Vocal Fold Scar

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Current Concepts and Management

Edited by

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Introduction

Jaime Eaglin Moore and Robert T. Sataloff.

The vocal fold is a complex layered structure as described by Minoru Hirano[1]. When vocal fold (VF) scar formation occurs, it disrupts the normal architecture of the lamina propria, and the mucosal wave is distorted affecting the quality of phonation. Alterations in the extracellular matrix, and particularly changes in the distribution of collagen, occur with scar formation. These changes affect the viscoelasticity of the vocal fold impairing the mucosal wave.

Many etiologies may cause scar including trauma (iatrogenic and non-iatrogenic), radiation, and inflammatory responses. Of particular concern to the otolaryngologist are iatrogenic causes. Gone are the days when vocal fold stripping was employed commonly. With advancements in phonosurgical techniques and increased understanding of the physiology of the vocal fold, post-operative voice outcomes have improved in the hands of experienced surgeons; but poor results do occur even in the best of hands.

Regardless of advancements in laryngeal microsurgery, vocal fold scar is still common in hoarse patients who present to the otolaryngologist's office. It is often missed on standard laryngoscopy, and the patient is frequently told his or her VFs are "normal on examination". Proper equipment such as strobovideo-laryngoscopy and a trained practitioner are important in establishing the diagnosis of VF scar. Accurate diagnosis is crucial to devise an appropriate treatment plan that may include voice therapy and/or surgery. Accurate diagnosis also is needed to establish realistic expectations for the patient and surgeon.

Many treatment options are available for vocal fold scar from voice therapy to vocal fold reconstruction. Despite the advances in surgical technique and tissue engineering, vocal fold scar is a difficult disorder to treat, and outcomes of all treatments vary widely. The appropriateness of each management option for a patient depends upon the severity of the scar, dysphonia, and vocal effort, as well as the patient's needs. Knowledge of all treatment options and an understanding of the direction of future research are important for the clinician when approaching and counseling these challenging patients. We hope that the material summarized in this book will provide a broad perspective of the state-of-the-art in the diagnosis and treatment of scar that will be of practical value for the clinician.

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Section I

1

Vocal fold anatomy and pathophysiology of scar

Jeanna M. Stiadle and Susan L. Thibeault

Introduction

According to estimates, at any given moment 20.7 million Americans have voice difficulties[1], and one of the primary reasons for dysphonia is vocal fold scar[2]. Vocal fold scar can cause vocal fatigue, hoarseness, and difficulty controlling the voice[3]. Treatment for vocal fold scar is limited secondary to a poor understanding of the biological mechanisms involved in this disorder. Scientists have begun investigating the cellular and molecular mechanisms involved in vocal fold scarring to better understand the complex processes involved in its manifestation[4]. More importantly, understanding these processes is necessary for clinicians as they strive to deliver effective treatments to ensure positive outcomes for their patients.

The introduction of scar to the lamina propria of the vocal fold has several complicated, lasting effects on its structure and function. Several main causes of scarring in the vocal fold have been identified. According to Benninger and colleagues[2], vocal fold scarring may be secondary to traumatic, neoplastic, iatrogenic, inflammatory, and miscellaneous etiologies.

Traumatic injuries are characterized by either blunt trauma or a type of penetrating injury. Neoplastic etiologies most commonly refer to scarring as a

result of carcinoma on the vocal fold. Scarring due to a medical procedure such as an injection, prolonged intubation, or tracheotomy surgery is classified as iatrogenic. Inflammatory etiologies refer to scarring resulting from inflammatory conditions including gastroesophageal reflux disease and necrotizing infections. Other causes of vocal fold scar that do not correspond to the previous categories are noted as miscellaneous etiologies. The etiology can affect the scar's appearance and severity level[2].

Stages of wound healing

To be able to understand wound healing in the vocal folds, a review of the stages is presented. It should be noted that the majority of what is known regarding the stages of wound healing has been extrapolated from the skin literature. When injury occurs, tissue immediately begins the process of wound healing. The recognized stages of wound healing include coagulation, inflammation, mesenchymal cell (MSC) proliferation, angiogenesis, epithelization, protein synthesis, and contraction and remodeling. All of these stages contribute to changes in the epithelium and lamina propria leading to the eventual development of scar tissue. Although these stages are described separately, all are dependent on one another and can overlap[5].

