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Electrospun fiber constructs for vocal fold tissue engineering: Effects of alignment and elastomeric polypeptide coating

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ABSTRACT

Vocal fold lamina propria extracellular matrix (ECM) is highly aligned and when injured, becomes disorganized with loss of the tissue's critical biomechanical properties. This study examines the effects of electrospun fiber scaffold architecture and elastin-like polypeptide (ELP4) coating on human vocal fold fibroblast (HVFF) behavior for applications toward tissue engineering the vocal fold lamina propria. Electrospun Tecoflex™ scaffolds were made with aligned and unaligned fibers, and were characterized using scanning electron microscopy and uniaxial tensile testing. ELP4 was successfully adsorbed onto the scaffolds; HVFFs were seeded and their viability, proliferation, morphology and gene expression were characterized. Aligned and unaligned scaffolds had initial elastic moduli of ~14 MPa, ~5 MPa and ~0.3 MPa, ~0.6 MPa in the preferred and cross-preferred directions, respectively. Scaffold topography had an effect on the orientation of the cells, with HVFFs seeded on aligned scaffolds having a significantly different ($p < 0.001$) angle of orientation than HVFFs cultured on unaligned scaffolds. This same effect and significant difference ($p < 0.001$) was seen on aligned and unaligned scaffolds coated with ELP4. Scaffold alignment and ELP4 coating impacted ECM gene expression. ELP4 coating, and aligned scaffolds upregulated elastin synthesis when tested on day 7 without a concomitant upregulation of collagen III synthesis. Collectively, results indicate that aligned electrospun scaffolds and ELP4 coating are promising candidates in the development of biodegradeable vocal fold lamina propria constructs.

1. Introduction

Voice disorders affect up to 9% of the population at any time, resulting in hoarseness or complete voice loss [1] and can be caused by misuse, hyperfunction, medical conditions or as a result of surgery [2]. The response of the vocal fold to an injury can cause scar tissue, changing the organization and composition of the vocal fold lamina propria, altering the biomechanical properties of the tissue and subsequent production of mucosal wave, resulting in abnormal voice quality [2]. At the present time, there are few treatment options for vocal fold scarring and research utilizing tissue engineering strategies to design effective treatments for vocal fold scarring have included growth factor therapies, cell therapies and biomaterials [3–9]. Biomaterials aim to be biocompatible and biodegradable, improve matrix production and reduce scar tissue, as well as have biomechanical properties that closely mimic native vocal fold tissue.

Electrospinning, a versatile technique, produces nano to microfibers by applying electrical fields to an ejected polymer solution. Electrospun biomaterials provide a structural network for the cells to grow on during repair and regeneration. The topographic alignment of nanofibers has been demonstrated to influence cell orientation in the fiber direction, morphology and proliferation. Moreover, because many of the body's tissues, including the vocal fold, have aligned structured ECM networks, the ability for electrospinning to produce scaffolds with highly aligned fiber formations is being widely utilized [10]. Fiber alignment and pretreating scaffolds with fibroblast cells improve embryonic stem-cell-derived cardiomyocytes differentiation, indicating that fiber alignment may be an important parameter for engineering myocardial tissue [11]. Further, higher fibroblast cell adhesion has been shown to be greater on randomly aligned electrospun collagen scaffolds than aligned scaffold after 7 h; however, there was greater proliferation on the aligned scaffold over a 7-day period; morphology also followed the alignment of the fibers [12]. Fiber alignment of scaffolds has been shown to affect gene expression, and morphology of Schwann cells [13], as well as morphology

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and proliferation of fibroblast cells and smooth muscle cells [12,14]. Due to the highly aligned and organized nature of the vocal fold lamina propria [15], growth on an aligned scaffold may affect the morphology and gene expression of vocal fold fibroblasts, in turn promoting matrix synthesis and matrix organization improving healing, and resulting in improved biomechanical properties of the tissue.

Elastin protein makes up ~9% of the total protein component in the vocal fold lamina propria, which is greater than that of skin, but less than that of the lungs and arteries [16]. Elastin protein is typically only produced during the pre- and neonatal stages of development, with fibroblasts secreting minimal amounts during adult years [17]. The structure of elastin needs to support numerous expansions and recoil for tissue function, and as such elastin fibers are often combined with collagen fibers to regulate stretching and to prevent tissue damage [16]. In healthy vocal folds, elastin is organized in long, somewhat parallel fibers with collagens whereas elastin fibers in scarred vocal folds are disorganized and collagen bundles are dense and thick, while the elastin fibers are sparse [18,19]. Elastin-like polypeptides (ELPs) derived from the elastin gene and produced using recombinant methods have been shown to have similar properties to native elastin, including the ability to self-assemble [20,21], and can be modified to contain specific amino acid sequences for the desired functionality. A family of ELPs has been purified, expressed and designed to contain alternating crosslinking domains and hydrophobic domains similar to those of tropoelastin [20,21]. Specifically, ELP4 has a 20-24-24-24-24 gene sequence, with the crosslinking domains 21 and 23 between the hydrophobic domains. The sequencing in exon 24 contains the repeating sequence VGVAPG.

