex fibers, respectively (Figure 3). These are significantly lower temperatures than the observed T_m of pure dexamethasone (260 °C). This downward shift is consistent with what has been previously reported for PCL blended with dexamethasone.[38]

Taken together, these results suggest that dexamethasone has a strong interaction with PLLA and possibly interacts with PCL in the PLLA/dex and PCL/dex fibers, respectively.

In Vitro Release of Dexamethasone. The in vitro release of dexamethasone varied greatly between the PCL/dex and PLLA/dex fibers. Over half of the dexamethasone released was released from the PCL/dex fibers in the first 20 min, almost 90% after 40 min, and nearly 100% after 90 min (Figure 4A). In contrast, the PLLA/dex fibers displayed a sustained release of dexamethasone for over 1 month. This sustained release followed a much less severe burst release of ∼25% of the dexamethasone (Figure 4B). The hypothesis of strong intermolecular interactions existing between dexamethasone and PLLA in the PLLA/dex fibers and possibly weaker interactions existing between dexamethasone and PCL in the PCL/dex fibers is consistent with these observations. Strong intermolecular interactions would cause the release of dexa-
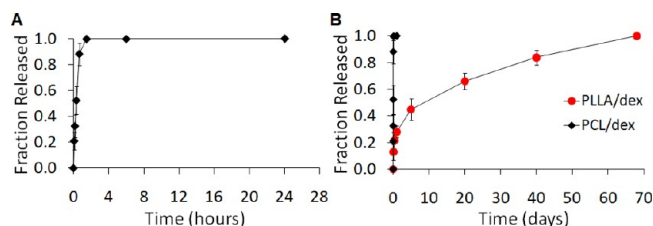

Figure 4. Dexamethasone released normalized to total dexamethasone released from PCL/dex and PLLA/dex fibers in PBS. Measurements acquired until release ceased or fibers degraded. (A) Released from PCL/dex fibers. (B) Released from PCL/dex and PLLA/dex fibers. Error bars indicate mean ± s.d. ($n = 3$ for each data point).

methasone from the PLLA/dex fibers to be more dependent on the rate of material degradation than on free diffusion, whereas the absence of these strong interactions may explain the severe burst release of dexamethasone observed from the PCL/dex fibers.

Foreign Body Response to Subcutaneously Implanted Fibers. To determine if the controlled release of dexamethasone could mitigate an in vivo host response, we performed a subcutaneous implant study in Lewis rats. Upon harvest, the retrieved implants were nonadherent to the dermis or subcutaneous muscle tissue, rather they moved freely within the intermediate connective tissue. Therefore, harvesting the samples with the surrounding tissues of their original in vivo environments was not feasible, and only the implants and adherent tissues were harvested. Histological analysis revealed frayed inflammatory capsules on a portion of the samples, and all samples examined displayed some degree of cell infiltration.

The early stages of the foreign body response are marked by the presence of inflammatory cells such as monocytes and differentiated macrophages. If the inflammatory stimuli persists, then the implant is walled off by a fibrous capsule.[39] As mentioned in the Materials and Methods section, the early stage foreign body responses were assessed by measuring cell infiltration into the subcutaneous implants, and the later stage responses were assessed by measuring the thicknesses of the inflammatory capsules.