During the first stage, coagulation, the body responds to extensive bleeding by forming a blood clot (hemostasis) at the site of the injury. After the clot is formed, the tissue undergoes alternating periods of vasoconstriction and vasodilation indicating the beginning of the inflammation stage. In the inflammation stage, neutrophils, albumin, and globulin infiltrate the matrix at the site of injury. Neutrophils are especially necessary to monitor and absorb foreign materials. Macrophages also emerge in the matrix and aid in tissue breakdown by secreting enzymes. MSC proliferation occurs days after coagulation and inflammation. This stage is characterized by the presence of fibroblasts at the site of the developing wound. Fibroblasts migrate across the wound site by binding and releasing substances such as fibronectin. Some fibroblasts undergo a change in phenotype to myofibroblasts to aid in the process of tissue repair. New blood vessels form at the site of the wound when several capillaries bind together signaling angiogenesis. The process of epithelialization results in a newly reformed epithelial barrier. At the edge of the wound, basal cells become thicker and migrate toward the collagen fibers present at the wound. Afterward, basal cells are restored to their previous phenotype.

The 'new' epithelium typically appears abnormal at the level of the dermis and epidermis as compared to an uninjured model. During protein synthesis, fibroblasts produce collagen at the wound site. Collagen forms a matrix to replace the previous fibrin scaffold allowing for cell movement. The last stage, contraction and remodeling, is the one in which scar tissue develops. This advanced stage can occur up to 12 months after the original injury.

Tissue is characterized by an increase in collagen density along the stress lines of the injury and the presence of metalloproteinases and enzymes at the site. As the remodeling stage progresses, collagen becomes less dense, and deposits of collagen bundles appear disorganized throughout the lamina propria. Elastin density also decreases, and fibers present as more short and compact than previously noted. Elastin, which promotes strength and flexibility, has an infrequent distribution in mature scars, never returning to baseline amounts[2].

Wound healing in the vocal fold epithelium

The layers that constitute the vocal mucosa are the epithelium, lamina propria, and deep muscle. Vocal fold epithelium is comprised of stratified squamous cells on the edge of the adducting folds[6]. The epithelium can be further subdivided into the suprabasal and basal layers. The epithelium contains a series of junctions that serve to connect epithelial cells to each other or to the matrix of the epithelium. There are three main types of cell junctions making up the epithelium: occluding (i.e. tight), anchoring, and communicating. Tight cell junctions are located near the edges of epithelial cells to seal the space between adjacent cells. These junctions mediate the cell's permeability. Anchoring junctions function similarly, in that they maintain strong bonds between cytoskeletons of adjacent cells or between cells and the basement membrane. Both tight and anchoring cell junctions serve to provide the epithelium with a barrier for protection of the vocal folds. Finally, communicating junctions allow electrical signaling between adjacent cells through ion transport. All three of these junctions are necessary to a functional epithelial layer[6].

In healthy human vocal folds, the epithelial cell layer is subjected to insult during everyday voice use. Savelli *et al.*[7] determined that the epithelium undergoes turnover once every 30 hours in a rat model under normal conditions. In order to maintain homeostasis and continue to provide protection to the vocal folds beneath it, the epithelium must be restored as quickly as possible. Leydon and colleagues[8] sought to determine the mechanism by which the epithelium

maintains homeostasis and regenerates by identifying the density and location of stem cells within the epithelium. They found stem cells primarily in the stratified squamous epithelium along the length of the fold, suggesting that their principal function is to restore the cell layer along the part of the vocal fold most susceptible to damage[8].

