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Three-dimensional piezoelectric fibrous scaffolds selectively promote mesenchymal stem cell differentiation

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ABSTRACT

The discovery of electric fields in biological tissues has led to efforts in developing technologies utilizing electrical stimulation for therapeutic applications. Native tissues, such as cartilage and bone, exhibit piezoelectric behavior, wherein electrical activity can be generated due to mechanical deformation. Yet, the use of piezoelectric materials have largely been unexplored as a potential strategy in tissue engineering, wherein a piezoelectric biomaterial acts as a scaffold to promote cell behavior and the formation of large tissues. Here we show, for the first time, that piezoelectric materials can be fabricated into flexible, three-dimensional fibrous scaffolds and can be used to stimulate human mesenchymal stem cell differentiation and corresponding extracellular matrix/tissue formation in physiological loading conditions. Piezoelectric scaffolds that exhibit low voltage output, or streaming potential, promoted chondrogenic differentiation and piezoelectric scaffolds with a high voltage output promoted osteogenic differentiation. Electromechanical stimulus promoted greater differentiation than mechanical loading alone. Results demonstrate the additive effect of electromechanical stimulus on stem cell differentiation, which is an important design consideration for tissue engineering scaffolds. Piezoelectric, smart materials are attractive as scaffolds for regenerative medicine strategies due to their inherent electrical properties without the need for external power sources for electrical stimulation.

1. Introduction

Tissue engineering or regenerative medicine offers a promising approach to repair damaged tissues by combining cells with biomaterials that act as scaffolds to facilitate tissue growth. The biomaterial can be designed to mimic the native tissue extracellular matrix providing appropriate cues for desired cell function. Endogenous electrical fields have been well established during embryonic development, wound healing and limb regeneration (Reviewed in Ref. [1]). The electrical activity generated can be associated with extracellular matrix materials, such as collagens [2] and glycosaminoglycans (GAGs) [3], which display piezoelectric activity. Specifically, they are capable of converting mechanical strain into electrical output. Tissues, such as bone and cartilage, which contain these materials, have been known to display electrical behavior when subjected to loading or deformation [4–6]. Yet, this phenomena of piezoelectricity has largely been unexplored as a potential scaffold strategy in the tissue engineering field (Reviewed in Refs. [7,8]).

The development of smart materials for biological and biomedical applications is an emerging field. Combining biological entities such as DNA, cells or tissues with soft or flexible piezoelectric materials can yield devices that can dynamically sense and adapt to environmental cues with or without the use of external stimuli. Yet flexible, piezoelectric biomaterials have only been in the form of thin-films, tubes, non-woven or aligned fiber membranes, and isolated fibers (Reviewed in Refs. [7,8]), which limits their use for tissue regeneration applications or as *in vitro* tissue

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models. For the growth of three-dimensional (3-D) tissues, we have fabricated 3-D piezoelectric fibrous scaffolds made of poly(vinylidene fluoride − trifluoroethylene) (PVDF-TrFE), which displays the greatest piezoelectric activity of known polymers [9,10], are biocompatible [11,12] and have been shown to stimulate cell function in a variety of cell types [12–19]. Previous studies have reported the fabrication of electrospun PVDF-TrFE fiber based nanogenerators and characterized their piezoelectric properties under compressive loads or displacements [20,21], making them suitable for self-power generators for energy harvesting applications. This level of output also may induce biological activity. The advantage of using PVDF-TrFE fibrous scaffolds is that externally applied electrodes are not needed wherein electrical stimulation can be generated through physiological movement.

In this study, piezoelectric PVDF-TrFE fibrous scaffolds were evaluated for promoting stem cell differentiation and *in vitro* tissue growth as a first study in demonstrating the potential of 3-D flexible smart materials as tissue engineering scaffolds. PVDF-TrFE scaffolds were either electrospun (as-spun) or electrospun and subsequently heat-treated (annealed) to increase the polar/piezoelectric β-phase crystal content and piezoelectric properties. Mesenchymal stem cell (MSC) differentiation towards chondrogenic and osteogenic lineages on these scaffolds was examined in a dynamic bioreactor where cyclic compression was applied at a physiological frequency, activating the piezoelectric properties of the scaffold. Polycaprolactone (PCL), a non-piezoelectric control, was used since PVDF-TrFE cannot be processed into a non-piezoelectric form due to its piezoelectric β-phase content. Although PCL has a different surface chemistry from PVDF-TrFE, PCL was chosen primarily due to its slow degradation rate and ease in fabricating fibrous scaffolds similar in morphology and size to PVDF-TrFE scaffolds. Additionally, PCL is well known for its biocompatibility with many cell types and is in clinical use [22]. We hypothesized that piezoelectric scaffolds undergoing dynamic compression will enhance chondrogenesis and osteogenesis and *in vitro* tissue formation. Findings demonstrated that MSC differentiation was dependent upon the level of piezoelectric activity of the scaffold, where lower levels promoted chondrogenesis and higher levels promoted osteogenesis. Piezoelectric activity promoted greater differentiation as noted by both matrix synthesis and gene expression than mechanical loading alone, demonstrating the effect of electromechanical stimulation on MSC differentiation.

