Decellularized Human Vocal Fold as a Scaffold for Laryngeal Tissue Engineering

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Statement of Purpose: Dysphonia due to vocal fold scarring is one of the most difficult voice disorders to treat. The scar tissue is caused by a change in extracellular matrix (ECM) composition that increases the stiffness of the vocal fold and decreases its ability to vibrate. Treatments such as voice therapy, fat augmentation, or ECM injections are limited because they do not restore the normal ECM composition and so the mechanical properties of the vocal fold remain stiff. More radical approaches, such as a tissue engineered vocal fold, may provide successful therapy by completely replacing the scarred tissue. In the present study, we developed a scaffold from decellularized human vocal folds to serve as a potential, tissue engineering therapy for dysphonia.

Methods: Human vocal fold tissues (n=4) were dissected from cadaveric larynges and then decellularized by immersing them in 1% sodium dodecyl sulfate (SDS) for 96 hours under agitation. The vocal folds were indented before and after SDS treatment to determine the Young's modulus *E*. The decellularized vocal folds and untreated (control) samples were then sectioned and stained with hematoxylin and eosin (H&E), Verhoeff- Van Gieson (EVG), Masson's trichrome (MT), and fluorescent antibodies to laminin to assess for decellularization and preservation of elastin, collagen, and laminin.

Results: After 96 hours of SDS treatment, we obtained a translucent scaffold that was devoid of cells yet retained the original shape of the vocal fold. Below (Fig. 1) is a panel that shows day by day decellularization progress with both the cover (epithelium and lamina propria) and thyroarytenoid (TA muscle) facing up.

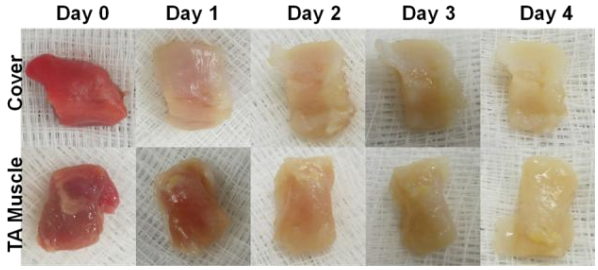

Figure 1. Decellularization of human vocal folds.

The vocal folds were also indented before and after decellularization to obtain the Young's modulus. It is important to maintain the Young's modulus within an optimal range because the Young's modulus determines the patient's fundamental frequency and the subglottic pressure, or effort, needed to initiate phonation. The decellularized vocal folds remained within a physiological stiffness range and did not change significantly: 7.0±2.0kPa (untreated) to 6.0±0.6kPa (treated; p=0.54, Fig. 2).

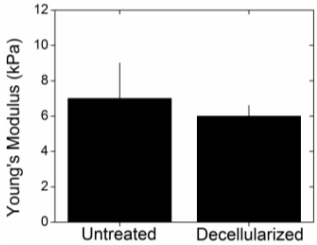

Figure 2. Young's modulus before and after decellularization.

To assess for complete decellularization, the sections were stained with H&E and MT, which revealed an absence of cells in the treated sample throughout the vocal fold cover and TA muscle layer (Fig. 3). MT also showed preservation of collagen fibers (blue), which will allow the scaffold to maintain tensile strength, transmit tension during phonation, and protect elastin from overextension.

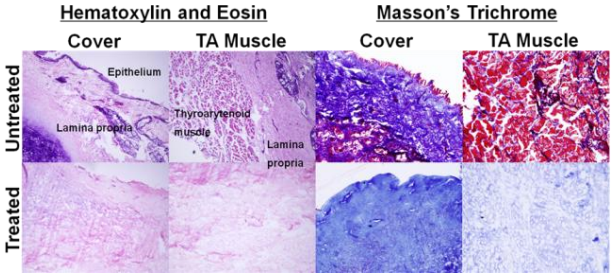

Figure 3. H&E and MT staining before and after decellularization.

Next, we investigated whether elastin and laminin were preserved. Elastin was found in the intermediate layer of lamina propria in the untreated folds (black), and qualitative inspection of decellularized vocal folds revealed preservation of elastin content, fiber length, and organization, e.g. running parallel to the mucosa. Elastin preservation is essential because it supplies elastic recoil and allows for repeated cycles of vibration. Laminin (red) was found in the basement membrane and muscle layer, and was also preserved after treatment. Along with collagen, laminin allows the potential for cell adhesion and infiltration when our scaffold is reseeded with cells.

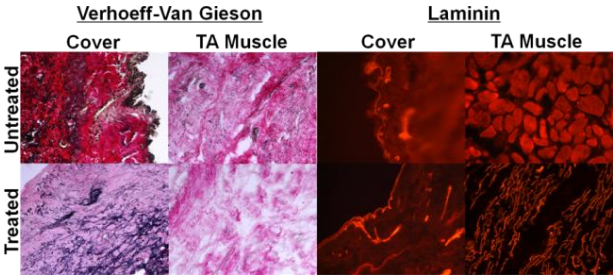

Figure 4. EVG and laminin immunofluorescence staining before and after decellularization.

Conclusions: We have developed a naturally derived scaffold from human vocal fold tissue that preserves the Young's modulus, collagen, elastin, and laminin. Because the above characteristics are important for vocal fold function, these results are promising for its tissue engineering applications. Plans for the immediate future include further characterization of the scaffold (e.g. quantifying DNA before and after decellularization), reseeding the scaffold with adipose-derived stem cells, and testing the scaffold's phonation properties.