

Development of the Human True Vocal Fold: Depth of Cell Layers and Quantifying Cell Types Within the Lamina Propria

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Objectives: We sought to further describe the development of the 3-layered human vocal fold in children and to quantify macrophage and myofibroblast concentrations in each layer.

Methods: We used an optical analysis software package to examine 8 longitudinally sectioned human vocal folds that had been fixed in formalin (ages 2 days to 14 years).

Results: The 2-day-old vocal fold contained only a monolayer of cells. This became a bilayer by 5 months, and a trilayer began to become evident by 7 years. The percent of total depth represented by the superficial layer of the lamina propria (SLP) gradually decreased with age. The SLP made up 22% of the total lamina propria by age 7 years; this percentage approximates that in the adult vocal fold. Macrophages and myofibroblasts were predominately found in the SLP, and began to be apparent by 11 months of age.

Conclusions: These results help describe the development of human voice and may have implications as to when phonosurgical therapy can be considered for children.

Key Words: development, lamina propria, larynx, phonosurgery.

INTRODUCTION

The modern description of the microanatomy of the human vocal folds has been properly attributed to Minoru Hirano. It was in his seminal work entitled "Phonosurgery: Basic and Clinical Investigations" that he defined the trilaminar structure of the human vocal fold.¹ These layers were labeled the superficial (SLP), intermediate (MLP), and deep (DLP) layers of the lamina propria. The lamina propria is found between the overlying surface epithelium and the underlying vocalis muscle.

Hirano defined the 3 lamina propria layers by examining the amounts of fibrous proteins that were found within each. The SLP lacks elastic and collagenous fibers and appears loose and pliant. This layer is also known as Reinke's space.¹⁻³ The MLP is composed of primarily elastic fibers and is "stiffer" than the superficial layer. The DLP is composed primarily of collagen fibers and together with the MLP is called the vocal ligament.¹⁻³

The lamina propria levels have previously been defined by staining for elastin and collagen content within each layer. This has been done with both light^{4,5} and electron microscopy.⁶ Hirano et al found that newborns did not have a true lamina propria, but had 2 distinct cellular regions consisting of the

anterior and posterior maculae flavae. Within these regions they found numerous immature fibroblasts, elastic fibers, collagenous fibers, and ground substance.^{7,8} One theory is that phonation after birth stimulates these cells to form the vocal ligament.⁷

The lamina propria is instrumental in producing voice. This function is often explained by use of the cover-body theory of phonation. Hirano¹⁻³ has defined the cover as the surface epithelium and the SLP. He includes only the vocalis muscle as the body, and labels the MLP and DLP the transition region. Hammond et al,⁴ on the other hand, include a portion of the MLP in their definition of the cover. Regardless of one's definition, the important message is that voice production is reliant on a pliable superficial layer of the vocal fold moving over a more rigid, stationary deeper layer.¹⁻³

The SLP is of particular interest because of its role in voice production and also because of the attention needed to avoid potential injury to this layer during phonosurgical procedures. The age at which this layer is fully developed has yet to be elucidated. Gray et al⁹ and colleagues have suggested that the SLP in the normal adult vocal fold consists of about 25% to 35% of the total depth of the lamina propria. This estimate was based on the elastin fiber concen-

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