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Characterization and *in vitro* cytocompatibility of piezoelectric electrospun scaffolds

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ABSTRACT

Previous studies have shown that electrical charges influence cell behavior (e.g. enhancement of nerve regeneration, cell adhesion, cell morphology). Thus, piezoelectric scaffolds might be useful for various tissue engineering applications. Fibrous scaffolds were successfully fabricated from permanent piezoelectric poly(vinylidene fluoride–trifluoroethylene) (PVDF-TrFE) by the electrospinning technique. Scanning electron microscopy and capillary flow analyses verified that the fiber mats had an average fiber diameter of 970 ± 480 nm and a mean pore diameter of 1.7 μm, respectively. Thermally stimulated depolarization current spectroscopy measurements confirmed the piezoelectric property of the PVDF-TrFE fibrous scaffolds by the generation of a spontaneous current with the increase in temperature in the absence of an electric field, which was not detected in the unprocessed PVDF-TrFE powder. Differential scanning calorimetry, thermogravimetric analysis, X-ray diffraction and Fourier transform infrared spectroscopy results showed that the electrospinning process increased the crystallinity and presence of the polar, beta-phase crystal compared with the unprocessed powder. Confocal fluorescence microscopy and a cell proliferation assay demonstrated spreading and increased cell numbers (human skin fibroblasts) over time on PVDF-TrFE scaffolds, which was comparable with tissue culture polystyrene. The relative quantity of gene expression for focal adhesion proteins (measured by real-time RT-PCR) increased in the following order: paxillin < vinculin < focal adhesion kinase < talin. However, no differences could be seen among the TCPS surface and the fibrous scaffolds. Future studies will focus on possible applications of these cytocompatible PVDF-TrFE scaffolds in the field of regenerative medicine.

1. Introduction

Electrospinning of polymers into fibrous scaffolds is a promising approach to mimicking the extracellular matrix of cells [1]. Electospun meshes have fiber diameters which can range from ~100 nm up to several micrometers, depending upon the polymer and spinning conditions, and provide a beneficial structural feature for cell adhesion and growth owing to their large surface-to-volume and high aspect ratios [1]. Scaffolds produced using this process are of interest for various tissue engineering applications, such as blood vessel [2], cartilage [3], nerve [4] and heart muscle regeneration [5]. Recent advances in the electrospinning process have resulted in scaffolds that are highly porous with large pore structures for potential use in the reconstruction of more complex, three-dimensional tissues, such as bone, which require vascularization and cellular infiltration [6–8].

A number of earlier studies have shown that electrically charged surfaces influence cell behavior, e.g. growth of different cell types [9], enhancement of nerve regeneration [10] and cell adhesion and morphology [11,12]. All these experiments have been performed on two-dimensional polymer films and coatings. However, it is known that a three-dimensional environment is necessary to ensure that the cells are able to organize into complex tissues [13]. Thus, an electrical charged fibrous scaffold could be a promising approach for a number of tissue engineering applications (e.g. nerve, bone, cartilage regeneration). Commonly used piezoelectric polymers (e.g. poly(vinylidene fluoride) (PVDF)) have to be mechanically stretched followed by corona poling in order to induce a net dipole in the materials. Unlike PVDF, its piezoelectric copolymer, poly(vinylidene fluoride–trifluoroethylene) (PVDF-TrFE) (Fig. 1) does not need mechanical stretching or corona poling to achieve a dipole-containing crystal structure which provides permanent polarization in the polymer [14]. Electrical stimulation of cells via attachment to these fibrous meshes might lead to improved cellular interaction and tissue growth compared with non-piezoelectric scaffolds. Hence, the permanent piezoelectric polymer PVDF-TrFE was chosen for this study and explored for its potential to form electrospun fibrous scaffolds. This report describes the successful fabrication and characterization of PVDF-TrFE fibers. Moreover, the *in vitro* cytocompatibility of these fibrous scaffolds relative to the tissue culture polystyrene (TCPS) surface is investigated. TCPS is widely used in expanding and maintaining cell cultures and can be considered the gold standard for *in vitro* cell experiments.

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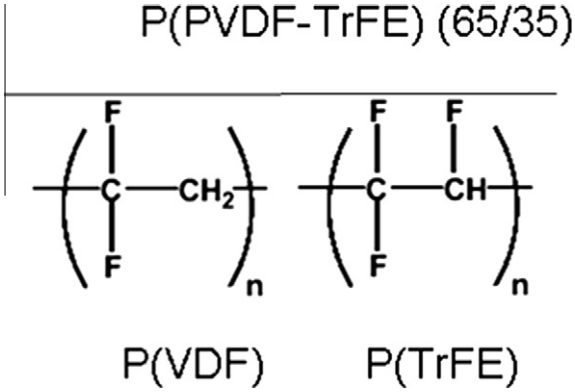

Fig. 1. Chemical structure of piezoelectric PVDF-TrFE copolymer.