The anatomy of the epithelium dictates its response during each stage of wound healing. Branski and colleagues[5] investigated the changes in the epithelial tissue during stages of early wound healing in a rabbit model. Immediately after the injury, the epithelium was absent; however, by the third day post-injury, proliferating inflammatory cells and fibroblasts at the site of the wound were observed, indicating the beginning of the inflammation stage. Next, new epithelial cells began proliferating at the site of the injury along with dense deposits of collagen. These observations are presumed to be part of the MSC proliferation and angiogenesis stages and were followed by dead epithelial cells being replaced by a new layer, signaling the epithelialization period.

Other studies have also targeted changes in the epithelium post-injury. Puchelle *et al*.[9] defined three main stages of epithelial regeneration following injury in airway epithelia: cell adhesion and migration, proliferation and stratification, and differentiation. Leydon *et al*.[10] later confirmed using a rat model that vocal fold epithelia follow this same pattern during post-injury regeneration. According to the study, cell adhesion and migration was evident 3 days post-injury. Epithelial proliferation was first observed one day after injury and continued through 5 days post-injury. Finally, a regenerated epithelium with differentiated cells was noted by day 15 post-injury. During the early stages of wound healing, Leydon and colleagues[10] noted the presence of EGF and TGFβ1 in the epithelial cells, suggesting that epithelial cells secrete these growth factors to mediate the process of regeneration. Leydon *et al*.[10] observed a restored epithelial barrier with intact intercellular junctions 3 days post-injury. However, a complete basement membrane was not observed until 5 weeks post injury. Despite the rapid structural restoration of the epithelial barrier, adequate functional restoration required additional time. Leydon and colleagues[10] observed leakiness in the epithelia until 2–5 weeks post-injury. The functional properties of the epithelial barrier are necessary for preventing the invasion of viruses, particulates, and bacteria into the vocal folds. Therefore, although structural aspects of the epithelial barrier may recover relatively soon after injury, more time may be important for functional properties to be restored.

Table 1.1 Stages of wound healing of the vocal fold—epithelium

Time point	Tissue changes	Corresponding stage
Immediately after	Epithelium appeared absent from sample[5]	N/A
3–5 days post-injury	Emerging epithelium Confluent, multilayered epithelium[10]	Cell adhesion and migration
1–15 days post-injury	Epithelial cell proliferation began 1 day after injury but was noted as sparse Proliferation peaked at 3 days and continued through day 5 New epithelial cells and collagen deposits at site Proliferation was complete by day 15	Proliferation and stratification MSC proliferation
5–15 days post-injury	Differentiated cells observed throughout epithelium Appears similar to uninjured control Full permeability of lamina propria still developing[10]	Differentiation

Wound healing in the lamina propria

The basement membrane of the epithelium separates it from the lamina propria (also known as Reinke's space), arguably the most complex section of the vocal folds. The uninjured lamina propria consists of fibrous proteins, such as collagen and elastin, and interstitial proteins. The extracellular matrix (ECM) forms the structure of the lamina propria. Each of the layers of the lamina propria have been characterized by unique concentrations of elastin and collagen. These layers include the superficial, intermediate, and deep layers. The superficial space is characterized by a predominance of reticular, elastic, and collagen fibers with relatively infrequent vocal fold fibroblasts. Macrophages and myofibroblasts are also present in this level of the lamina propria. The intermediate layer contains hyaluronic acid (HA) and fibromodulin. The number of elastic and collagen fibers increases moving toward the intermediate and deep layers of the lamina propria. The deep layer contains slightly less elastin than the intermediate layer and a similar concentration of collagen fibers. This layer also contains fibromodulin and HA.

The structure and function of interstitial proteins in the vocal folds are less well understood than the roles of collagen and elastin. However, these interstitial proteins may have a prominent role in oscillating the vocal folds. A proteoglycan has the ability to link to various types of molecules important to the function of vocal folds, such as water molecules. These proteins can control the concentration of carbohydrates and lipid molecules in the ECM thus affecting its biological properties. In addition, according to Gray, Titze, Chan, and Hammond[11], these proteins can support or suppress growth and modulate wound healing within the ECM. Large chain proteoglycans, such as HA, are important to viscosity, while small proteoglycans regulate collagen organization.

