3 Microanatomy and Cellular Physiology
Kimberly N. Vinson and C. Gaelyn Garrett

The human vocal folds are unique structures. The microanatomy of these structures dictates the fluidity of the vibration and the quality of sound they produce. An understanding of vocal fold histology and cellular physiology is necessary to understand, diagnose, and treat pathology of the vocal folds.

Vocal Fold Histology
Hirano elegantly described the unique multilayered structure of the vocal folds in 1974 (Fig. 3.1). This layered structure has relevance not only in the maintenance of the mechanical properties of the vocal folds but also in the development of benign vocal lesions. These layers include the epithelium, the lamina propria, and the vocalis muscle. The lamina propria is further subdivided into superficial, intermediate, and deep layers. Each layer has distinct structural and mechanical properties that contribute to the characteristic vibration seen on laryngeal videostroboscopy or high-speed videography. An understanding of the structure and the varying mechanical properties of each layer is necessary to fully understand vocal fold vibration.

Epithelium
The epithelium of the vocal folds is composed of stratified squamous cells that maintain the shape of the vocal folds. The remainder of the glottis, however, has ciliated pseudocolumnar epithelium. Unlike most squamous cells, such as normal skin, the vocal fold epithelium remains metabolically active throughout its life cycle. The surface cells slough off into the laryngeal lumen as new cells migrate superiorly and medially to replace them. The luminal cells exhibit a "microridge" pattern, thus increasing the surface area to better maintain the mucus blanket that covers the vocal fold surface. This bilayer mucus blanket is vital to ensure adequate moisture and lubrication of the vocal folds. The luminal layer of the blanket is mucinous. This layer, which is primarily composed of mucin molecules, is thicker than the two layers and acts to prevent dehydration of the surface epithelium. The inner, serous layer is thinner due to its higher water content. This layer is in direct contact with the squamous cells of the vocal fold epithelium and the cilia of the pseudocolumnar cells of the glottis and facilitates the mucociliary transport of secretions up from the trachea and through the glottis for expectoration. Inhibition of mucociliary clearance can lead to impaired function of the upper respiratory tract.

The epithelium is secured to the lamina propria by the basement membrane zone (BMZ). This zone is a collection of protein and nonprotein structures and is composed of two layers, the lamina lucida and the lamina densa. The lamina lucida is the low-density, clear zone medial to the basal epithelial cells, while the lamina densa is a zone of increased type IV collagen fibers adjacent to the lamina propria. These two layers provide structural support, connecting the epithelium to the underlying lamina propria through anchoring fibers. The BMZ is also essential for repair and maintenance of the epithelium. Chronic disruption of this layer may lead to the formation of a vocal fold nodule.

Clinical Insight
The BMZ is an area of clinical importance to differentiate between carcinoma in situ (CIS) and invasive carcinoma of the vocal folds. CIS of the vocal fold is defined as the presence of atypical cells within the epithelial layer of the vocal fold without evidence of invasion through the BMZ. Once cancerous cells infiltrate the BMZ, the process becomes invasive and the risk of regional and distant metastases increases. Therefore, great care must be taken to dissect below the BMZ when performing a biopsy of a suspicious vocal lesion to ensure that the correct diagnosis is made.
Superficial Layer of the Lamina Propria

The superficial layer of the lamina propria (SLLP), or Reinke space, is a potential space composed of loose, fibrous tissue in an extracellular matrix (ECM). It is extremely pliable and often compared to soft gelatin. Owing to its structure, the SLLP offers little resistance to vibration. The ECM is the key component of the SLLP that allows this motion. The ECM is a mixture of fibrous and interstitial proteins that serve as a scaffold for the SLLP. It provides strength and resilience in allowing the vocal fold to vibrate freely, while allowing the SLLP to maintain its structure. The fibrous proteins, namely collagen type I and elastin, provide the tensile strength needed to maintain the SLLP, while the interstitial proteins, such as hyaluronic acid, decorin, and versican, provide the viscoelastic characteristics of the vocal fold for vibration. These interstitial proteins also give the vocal fold the ability to absorb the shock of repeated impact from phonation. Understanding the microanatomy and the mechanical properties of this layer is extremely important as the majority of benign vocal fold lesions occur in this layer. Vocal fold polyps, nodules, cysts, and polypoid corditis, or Reinke edema, all form in the SLLP.

Hyaluronic acid, or hyaluronan, is perhaps the most important of the ECM proteins. It is a glycosaminoglycan polymer of disaccharides composed of repeating units of glucuronic acid and acetylglucosamine. Owing to its high charge at physiologic pH, hyaluronic acid readily binds water to form a gel-like substance that affects the viscoelasticity of the lamina propria, determining tissue viscosity, osmosis, and flow resistance. This most likely determines the biomechanics of vocal fold vibration.

