Abstract

Optical coherence tomography (OCT) is a promising technology for imaging laryngeal tissue, however, very little is known how the components of vocal fold tissue contribute to OCT images. We sought to describe the optical characteristics of collagen by selectively removing it from vocal fold tissue via enzymatic degradation. Swine larynges were split along the midline and either a collagenase-containing solution or control solution without collagenase was injected into the lamina propria of each hemilarynx. OCT imaging was performed before injection and up to 3 hours after injection. Mean pixel intensity (MPI) as a function of image depth was extracted using the ImageJ analysis software at two points per vocal fold: one at the injection site and another not at the injection site. Two-tailed P values were calculated using paired t-tests to compare MPIs. Collagen disruption was confirmed using Picrosirius Red staining following imaging. There was a significant increase in MPI below the lamina propria after 3 hours at the injection site in samples treated with collagenase (p-value < 0.05). At non-injection sites, there was no significant change in MPI (p-value = 0.4519). In vocal folds injected with control solution, there were no statistically significant changes in MPI at injection sites or non-injection sites (p-values = 0.8432, 0.7093, respectively). These results suggest collagen content limits OCT image depth in vocal fold tissue.

Introduction

Optical coherence tomography (OCT) noninvasively creates 2-dimensional images using infrared light [1]. This technology provides a potentially useful technique for assessing laryngeal tissue without the risk of scar development associated with diagnostic biopsy. Previously, OCT has been used to describe normal and abnormal vocal fold pathology in adults and children [2]. And OCT has also been used to demonstrate changes in vocal fold structure as a function of age [3]. However, very little is known how the normal components of vocal fold tissue contribute to OCT images. Collagen, elastin, and hyaluronic acid are major extracellular matrix components of the lamina propria [4]. Since collagen makes up to 40% of the lamina propria, we sought to explore the optical characteristics of collagen by selectively removing it from vocal fold tissue through enzymatic degradation.

Materials & Methods

Five fresh swine larynges were divided along the midline. India ink dots were placed along the true vocal fold for reference. Collagenase was injected into the midmembranous lamina propria of five hemilarynges between the two ink dots. Collagenase-free solution was injected into another five hemilarynges as controls. A VCSEL swept source-OCT system was used to obtain images pre- and post-injection and after 45, 90, and 180 minutes of incubation.

The ImageJ software was used to calculate MPI at two regions of interest below the lamina propria: one below the injection site and another located elsewhere to be used as an internal control for each hemilarynx. India ink dots were used as reference points to ensure consistency in the location where data extraction was performed across images of the same sample. Two-tailed P values were calculated using paired t-tests to compare MPIs.

Results

Example hemilarynges 3 hours after injection with collagenase solution (Fig. 1a) and control solution (Fig. 1b) are shown. Ink dots, true vocal fold (TVF), and injection sites are indicated for reference. Example OCT image series for collagenase injections and control injections are shown in Fig. 1c and 1d. Epithelium (Epi), lamina propria (LP), and thyroarytenoid muscle (TA) are indicated in the first pane of Fig. 1c for reference. Ink dots are indicated in first pane of Fig. 1d for reference. Injection sites (IS) and Non-injection Sites (NS), shown throughout Fig. 1c and 1d, indicate where MPI was calculated. MPI extraction points were placed with reference to the ink dots to maintain consistency across images.

MPI below the lamina propria at injection sites in hemilarynges injected with collagenase were higher at 3 hours than before injection, p-value = 0.0411 (Fig. 2). MPI below the lamina propria at non-injection sites in hemilarynges injected with collagenase were not significantly different at 3 hours, p-value = 0.4519. MPI below the lamina propria at both injection sites and non-injection sites in hemilarynges injected with control solution were not significantly different at 3 hours, p-values = 0.8432, 0.7093 respectively.

Discussion

These results suggest collagen content limits OCT image depth in vocal fold tissue. Although the conclusions from this study are limited by the fact that the collagenase did not remove all the collagen in the tissue samples, these results may be useful in interpreting OCT images during the management of laryngeal disorders defined by altered collagen content such as vocal fold scarring.

References


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