HA is an interstitial protein involved in the regulation of viscosity, flow, and dampening in the vocal folds[12]. In addition, it is recognized as contributing to wound healing without scar in models of fetal ECM[13]. However, not all studies have supported this proposed role of HA in wound healing. Thibeault *et al*.[3] investigated levels of collagen, elastin, and HA in normal and scarred vocal folds in a rabbit model. The investigation revealed less collagen and elastin in the scarred model, as expected. HA levels were not significantly different between the two models, suggesting that HA may not be as essential in wound healing of the vocal folds as originally hypothesized. On the other hand, HA derivative injectables have been shown to improve viscoelastic properties of the ECM when injected after surgery[14]. The exact function and contributions of HA in wound healing have yet to be fully understood.

Small proteoglycans include decorin, biglycan, and fibromodulin. Decorin is distributed in the superficial layer of the lamina propria, while fibromodulin is distributed in the middle and deep layers. Gray *et al*.[11] proposed that decorin may play a major role regulating fibroblasts as a response to injury. This theory would explain why superficial layers tend to show less overall damage after injury/surgery than the middle and deep layers where less decorin is present. In contrast, fibromodulin is responsible for the structure and function of tendons and ligaments during the wound-healing process. Glycoproteins are also involved in the regulation of the ECM during the wound-healing process. Fibronectin binds proteins and supplies strength to other cells in the ECM. This glycoprotein is present in normal vocal folds, but exists in elevated concentrations in scarred tissue.

Other cells in the ECM essential to wound healing include myofibroblasts and macrophages, both of which have been associated with synthesizing proteoglycans. Catten and collegues[16] found that in human vocal folds, myofibroblasts

and macrophages appeared most frequently in the superficial portion of the lamina propria suggesting that these cells are involved with maintenance and repair before and after injury. Macrophages are specifically involved in conducting an inflammatory response. The authors of this study postulated that myofibroblasts contribute differently in the process of wound healing. Myofibroblasts were more prevalent than macrophages in the injured model and are linked to the processes of reorganization and repair in the vocal fold. The area of highest stress in the vocal folds (and likely the section most affected during injury) is the superficial layer of the lamina propria, which also has the highest concentration of myofibroblasts and macrophages.

Branski *et al*.[5] examined changes in the lamina propria throughout the wound-healing process using a rabbit model. Immediately after injury, the lamina propria appeared to be absent from the sample. A fibrinous blood clot formed 1 to 3 days after on the bed of the exposed muscle tissue, signaling the beginning of the coagulation stage. Evidence of a newly developing lamina propria was also observed in this area. By 3 to 10 days after injury, vascular channels appeared to form inside the lamina propria, signaling angiogenesis.

Hu *et al*.[15] described the process of wound healing in a canine model and specifically examined changes in concentrations of proteins in the ECM of the lamina propria using fluorescence. On day 15, increased collagen was observed in the ECM and remained until 6 months after injury. Elastin was observed throughout the lamina propria, but amounts decreased after the 15 day marker. By 6 months after injury, this decreased amount of elastin was noted in an irregular distribution in the lamina propria as compared to the control specimen. HA was distributed in the superficial and middle layers of the lamina propria in normal tissue[9], but was found throughout the lamina propria in the injured tissue at day 15. Similarly to elastin, amounts of HA decreased with time and by the 6-month marker less HA was noted in the injured tissue than in normal tissue.

Decorin was noted in the superficial layer of the lamina propria in the normal tissue, but was distributed throughout all layers of the lamina propria in the injured tissue. The concentration of decorin also decreased as the process of wound healing continued. By the 6-month time period, decorin in the injured tissue was significantly decreased as compared to the control[15].

Finally, fibronectin levels were found to be slightly increased in the injured tissue as compared to the control group. These changes in protein levels correspond to the different stages of wound healing. The increase in all of these proteins by day

15 suggests proliferation in the tissue related to the early wound-repair process. The increase in collagen noted at day 40 may correspond to the remodeling stage of the wound-healing process. Similar changes in these protein levels were described in a review by Hansen and Thibeault[17] who reviewed literature on rabbit, canine, rat, and pig models.