2. Results

2.1. 3-D piezoelectric scaffold fabrication and characterization

3-D scaffolds were prepared using the electrospinning technique. Electrospinning is a dynamic process where an electric field is applied to an ejecting polymer solution resulting in the formation of fibers that collect on a grounded plate. Conventional electrospinning has the limitation of producing two-dimensional (2-D) sheets or membranes however, we were able to fabricate thick, continuous electrospun scaffolds (Fig. 1A) using a two power supply setup, in contrast to the commonly used one power supply setup, where both the spinneret and grounded plate are charged. Electrospun scaffolds can mimic the fibrous extracellular matrix and provide a large surface area, which has been shown to influence cell attachment and protein adsorption [23] (Fig. 1B–D). Depending on the application, scaffolds properties such as porosity, fiber size and orientation can be customized by modifying the electrospinning process parameters and polymer concentration. Micron sized fibers with large interfiber spacing and porosity for cellular infiltration and tissue growth were achieved for both as-spun and annealed PVDF-TrFE and PCL. No statistical differences were detected between the three scaffold groups for fiber diameters, inter-fiber spacing and porosities (Table 1). In addition, air-water contact angle measurements were performed to assess hydrophobicity and no statistical differences were detected between the three scaffold groups.

Scaffolds were characterized for material properties. No significant differences were detected for the tensile Young's moduli between all three groups, however, the ultimate tensile stress was significantly lower for PCL scaffolds compared to PVDF-TrFE scaffolds (Table 1). The ultimate tensile strain was significantly higher for as-spun PVDF-TrFE (125.17 ± 0.21%) compared to other groups demonstrating its relatively high flexibility. All three groups had compressive Young's moduli on the order of kPa demonstrating their relative softness. As determined by fourier transform infrared (FTIR) spectroscopy, x-ray diffraction (XRD), and differential scanning calorimetry (DSC) (Table 1), the annealed PVDF-TrFE had significantly higher piezoeolctric β-phase fraction and crystallinity than as-spun PVDF-TrFE. Annealing is used with PVDF-TrFE films and recently demonstrated with fibers to enhance degree of crystallinity of the β-phase by choosing annealing temperatures above the Curie temperature (T_c) but below the melting temperature (T_m) [24–26]. In this study, 135 °C was chosen as the annealing temperature, which was between T_c at 113 °C and T_m at 147.4 °C for electrospun as-spun PVDF-TrFE scaffolds as determined by DSC.

2.2. Piezoelectric characterization of scaffolds

Electrical output from the bulk scaffold was determined by applying dynamic compression (Fig. 1E–G). In dry conditions, the voltage output increased linearly with increasing level of deformation and increased with frequency for both PVDF-TrFE scaffolds (Fig. 1H&I). Annealed scaffolds had a higher voltage output than as-spun PVDF-TrFE. The electric fields were approximately 20 mV/mm for as-spun and 1 V/mm for annealed PVDF-TrFE, which corresponds to endogenous electrical fields during early development [27]. PCL did not generate any electrical output. For wet conditions, we immersed the scaffold in saline and measured the streaming potential [28] of the surrounding fluid when the scaffolds were subjected to dynamic sinusoidal compression at 1 Hz and a deformation of 10%, which was the same loading condition as the bioreactor. The streaming potential for the annealed PVDF-TrFE was 61.1 ± 1.5 μV, which was significantly higher than the as-spun PVDF-TrFE scaffolds having a streaming potential of 25.2 ± 2.5 μV. No streaming potential was detected for PCL. To our knowledge, this is the first study characterizing the streaming potential generated by fibrous piezoelectric scaffolds. The streaming potentials were similar to values determined for bone [29].

Piezoresponse force microscopy (PFM) was used to demonstrate piezoelectric behavior in the electrospun fibers. We used PFM switching, with the AC excitation voltage applied on top of a DC bias, to examine the ferroelectric polarization switching at three different locations in as-spun and annealed PVDF-TrFE and PCL fibers. As shown in Fig. 2A–D the displacement amplitude and phase response of the PVDF-TrFE fibers manifest the well-known butterfly loop and hysteresis, which is the signature of ferroelectric materials. The annealed fibers exhibited a higher piezoresponse than that of as-spun ones, as shown by the piezoelectric displacements for the annealed (Fig. 2C) and as-spun samples (Fig. 2A). The control non-piezoelectric PCL fibers do not show the characteristic behaviors as expected (Fig. 2E&F).

2.3. MSC chondrogenic differentiation promoted on piezoelectric scaffolds

The piezoelectric scaffolds, as-spun and annealed PVDF-TrFE,