Cell infiltration into the PLLA/dex fibrous membranes was, on average, an order of magnitude less than that into the PLLA fibrous membranes after 3 days (Figure 5B). This difference can be seen in representative images of the H&E stained cross sections of the PLLA and PLLA/dex fibrous implants harvested at this time (Figure 6A,B). Very little difference in cell infiltration was observed between the PCL and PCL/dex fibrous membranes (Figure 5B). Representative images of the H&E stained cross sections of the harvested PCL and PCL/dex
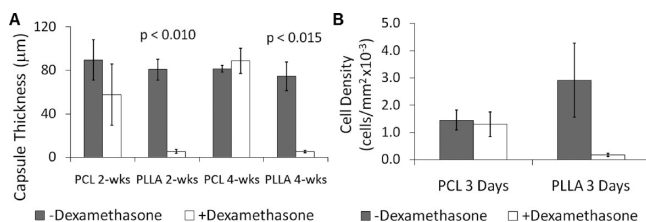
Figure 5. (A) Inflammatory capsule thickness of harvested subcutaneous implants 2 and 4 weeks post implantation. The p values were obtained from two-tailed, unequal variance, Student's t tests. (B) Cell infiltration into harvested subcutaneous implants 3 days post implantation. Error bars indicate mean ± s.d. ($n = 3$ for each data point, except $n = 2$ for PLLA 3 Days + Dexamethasone).
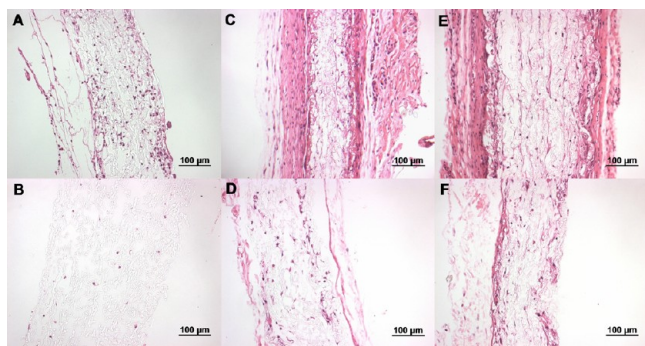

Figure 6. Representative images of H&E stained cross sections of PLLA and PLLA/dex fibrous membranes harvested after subcutaneous implantation. (A) PLLA 3 days. (B) PLLA/dex 3 days. (C) PLLA 2 weeks. (D) PLLA/dex 2 weeks. (E) PLLA 4 weeks. (F) PLLA/dex 4 weeks.

fibrous implants can be seen in the Supporting Information as Figure S1.

The PLLA/dex fibers induced the formation of a much thinner inflammatory capsule than the PLLA fibers after 2 and 4 weeks post implantation. Statistical significance was shown using a two-tailed, unequal variance, Student's t-test and obtaining p values of less than 0.015 for both time points. No significant difference was observed between the PCL and PCL/dex fibrous implants (Figure 5A and Supporting Information, Figure S1). The variation in capsule thickness can also be seen in representative images of the H&E stained cross sections of the PLLA and PLLA/dex fibrous implants harvested after 2 and 4 weeks (Figure 6C−F).

Because the formation of thicker inflammatory capsules[29−33] and higher infiltrating inflammatory cell densities[28] are indications of poorer biocompatibility, the results of these subcutaneous implant studies provide evidence that the release of dexamethasone from the PLLA/dex fibers was effective at reducing the inflammatory response after 3 days, 2 weeks, and 4 weeks post implantation (Figure 5). Furthermore, these results suggest that a controlled release of dexamethasone is required to reduce effectively the foreign body response because the PCL/dex fibers, which displayed a severe burst release in vitro, did not elicit a less severe inflammatory response than the PCL fibers.

In Vitro Cell Viability. Assessing the viability of proliferating cells on the fibers fabricated in this study was important because these fibers are intended to serve as scaffolds for regenerating tissues. Human mesenchymal stem cells were

selected as model regenerating cells, and the alamarBlue viability assay was used to assess their viability in vitro.

The fluorescence emission at 610 ± 10 nm by the samples collected from the alamarBlue viability assay are reported in Figure 7 as the increase in intensity of emission by the
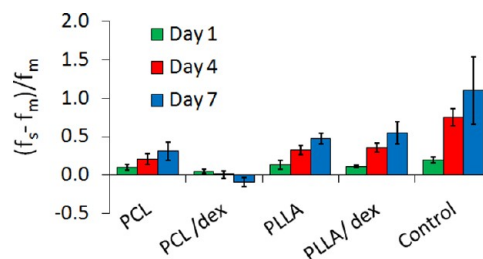

Figure 7. AlamarBlue viability assay results. f_s is the intensity of emission from the supernatant of the cells in culture on the fibrous membranes and f_m is the intensity of emission from the culture medium alone. Emissions were measured at 610 ± 10 nm. Error bars indicate mean ± s.d. ($n = 4$).

supernatant of the culture wells (f_s) from the intensity of emission by the culture medium alone (f_m), normalized by the intensity of emission by the culture medium alone. The intensity of emission by the supernatant of a culture well was taken as an indicator of the quantity of viable cells present in that well.

