Bioengineering of the Vocal Folds

An Essay
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Anatomy of the Vocal Fold

The normal structure of the vocal folds, especially the fine structure of the vocal folds and their movements, is essential to be clear to whoever is dealing with disorders of voice.

Glottic region:

Hirano, (1991) has stated that the glottis is divided into two parts the intermembranous portion, or anterior glottis, and the intercartilagenous portion, or posterior glottis. These two parts are separated by an imaginary line between the tips of the vocal processes. The anterior glottis is bounded by the two vocal folds and assumes a triangular shape. It extends from the anterior commissure anteriorly to the tips of the vocal processes posteriorly. The term "posterior commissure" (i.e. joining together) incorrectly describes the posterior part of the glottis, because the bilateral vocal folds never join posteriorly. It is more correctly called the posterior glottis ***(Mcllwain, 1991).***

Vocal fold structure:

In humans, vocal folds are responsible for the great variety of sounds that can be articulated by humans are produced when the vocal folds move together and apart in a wavelike motion at frequencies of 75 to 1000 Hz ***(Titze, 2007).***

The vocal fold is defined as “a fold-like structure between the anterior commissure and the vocal process of the arytenoid cartilage”. It does not include cartilaginous structures ***(Hirano, 1991).***

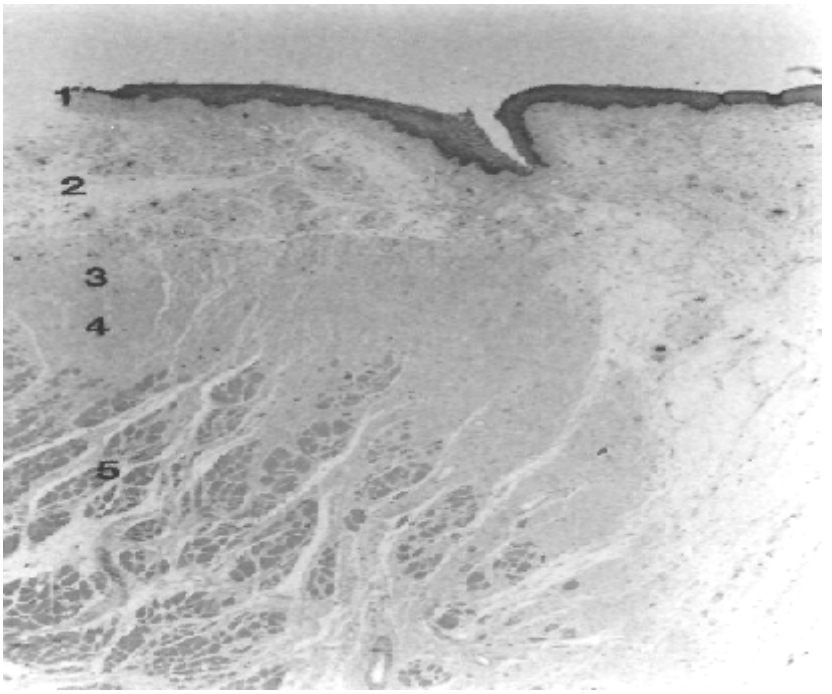

Figure 1. Hematoxylin and eosin - stained coronal section of vocal fold showing normal vocal fold mucosa. Numbers 1, 2, 3, 4, and 5 refer to the stratified squamous epithelium, superficial layer of the lamina propria (Reinke's space), intermediate layer of the lamina propria, deep layer of the lamina propria, and vocalis muscle, respectively. ***(Nicolai et al., 1990)***

Each vocal fold is a laminated structure consisting of a pliable vibratory layer of connective tissue, known as the lamina propria, sandwiched between a membrane (epithelium) and a muscle (vocalis muscle) ***(Hirono et al., 1983).***

The most superficial layer of the vocal fold consists of pseudostratified ciliated columnar epithelium (respiratory epithelium). However, the adducting edge of the vocal fold is lined predominantly with stratified squamous epithelium. Seromucinous glands are found throughout the mucosa, except along the adducting surface. The epithelium rested on a continuous basement membrane ***(Benninger et al., 1996).***

Vocal fold lamina propria:

The lamina propria is multilayered connective tissue, composed of fibroblasts and their secreted extracellular matrix containing elastin, collagen, hyaluronic acid, and proteoglycans come together to form the structural framework that stabilizes tissues and provides mechanical support for cell attachment. The extracellualr matrix plays a very important functional role in cell behavior, including cell proliferation, migration, shape, orientation and, most importantly, cell differentiation. The maintenance and induction of cell differentiation is governed by regulation

of gene expression, which occurs in a tissue-specific manner and dictates how cells carry out their necessary functions. When the composition of the extracelluar matrix of the lamina propria is altered, vocal fold vibratory function can be severely disrupted due to alterations in tissue viscoelasticity ***(Gray et al., 2000)***.

