An Electrospun Biomaterial for the Application of Vocal Fold Constructs

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Introduction:

Voice disorders can have drastic negative effects on the quality of a person's life, including depression, inability to perform in the workplace, and poor self-image. Currently there are no viable treatment options for vocal fold scarring that address the underlying cause of the scar tissue and the changing biomechanical properties of the vocal fold. Additionally, many biomaterials have inadequate biomechanical properties in vocal fold applications. The main cause of suboptimal biomechanical properties is the composition and organization of the matrix during wound healing, resulting in stiff scar tissue. This work focuses on developing a novel biomaterial using a non-biodegradable polyurethane (Tecoflex®) coated in an elastin-like polypeptide (ELP) to promote growth, proliferation, and matrix synthesis of human vocal fold fibroblast cells (HVFFs). ELP4 is an ELP previously designed by the Woodhouse lab that has properties similar to native elastin, and reduces platelet activation. Initially, aligned and unaligned scaffolds are made using the process of electrospinning, and characterized using SEM images and mechanical testing. Lastly the viability and matrix synthesis of immortalized human vocal fold fibroblast cells are evaluated with proliferation assays, qPCR, and confocal microscopy.

Materials and Methods:

Electrospun scaffolds were made using a solution of 10% Tecoflex® and dichloromethane. Two scaffolds structures were made; an aligned fiber scaffold and an unaligned fiber scaffold. The fiber diameters and angles were measured on SEM images using ImagePro software. The scaffolds were tested uniaxial in two directions to ASTM D1708 standards, and stretched to break to determine initial modulus, tensile stress, and strain at break. Scaffolds were coated in 50ug/mL fibronectin overnight. Before seeding half of the scaffolds were coated in 1mg/mL ELP4 in a 70% EtOH solution for one hour. VFFs were seeded onto 6mm diameter scaffolds at a cell density of 50,000 and tests were conducted on days 3 and 7 during culture. Cell viability was determined using Live/Dead® staining. Proliferation was determined with PicoGreen and AlamarBlue assays. Morphology was determined with DAPI and TRITC conjugate phalloidin, as well as alpha-smooth muscle actin (α-SMA) and COL1. For gene expression studies, the scaffolds used are 5mm x 1.5mm, seeded with 500,000 cells. Gene expression levels of fibronectin, collagen I and III, elastin, decorin, and fibromodulin were determined with qPCR after 7 days. Statistical analysis was performed with a two-tailed independent t-test or ANOVA with Bonferroni post-hoc analysis.

Results:

The scaffolds have been successfully produced with both aligned and unaligned fibers. The fiber diameters are very similar between the two alignments, and there is minimal fusion and beading of the fibers. The Fastin Elastin assay positively concluded that the ELP4 successfully coated onto the scaffolds. Cell passages between 7 and 13 were used for seeding, and were determined to be viable from the Live/Dead images (Figure 1) (n=3, N=3), and confluence occurred by day 7. The cells on the scaffolds were shown to proliferate over the 7 day period, and the ELP4-coated scaffolds appeared to have a higher amount of cells with the unaligned ELP4 coated scaffold having the highest (n=3, N=6). The scaffold alignment appears to affect cell morphology (Figure 2). The cells seeded on the aligned scaffolds seem to have the same axis angle as the scaffold fibers, with little

variation. The cells on the unaligned scaffold appear to have more variation in the angle of cell axis, however by day 7 the cells appear to align with each other, and look similar to what is seen on TCP at confluence (n=3, N=3). There is no evidence of α-SMA on any conditions. The gene expression studies show relatively little difference between the gene levels on all conditions, however all levels are elevated compared to the housekeeping gene B-actin (n=3, N=3).

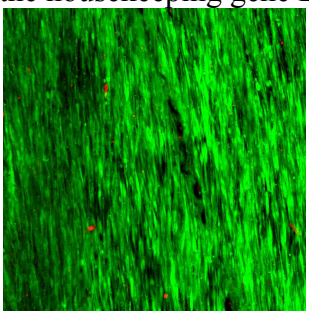
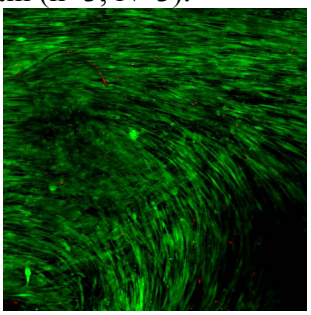
a)  b) 

Figure 1: Live/Dead images (40x) of a) aligned scaffold and b) unaligned scaffold on day 7 (green: live cells, red: dead cells)

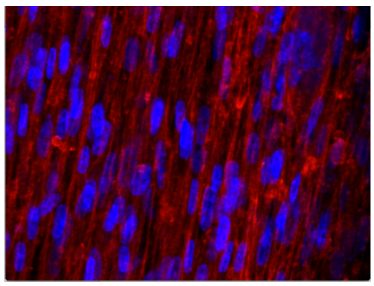
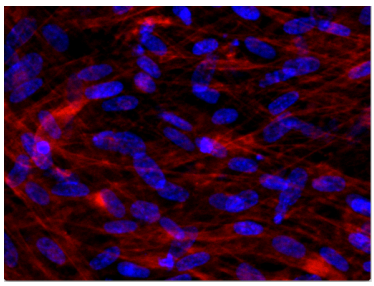
a)  b) 

Figure 2: Focal adhesion and DAPI (40x) of a) aligned and b) unaligned scaffold on day 7 (red: actin filaments, blue: nucleus)

Discussion:

As vocal fold scarring is very difficult to treat, alternative treatment options that will address the underlying changes in the tissue are of great need. Aligned and unaligned fiber scaffolds have been successfully created using an electrospinning technique, and cells have been shown to be viable and grow to confluence on the surface. Scaffold fiber alignment can be used to affect the morphology of HVFF cells, which may improve vocal fold tissue properties. It is anticipated that an ELP4 coating may provide better adhesion properties and promote cell seeding and proliferation onto scaffolds. The scaffolds may also hold the potential to provide an environment for enhanced gene expression of matrix components, improving wound healing. Overall, this study develops a scaffold that may promote HVFF proliferation, matrix gene expression, as well as optimal cell morphology. Further studies are required to develop a biodegradable scaffold with similar properties in an effort to reduce scar tissue in vocal folds, and improve biomechanical properties.