Introducing elastin as a coating on biomaterials could provide cell signaling for improved cell growth and regeneration. ELPs have been investigated as a coating for vascular applications, reducing platelet activation and thrombogenicity on synthetic materials in vitro [22,23] and as elastin coatings on poly(glycerol sebacate) scaffolds, which have been shown to promote endothelial progenitor cell adhesion and proliferation [24]. Human skin fibroblasts have an elastin receptor, a 67 kDa glycoprotein and elastin derived polypeptides (EDPs) coated on Petri dishes, or added to media to promote cell growth [25]. Further, EDPs promote MMP-2, which degrades collagen type IV and has some elastinolytic ability [26]. Such activity could aid in tissue remodeling of disorganized elastin and collagen fibers evident in vocal fold scar tissue.

Despite the potential uses of nanofibers for vocal fold tissue engineering applications, little information is available on the influence of aligned and unaligned constructs on cell behavior, as well as the addition of an elastin coating on these nanofibers, for a soft tissue whose biomechanical properties are vital for function. The aim of this study is to use an elastomeric electrospun biomaterial seeded with vocal fold fibroblasts as an in vitro model system to evaluate morphology, viability, proliferation and gene expression in response to material architecture (aligned and unaligned) with and without an elastin polypeptide (ELP) surface coating. Results will guide the future development of constructs for vocal fold tissue engineering.

2. Materials and methods

All chemicals were from Sigma-Aldrich, St Louis, MO, unless otherwise stated.

2.1. Electrospinning Tecoflex™ scaffolds

Tecoflex™ SG-80A beads (Thermedics, Wickliffe, OH) were first dissolved in chloroform (3% w/v), then filtered through Whatman 40 paper, and cast into a mold that was placed in a vacuum oven overnight then stored in a desiccator until use. In preparation for electrospinning, solutions of 10% w/v concentration were made by dissolving the mold in dichloromethane (DCM) (Fisher Scientific, Ottawa, ON). Electrospun scaffolds were made using a 10 w/v.% solution and loaded into a 5 ml plastic syringe (Becton Dickenson, Franklin Lakes, NJ) with a 1" 22 gauge blunt end needle (Kontes Glass Company, Vineland, NJ) and placed on a syringe pump (KD Scientific, Holliston, MA) running at 1 ml h^{-1}. Electrospinning was carried out under ambient conditions in a ventilated area on a custom-made electrospinning apparatus. The grounded collection mandrel was positioned at a distance of 25 cm from the needle tip. A high power voltage supply (Gamma High Voltage Research Inc, Ormond Beach, FL) applied a voltage of 15 kV to the needle tip. To make the aligned scaffold, the mandrel was set at a translation speed of 3 m s^{-1} and a rotation of 410 cm s^{-1}. To make the unaligned scaffold, the mandrel was set at a translation speed of 3 m s^{-1} and a rotation of 5 cm s^{-1}. Scaffolds were made to a thickness of ~50–80 μm (~45 h), measured by a caliper. Completed scaffolds were left on the mandrel for 2 days to ensure solvent removal, and then placed in a desiccator until use.

2.2. Scaffold characterization

Scaffolds were characterized by imaging with a scanning electron microscope (SEM). Disks of the scaffold were punched out using a 6 mm biopsy punch, and placed on metal stubs using carbon tape. Punches were gold coated for 10 min on a pulse setting. Images were taken using an SEM (Model Jeol JSM-840, Queen's University, Mechanical and Materials Engineering Department) with an accelerating voltage of 10 kV. Using ImagePro Premier the fiber diameters and angles for the aligned and unaligned scaffolds were measured manually from 300 fibers using a random dot generator from at least three different images taken for each scaffold type. In order to determine pore sizes for each condition, SEM images were analyzed in Adobe Photoshop. Using the scale bar the number of pixels corresponding to 1 μm was determined for each pore.

2.3. Mechanical properties

Mechanical properties of the scaffolds were determined using uniaxial testing, and testing was performed under ASTM D1708. Scaffolds were preconditioned at ~24 °C and 55% humidity for 48 h. Aligned and unaligned samples were tested in the preferred and cross-preferred directions, with preferred being parallel to the mandrel rotation, and cross-preferred being perpendicular to the mandrel rotation. At least five samples were tested for each condition at a size of 1 cm wide and 2 cm long. To make handling of the samples easier for testing, paper windows were cut with an inner length of 1.5 cm. A strip of double-sided tape was placed on the top and bottom of the windows and scaffold pieces were placed lengthwise on top. Samples were then placed in the grips so that the gauge length was the 1.5 cm window length. Testing was done using a Mach-1 device (Human Mobility Research Center, Queen's University) with a 10 N load cell, and a crosshead speed of 9 mm min^{-1}. Samples were stretched until failure or the maximum displacement of the machine. The cross-sectional areas of the scaffolds were estimated using the mass of the sample, the density of the polymer and average fiber diameter to determine the number of fibers and in turn the cross-sectional area of all fibers [27]. Initial modulus was calculated from the stress–strain graphs, and statistical analysis was performed using one-way ANOVA with Bonferroni post hoc analysis.