The superficial lamina propria consists chiefly of an amorphous substance ***(Hirono et al., 1983),*** it is this layer that vibrates most significantly during phonation. If this layer becomes stiff, its vibrations are disturbed, and voice problems result ***(Hirano, 1991)*** whereas the intermediate layer and deep layers of the lamina propria are made of fibrous proteins ***(Hirono et al., 1983),*** the intermediate layer is primarily elastic fibers and the deep layer is primarily collagenous fibers. The intermediate and deep layers of the lamina propria together form the vocal ligament. Deep to the vocal ligament is the vocalis muscle that constitutes the body of the vocal fold ***(Hirano, 1991).***The epidermis is secured to the lamina propria through the basement membrane zone ***(Gray, 1999).***

Around the edge of the vocal fold, the elastic and collagenous fibers in the lamina propria, as well as the muscle fibers of the vocalis muscle, run roughly parallel to the edge, this arrangement facilitates vibratory movements of the fold, the

blood vessels in the mucosa run roughly parallel to the edge and all the vessels are very small. This vascular arrangement helps maintaining vibration. Also no glands are evident in the mucosa around the edge of the vocal fold. Glands here would add resistance and impede vibratory motion ***(Hirano, 1991),*** In addition to, absence of lymph vessels thus enhancing vocal fold vibration ***(Weir, 1997).***

At the anterior end of the vocal fold the intermediate layer of the lamina propria is thick and forms an oval mass called the anterior macula flava. At the posterior end of the membranous vocal fold, the intermediate layer of the lamina propria forms another oval mass, the posterior macula flava. The structures at both ends of the membranous vocal fold appear to serve as cushions to protect the ends from possible mechanical injury caused by vocal fold vibration ***(Hirano, 1991).***

Vocal fold muscle layer:

The muscle fibers comprising the vocal folds originate on the thyroid cartilage and insert at the arytenoid cartilages thus called consequence thyroarytenoid muscle or muscles. A "medial bundle" of fibers that arise from the inner surface of the thyroid cartilage just lateral to the medial line and about mid-way between the upper and lower edges of the cartilage is often designated as the vocalis muscle. Contraction of the vocalis and

the thyroarytenoid muscles has varying effects, depending on the positions of the arytenoid cartilages. These cartilages, and consequently the vocal folds, are approximated by contraction of the muscles of the folds ***(Moore, 1996)***.

The vocalis muscle receives its motor innervation via the recurrent laryngeal nerve. Its fibers end in a small number of motor fibers ***(Ruedi, 1959).***

Kotby and Haugen (1970) cleared that this low innervation ratio is suitable for the delicate action of laryngeal muscles, as the vocalis muscle serves mainly for fine isometric tension of the folds i.e., produces tension by rearranging its motor units.

Cover-body complex:

The five histological layers can be re-classified into three sections the cover, consisting of the epithelium and the superficial layer lamina propria, the transitional portion, consisting of the intermediate and deep layers of the lamina propria or the vocal ligament, and the body, consisting of the vocalis muscle ***(Hirano, 1991).***

The vocal fold is a multilayered vibrator, and this is its most important characteristic for vocal function. The "membrane cushion" model of vocal fold structure. This is a two-layer

model in which the surface mucosa of the vocal folds constitutes the "membrane" and the conus elasticus plus the vocalis muscle the "cushion" ***(Hammarberg, 2000)***

From the standpoint of mechanics, the epithelium can be considered as a thin capsule of the vibrator whose purpose is to keep the content of the vocal fold from being blown off during vibration. The superficial layer of the lamina propria can be likened to a mass of soft gelatin, where the elastic fibers are analogous to soft rubber bands and the collagenous fibers are analogous to cotton thread. The muscle, when contracted, is something like a bundle of rubber bands. Tissue stiffness thus increases from the very pliable superficial layer of the lamina propria to the contracting muscle ***(Hirano, 1991).***

Mucociliary blanket:

An important element of the layered structure of the vocal fold is one additional layer outside the vocal fold itself. This is the mucociliary blanket ***(Hirano and Bless, 1993).*** The mucociliary blanket refers to a layer of mucous which covers the vocal folds ***(Kobayashi and Wanner, 1993).*** Without this layer, the vocal fold surface is completely dry and cannot vibrate. Mucus is not created at the edge of the membranous vocal fold, for no glands are located there. Rather, mucus comes from the glands located superiorly, inferiorly, anteriorly and

posteriorly to the edge of the membranous vocal folds ***(Hirano and Bless, 1993)***

Basement membrane zone:

The basement membrane zone is collection of protein and non-protein structures, which together help the basal cells secure themselves to the rather amorphous mass of proteins present in the lamina propria. It is important to point out that some of the proteins in the basement membrane zone, such as collagen type (anchoring fibers), have been shown to be genetically influenced. For example, the average person may have between 80 and 120 anchoring fibers per unit area of their basement membrane zone while someone who has a recessive form of the gene which doesn't create as many anchoring fibers may have only between 40 and 60 anchoring fibers per unit area. Those who are homozygous for the recessive gene will have few or no anchoring fibers. This is makes us aware that genetics may play a role in vocal health ***(Gray, 1999).***

Biological components of vocal folds:

Another useful division of the lamina propria is by biologic components. The lamina propria can be divided into: cellular and non-cellular contents (extracellular matrix) ***(Lander, 1993).*** This is a useful division since the contents of the extracellular

matrix influence the properties of tissue oscillation.