As seen in Figure 7, the fluorescence emission intensity of the supernatant collected from cells seeded on PCL, PLLA, and PLLA/dex fibrous membranes and on the coverslips coated with gelatin (the control) increased over time. The fluorescence emission by the supernatant collected from the wells containing cells seeded on the PCL/dex fibrous membrane decreased to a value very near that of the culture medium alone. Because the intensity of fluorescence provided a measure of the quantity of living cells in each culture well, this indicates that cells seeded on PCL, PLLA, and PLLA/dex fibrous membranes and those in the control group proliferated (consistent with in vitro assays performed to examine cell viability on other dexamethasone releasing electrospun fibers[19,20]), whereas those seeded on the PCL/dex fibrous membranes died.

Images of fluorescently stained human mesenchymal stem cells attached to the fibers four days after seeding can be seen in Figure 8. No cells are seen in the image of the PCL/dex fibrous membrane (Figure 8B).

The alamarBlue viability assay showed that human mesenchymal stem cells will attach to and proliferate on the PCL, PLLA, and PLLA/dex fibers, where human mesenchymal stem cells were used as model cells for a potential regenerating tissue. The results of this assay suggest that a scaffold fabricated from these fibers will allow for cellular attachment and proliferation − both necessary components of regeneration. Cell death on the PCL/dex fibers may have been caused by high concentrations of dexamethasone in the culture medium due to the severe burst release. High concentrations of dexamethasone have been shown to be lethal to human glioblastoma cells in culture.[40]

To our knowledge, this is the first study to demonstrate, in vivo, that a controlled release, but not a burst release, of an anti-inflammatory drug from an electrospun scaffold reduces the degree of the host's foreign body response to the scaffold over a period of time relevant to tissue regeneration.
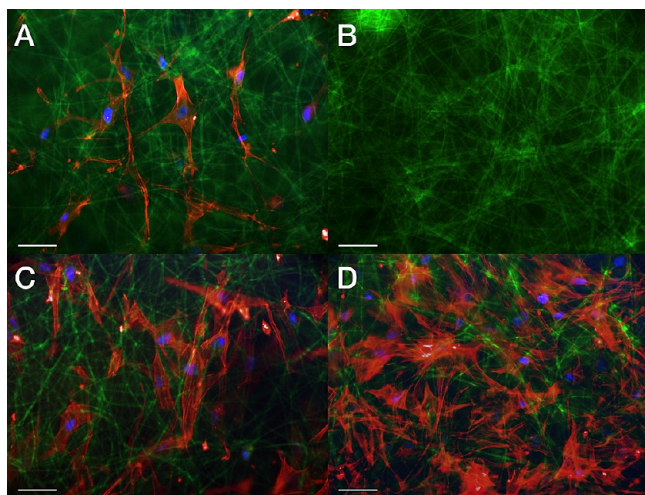
Figure 8. Fluorescence micrographs of human mesenchymal stem cells on the fourth day of culture seeded on the fibrous membranes. Actin filaments, nuclei, and scaffold fibers are shown in red, blue, and green, respectively. Scale bars represent 50 μm. Fibrous membranes are (A) PCL, (B) PCL/dex, (C) PLLA, and (D) PLLA/dex.

CONCLUSIONS

The biocompatibility of a material is a crucial consideration when selecting a tissue engineering scaffold. Failure of an implant may result from an inflammatory response much more severe than that observed under optimal conditions.[39] This study shows that the controlled release of dexamethasone from the PLLA/dex fibers can reduce the inflammatory response upon implantation in rats by delivering dexamethasone locally and that regenerative cells can attach to and proliferate on these fibrous scaffolds in vitro. These results demonstrate the great potential utility of these fibers in regenerative medicine applications where controlling inflammation is a crucial consideration.

ASSOCIATED CONTENT

Ⓢ Supporting Information

Images of H&E stained PCL and PCL/dex subcutaneous implants are provided. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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