Intermediate and Deep Layers of the Lamina Propria

The intermediate layer of lamina propria (ILLP) and the deep layer of lamina propria (DLLP) together comprise the vocal ligament, or the transition layer between the overlying epithelium and the SLLP and the underlying vocalis muscle. The ILLP comprises roughly one-half of the lamina propria and contains a high concentration of reticular type III collagen fibers. It is less densely organized than the DLLP, which is composed of type I and type III collagen fibers that penetrate the superficial bundles of the vocalis muscle. The vocal ligament is the free, superior margin of the conus elasticus, which is an elastic membrane that connects the cricoid cartilage to the thyroid and arytenoid cartilages. The ILLP is thickened at the anterior and posterior ends of the membranous vocal fold. These areas are known as the anterior and posterior macula flava. The anterior macula flava serves as a transition zone between the stiff thyroid cartilage and the pliable membranous vocal fold, while the posterior macula flava serves as a transition between the membranous vocal fold and the stiff vocal process of the arytenoid cartilage. This structure allows for the support of increased tensile stress that occurs in vocal tasks that require high-pitched sounds. The vocal ligament is a key landmark for the depth of dissection of a benign laryngeal lesion in laryngeal microflap surgery. Once the epithelium has been incised and the SLLP bluntly elevated, the vocal ligament appears laterally as a shiny, yellow-white strip of elastic tissue.
Studies have shown that 95% of the cells of the macula the lamina propria, that is, the areas of greatest vibration. CD44 is most concentrated in the vocal fold epithelium and in the maintenance of the ECM. The hyaluronic cell receptor signaling calls for fibroblasts to migrate into the wound bed to lay down collagen, elastin, and hyaluronic acid to establish a new ECM. Epithelial cells create a watertight seal over the wound bed. Finally, tissue remodeling occurs and persists up to 12 months after injury. Early scar formation occurs in the first 3 months following injury. During early scar formation in the first 3 months, the scar is thick and stiff. As it matures, it becomes more pliable as reorganization of collagen and elastin fibers occurs.\(^9\)

**Vocalis Muscle**

The vocalis muscle is composed of the most medial fibers of the thyroarytenoid muscle. Its medial insertion is the vocal ligament. The vocalis is stiff compared with the other layers of the vocal fold and has contractile properties that allow for the modification of the effective overall tension of the vocal folds.

**Cover-Body Theory of Vocal Fold Motion**

The five histological layers of the vocal fold work in three increasingly stiffer mechanical layers during vibration. The “cover” is composed of the epithelium and the SLLP. The intermediate and deep layers of the lamina propria comprise the “transition” layer, and the vocalis muscle act as the “body.”

The cover-body theory was proposed by Hirano in 1974 and describes the mucosal wave of the vocal fold. As air passes between the vocal folds, the loose “cover” moves in a wave-like motion over the stiffer “body.” The cover is pliable and elastic, while the body uses its contractile properties to allow for the adjustment of the stiffness of the vocal fold. The overall tension of the vocal fold is dependent on the coupling of cover to the adjustable, muscular body.\(^1\)

**Vocal Fold Wound Healing**

When considering the cellular physiology of the vocal fold, wound healing is perhaps the most important phenomenon to discuss. However, before examining the unique characteristics of vocal fold wound healing, a brief review of the general principles of wound healing should be done. Basic knowledge of this process is crucial for the voice clinician, as most of the treatment that is obtained is aimed at improving injured tissue.

The wound-healing process is a complex cascade that involves inflammation, proliferation, epithelialization, and remodeling. During the inflammation stage, several processes must take place. Hemostasis must be achieved at the wound site. Tissue deficits resulting from the injury must be filled and a matrix to facilitate migration of cells into the wound bed must be formed. All of these processes are achieved with the formation of a fibrin clot. Macrophages and neutrophils then invade the area to clear the wound of contaminants. Cell

Pearls and Pitfalls

Iatrogenic vocal fold scarring can be a devastating complication of vocal fold surgery, causing permanent dysphonia. It may result from over-resection of the SLLP along with surface epithelium during the excision of a benign vocal fold lesion. This most often occurs with a technique called vocal fold stripping. In this technique, the vocal fold epithelium and the SLLP are literally stripped away from the underlying vocal ligament to remove the benign lesion. As this vocal fold wound heals, remucosalization occurs over the area devoid of the SLLP. The result is scarring of the epithelium to the underlying vocal ligament (Fig. 3.3). With the loss of the loose vocal fold cover that typically vibrates over the vocal fold body and the resultant scar to the vocal ligament, the mucosal wave is disrupted. As there is then no way to adequately reconstruct the SLLP, the resulting dysphonia is often permanent.

To avoid iatrogenic scar when excising benign vocal fold lesions, it is imperative to preserve as much as possible all normal epithelium and superficial lamina propria and to avoid trauma to the vocal ligament. With microflap excision of a benign lesion, the epithelium overlying the lesion and the normal surrounding SLLP are preserved as the lesion is excised. This reduces the scarring observed with remucosalization after vocal fold stripping.