A. Cellular contents:

Catten et al., (1998) surveyed human vocal fold lamina propria for the population-density of cells in the cellular part of lamina propria. He noted that about one-third of humans have a moderate concentration of macrophages present just below the basement membrane zone and the superficial layer of the lamina propria. Their location, right below the epidermis, is suggestive that these cells are present to combat inflammatory agents which are crossing the epithelium. These agents may be bacteria, viruses, or environmental inhalants which are noxious to the tissue. Fibroblasts are cells which maintain the lamina propria. They replace old proteins and manufacture new proteins. They are present with a similar population density in all layers of the vocal fold. Myofibroblasts are fibroblasts which have differentiated into cells of repair. These cells are only present when injury or damage has occurred and repair and construction are needed. Following tissue injury, these cells will show up to provide extracellular matrix repair and construction. Interestingly, these cells are found in the majority of normal human vocal folds. They are at the highest population density in the superficial layer with a decreasing population as tissue depth becomes deeper. This would likely indicate that in the normal

human vocal fold some small amount of tissue injury is constantly present and this tissue which more serious forms of pathologies arise. The basement membrane zone is extremely competent in repairing itself within a 36-48 hour time frame. This is mainly true for microscopic injury.

B. Non-cellular contents:

The extracellular matrix has been divided by the class of molecules that makes up its composition. In the vocal fold lamina propria this division is usually as follows:

1) Fibrous proteins.

2) Interstitial proteins.

3) Interstitial molecules such as carbohydrates and lipids.

The most important fibrous proteins are collagens and elastin. Collagens provide strength and structure to the tissue. It is useful in bearing stress and resisting deformation when subjected to a force. Elastin fibers convey elasticity to the tissue. Elasticity is defined as the ability to be deformed and return to the original shape.

Pawlak et al., (1996) described many of the interstitial proteins. These proteins are listed in table (1).

Proteoglycans	**Function**	**Localization in vocal folds**
Hyaluronic Acid	Creates and is the means for control of tissue viscosity, effects tissue flow resistance and tissue osmosis, provides space filling and space occupying molecules.	Found throughout the ECM of the lamina propria, slightly more intense in the intermediate layer of the Lp, found in macrophages and fibroblasts of the LP.
Decorin	Binds to collagen fibers resulting in delayed fibril formation and thinner fibril formation, may help reduce fibrosis and scar following injury.	Found in the ECM of the lamina propria, may be more concentrated in the SLLP.
Fibromodulin	Binds to collagen fibers resulting in delayed and thinner collagen fibers, both decorin and fibromodulin may affect ligament performance	Found in the ECM of the lamina propria, found mainly in the intermediate and deep layers of the Lp, seems to be concentrated around the vocal ligament
Versican	Has ability like hyaluronic acid to fill space, bind and nize water molecules	Found being manufactured by the fibroblasts and macrophages in the LP
Heparan Sulfate Proteoglycan	Binds to fibronectin, collagen lV, and laminin, may play a role in tissue phogenesis.	Found in the basement membrane zone of the vocal folds, found in the fibroblasts and macrophages of the Lp.

Table l. Proteoglycans found in the human vocal fold ***(Gray, 1999).***

Lp: Lamina propria, SLLP: Superficial lamina propria, ECM: Extracellular matrix

Interstitial proteins affect the properties of viscosity. Viscosity is defined as how easy a substance flows. Interstitial molecules not only affect but often control the viscosity of the vocal folds. Interstitial molecules also convey shock absorption properties to the tissue. Distribution of the interstitial and fibrous proteins is influenced by age and gender and is maintained by the fibroblast. Collagen type IV and fibronectin are produced by fibroblasts and secreted into the extracellular matrix of human tissue. Fibronectin is a high-molecular weight glycoprotein involved in cell-to-cell adhesion, cell-to-substrate adhesion, migration and differentiation of cells, maintenance of cellular structure and blood clotting. As such, fibronectin is involved intimately in tissue response to injury and is found in increased concentration in healing and previously injured tissue. Therefore, fibronectin should be found in higher concentrations in vocal fold nodules and polyps, which represent a response of vocal folds to trauma. Collagen type IV is a supporting substance of both epithelial and endothelial basement membrane ***(Courey et al., 1996).***