2. Materials and methods

2.1. Fabrication of the scaffold by electrospinning

The electrospinning setup used for the fabrication of scaffolds consists of a syringe pump (Cole Parmer, Vernon Hills, IL), a syringe containing a polymer solution, a needle attached to the syringe, a grounded collector (aluminum plate) and a high voltage power supply (Gamma High Voltage, FL). The basic principle behind this process is that an electrical voltage sufficient to overcome the surface tension of the polymeric solution causes the polymer droplets to elongate and eject very fine fibers which, when deposited, form non-woven mats. Poly(vinylidene fluoride–trifluoroethylene) (65/35) (PVDF-TrFE; Solvay Solexis Inc., NJ, USA) was dissolved in methylethylketone (MEK; J.T. Baker, NJ, USA) and electrospun into fibers according to commonly used optimization procedures [15]. The parameters to optimize the fiber formation were applied voltage (15–35 kV) and polymer solution concentration (12–18%) (w/v). The meshes were kept under vacuum for at least 24 h to remove traces of the organic solvent from the polymer fibers.

2.2. Fiber morphology, fiber diameter and pore size

The fiber morphology and average fiber diameter of the scaffolds were evaluated by scanning electron microscopy (SEM) using a LEO 1530 Gemini (Germany) instrument. Mats were sputter-coated with carbon and viewed using an accelerating voltage of 8–10 kV and a working distance of 8–12 mm. The fiber size of electrospun scaffolds was determined from the SEM images using Image J software (National Institutes of Health, USA). Diameters of 30 fibers, 15 each from two randomly selected samples, were measured. The average fiber diameter was calculated using the Image J 1.38× software (National Institutes of Health, USA). The mean pore diameter was determined by capillary flow analysis (1100 AEX Capillary Flow Porometer, Porous Materials, Inc., Analytical Services Division, Ithaca, NY) using a sample 2 cm in diameter by 200 μm thick.

2.3. Thermal analysis

The amount of any residual solvent in the electrospun mat was determined by thermogravimetric analysis (TGA; TA Q50, New Castle, DE). TGA measures the amount and rate of weight change in a material as a function of temperature in a controlled atmosphere. These tests were conducted in an inert atmosphere (dry nitrogen, flow rate 40 mL min^{-1}) at a heating rate of 10 °C min^{-1} and maximum temperature 300 °C.

Differential scanning calorimetry (DSC; TA Q100, New Castle, DE) was used to determine the Curie temperature (T_c), melting temperature (T_m) and heat of fusion (ΔH_f) of electrospun mats compared with the unprocessed powder. The Curie temperature, or Curie transform, is unique to PVDF-TrFE, where it can transform from a ferroelectric (piezoelectric or beta crystal structure) to a paraelectric (non-piezoelectric or alpha crystal structure) state only by alternating its structural conformation at this temperature. DSC measures the heat flow associated with thermally active transitions such as crystallization, melting and glass transitions. A heat-cool-heat temperature programming protocol was used in DSC experiments with a heating rate of 10 °C min^{-1} and cooling rate of 5 °C min^{-1} in the range −70 °C to +250 °C. The crystallinity of samples was calculated using the following relation:

$$X_c(\%) = (H_{fs}/H_{ft}) \times 100 \quad (1)$$

where H_{fs} is the measured heat of fusion for melting of sample, and H_{ft} is the heat of fusion for 100% crystalline PVDF-TrFE. The H_{fs} takes into account both the ferroelectric and paraelectric crystal structures, since it is above the Curie temperature [16]. The 100% crystallinity value of PVDF-TrFE for calculating the crystallinity of the mats is 45 J g^{-1} [17].

Thermally stimulated depolarization current (TSDC) experiments were conducted with a TherMold TSC 9000 TSC/RMA instrument. Thermally stimulated polarization current protocol was used, which measures the current as dipolar structures orient as the temperature is increased [18]. The PVDF-TrFE electrospun mat or powder was sandwiched between two pieces of Teflon film and heated from −60 to 140 °C in the absence of an externally applied field at 7 °C min^{-1}.

2.4. X-ray diffraction

X-ray diffraction (XRD) patterns of electrospun and powder PVDF-TrFE were recorded on an X'pert Pro Diffractormeter (PW3050/60, Philips, Netherlands). The samples were irradiated with monochromatized Cu Kα (1.54056 Å) X-ray source with a step size (2-theta) of 0.05° and scan step time (s) of 1.0. The operating voltage and current used were 45 kV and 40 mA, respectively. The samples were scanned in the 2-theta range 10–30°.

2.5. Fourier transform infrared spectroscopy

Fourier transform infrared (FTIR) analysis was performed using the Perkin Elmer FTIR-ATR 100 series. The spectra were collected in the range 1600–200 cm^{-1} with a resolution of 4 cm^{-1} and 30 scans. The spectra were collected for electrospun and unprocessed powder PVDF-TrFE.

2.6. Cell culture

Human skin fibroblasts were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA) and were cultured in complete medium, consisting of Eagle's minimum essential medium (EMEM; ATCC) supplemented with 10% fetal bovine serum (Hyclone, UT, USA), and 1% antibiotic–antimycotic mix (Invitrogen). Cells were maintained at 37 °C and 5% CO_2 in T-175 culture flasks (NunC Nunclon) and harvested at 80–90% confluency, then 1×10^5 cells were seeded per well in 24-well plates. Cell culture on PVDF-TrFE scaffolds was performed in 24-well ultra-low attachment plates (Corning). Cell culture on 24-well TCPS plates (Beckon Dickinson Falcon) served as a control. Cell density and integrity were established at the time of plating by hemocytometer counts and trypan-blue dye exclusion.

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