**Figure 3.3** Sulcus vocalis. The image demonstrates sulcus vocalis of the right vocal fold in a patient who also has a left vocal fold polyp.
Human vocal folds are unique because they are subjected to nearly continuous mechanical trauma resulting from the phonation that takes place on a daily basis. It is thought that vocal fold tissues have developed specialized functions to withstand the constant stress of phonation. Recently, a great deal of research has been performed to characterize these functions. As it is difficult to study these processes in vivo in humans, animal models have been developed to study the effects of phonotrauma on cellular physiology of the vocal folds.

Changes to the ECM of the lamina propria have great impact on the voice. The lamina propria is maintained by the constant degradation and synthesis of ECM components. Gene expression of a variety of lamina propria proteins, including matrix metalloproteinases (MMPs) and cytokines, is being explored.

MMPs are enzymes that play an important role in wound healing by acting against certain ECM components. The two important MMPs are MMP-1 and MMP-9. MMP-1 is an interstitial collagenase that catalyzes the first step in the degradation of fibrillar collagen types I and III. MMP-9 is both a collagenase and a gelatinase that breaks down basement membrane collagens and gelatins. MMPs are essential for maintaining ECM homeostasis.11

Cytokines are cell-signaling proteins that attract inflammatory cells to an area of injury to initiate the inflammatory phase of the wound-healing cascade. Proinflammatory cytokines interleukin-1 (IL-1) and tumor necrosis factor α (TNF-α) and the pro-cytokine cyclooxygenase-2 (COX-2) play a role in the initiation of the inflammatory response in the vocal fold. IL-1 is activated by macrophages, neutrophils, and fibroblasts and induces the synthesis of procollagen types I and III. TNF-α is produced primarily by macrophages and stimulates the phagocytosis of contaminants in the wound bed. COX-2 is an enzyme that is undetectable in most normal tissues, but becomes abundant in activated macrophages during the inflammatory process to stimulate the conversion of arachidonic acid to prostaglandins.12,13

When considering wound healing in the vocal fold, the differences between acute and chronic phonotrauma should be considered. Acute phonotrauma typically manifests as vocal fold edema or laryngitis. With this type of injury, the vascular network, basement membrane, and ECM are all likely impacted. In 2008, Rousseau et al10 demonstrated an increase in the gene expression of MMP-1 in rabbits receiving acute experimental phonation compared with controls that did not. His group did not find a significant increase in the expression of MMP-9 of IL-1β. In 2010, Rousseau's laboratory demonstrated an increase in the expression of IL-1β, tumor growth factor β1 (TGF-β1), and COX-2 in the setting of acute raised-intensity phonation as compared with modal phonation and sham control in the rabbit model.14 Another group found IL-1α and prostaglandin E2 (PGE2) to be associated with acute wound healing in the rabbit model. Both markers were found to be significantly elevated in the injured vocal fold compared with normal vocal fold.15 Significant changes in TNF-α and MMP-8 have also been observed.16 As discussed previously, data also suggest that hyaluronic acid is a key cellular signal for the maintenance of the lamina propria in response to stress.

Chronic phonotrauma occurs in the setting of repeated episodes of acute phonotrauma that result in long-standing vocal fold damage. This damage could present in the form of vocal fold scarring as seen in benign vocal fold lesions, such as nodules, polyps, and cysts. Vocal fold nodules occur with repeated injury of the basement membrane. Kotby et al17 report intercellular junction gaps, disruption and duplications of the basement membrane, and focal collagen deposition in nodules. Fibronectin, an adhesion molecule primarily located in the basement membrane, is also thought to be increased in nodules. It is also believed that the disruption of the basement membrane places the nodule at an increased risk for repeated injury, leading to propagation of scar over time. Polyps, however, occur in the setting of repeated acute vascular trauma instead of disruption of the basement membrane and have less deposition of fibronectin. While nodules appear to be the end result of wound healing with the deposition of fibronectin as early scar, polyps may be an arrest of the wound-healing process following the inflammatory phase.

Controversy exists when considering vocal fold cysts as the result of the wound-healing process from chronic phonotrauma. Generally, two types of cysts are thought to exist, epithelial-filled cysts and mucus retention cysts. The epithelial-filled cysts that are found at the impact zone of the membranous vocal fold are likely due to repeated phonotrauma at that site.10

Biochemical markers involved in wound healing in the setting of chronic phonotrauma have been studied. In contrast to acute trauma, IL-1 is not upregulated. This supports the hypothesis that the products of chronic phonotrauma demonstrate the entire process of the wound-healing cascade. In the case of polyps, it demonstrates at least progression of the cascade beyond the acute inflammatory phase before arresting. PGE2, however, remains increased even in the setting of chronic phonotrauma, as it is an inflammatory mediator that is ubiquitous in wound healing.17

References