Table 1.2 Stages of wound healing of the vocal fold—lamina propria

Time point	Tissue changes	Corresponding stage
Immediately after–3 days post-injury	Lamina propria absent from sample[5] blood clot formed on surface of deep muscle over wound site[5]	N/A Coagulation
3–10 days post-injury	Inflammatory and fibroblast cells at site of injury[5] Vascular channels form within lamina propria[5]	Inflammation Angiogenesis
10–14 days post-injury	Disorganized pattern of collagen deposits and continued fibrosis[5]	Protein synthesis
15 days–6 months post-injury	Increased collagen observed in ECM Decreasing elastin throughout the lamina propria Increased HA[18,19] Increased decorin[19]	Contraction and remodeling
6 months–1 year post-injury	Decreased elastin[15]	

Other studies have shown contradictory results to Hu *et al.*[15] with regard to changes in HA and decorin during the wound-healing process. For example, a study investigating the role of HA in a rat model indicated that HA in scarred tissue remains at a higher level than normal tissue up to at least 2 months after injury[18]. In addition, Yamashita, Bless, and Welham[19] investigated gene concentrations in scarred and unscarred mouse vocal folds. They showed contradictory results in that HA and decorin both increased concentrations over time in scarred folds as compared to the controls. Therefore, despite Hu *et al.*[15] findings, more support exists for increases in HA and decorin rather than decreases[10].

Anatomy of a vocal fold scar

As previously stated, the final stage of the wound-healing process is scar formation. Scar tissue presents in a variety of anatomical forms with time: 1 to 3 months after the initial injury, early scar tissue develops. This type of scar is characterized by a stiff and thick quality. In contrast, a mature scar, one beyond 3 months old, is more thin and pliable than early scar. Injury to areas of tissue with higher levels of collagen and fibroblasts are more likely to lead to severe scarring[2].

In scar tissue, collagen, procollagen, and decorin increase in the ECM in an attempt to preserve the organization of collagen fibers. Fibronectin aids in adhesion and migration of these cells during repair. Finally, the increase of HA and loss of elastin contributes to increased stiffness and decreased viscosity in the lamina propria. Throughout the vocal fold, scar consists of disorganized collagen and elastin bundles, loss of constituents of the ECM, lower overall volume, and reduced pliability. The disorganized fibers contribute to distinct change in the biomechanical properties of voice[20]. The body–cover relationship is altered due to the consequences of the tissue injury, and the mucosal wave, which is essential to voice function, is severely compromised. The stiffer, less functional quality of the mucosal wave occurs as a result of the compromised concentration of elastin and collagen and the increased level of fibronectin.

Levels of viscoelasticity in vocal fold scar are especially important to treatment outcomes because the vocal folds are dependent on their ability to vibrate. Much research has examined properties of viscoelasticity in the vocal folds using a variety of models. Hertegerd and colleagues[21] examined properties of viscoelasticity in a rabbit model. They found that untreated vocal fold scar presented with longitudinally arranged fibrous bundles appearing similar to collagen. These bundles were not present in the uninjured vocal fold tissue. In addition, denser collagen was noted in the injured tissue than in the uninjured specimen. These numerous bundles and increased density of collagen likely contributed to an overall stiffer characterization in the scarred vocal fold.

Thibeault and colleagues[3] examined the changes in the lamina propria following the development of scar. Using a rabbit model, rheologic properties were examined to determine the modulus of elasticity and viscosity in the damaged tissue 2 months post-injury. Both stiffness and viscosity were significantly increased in the scarred model as compared to the uninjured model of the lamina propria. The decreased elasticity was presumably related to the scattered

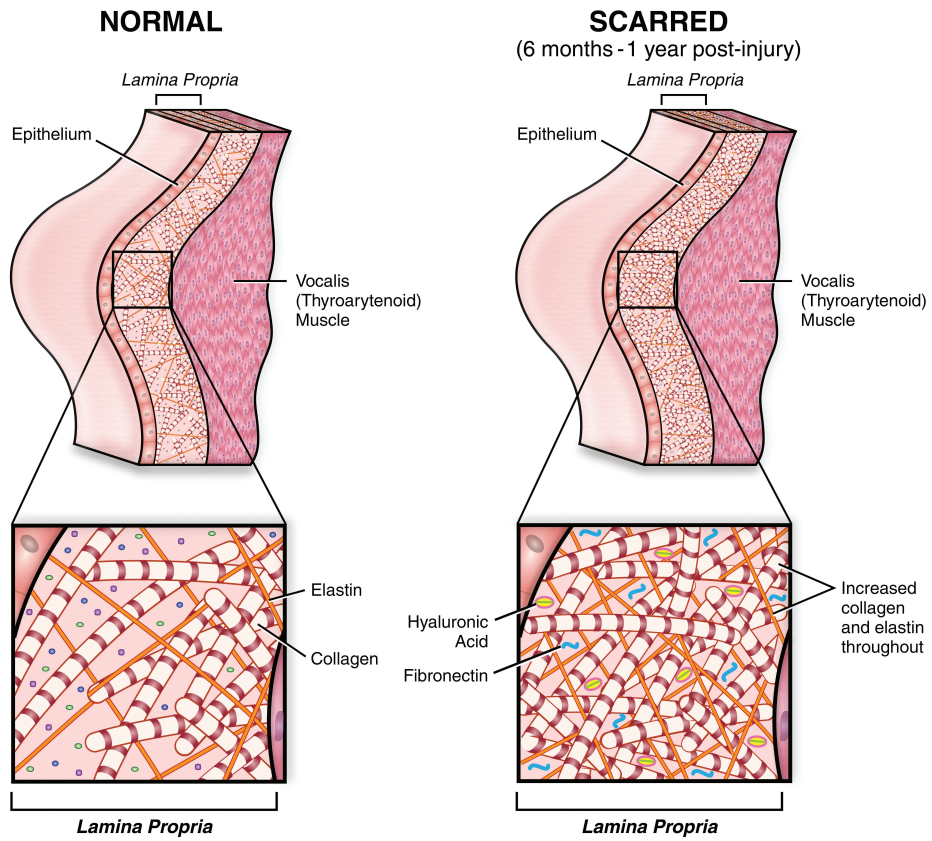

Figure 1.1 Schematic of normal and scarred vocal fold lamina propria. The scarred lamina propria is marked by an increase in collagen. The tissue is denser and thicker, causing decreased viscoelasticity and subsequent alteration of the mucosal wave propagation.

elastin fibers present in the scarred lamina propria. The difference in viscosity was also hypothesized as related to the change in the architecture of the fibers in the vocal folds. Hirschi and colleagues[22] also described fibronectin as a protein in the vocal folds that also may be related to increased viscosity in vocal fold scar.

Rousseau *et al.*[23] conducted a follow-up study to examine chronic scarring of the vocal folds in a rabbit model 6 months post-injury. Similarly, they found increased viscosity and increased modulus of elasticity in the scarred tissue as compared to normal tissue. Although other aspects of the tissue had returned to pre-injury levels (such as in collagen and procollagen), the disorganized elastin throughout the layers of the lamina propria persisted, likely contributing to the increased viscoelasticity in the scarred model. Rousseau *et al.*[23] also hypothesized

that the observed higher density of the fibrous collagen bundles also contributed to increased viscoelasticity.

Cellular and molecular investigations: implications for prevention and treatment

Following injury, the vocal folds sustain many changes at the molecular level. Many studies have investigated means for prevention and treatment of these changes through cellular and molecular methodologies. These are discussed in more detail in a later chapter. Hirano *et al*.[24], for example, examined the use of growth factor therapy in treating vocal scarring in a canine model. They used hepatocyte growth factor (HGF), a polypeptide known to be involved in tissue regeneration of the liver and kidneys, and noted its effects on vocal fold vibrations in the scarred tissue of the canines via injection. Hirano *et al*.[25] had previously determined that use of HGF in vocal folds suppressed collagen production and increased the production of HA, suggesting a possible role in vocal fold wound healing. They found that the scarred vocal fold canine samples treated with HGF had overall better vibration according to the mucosal wave amplitude, suggesting that this treatment could reduce the negative effects of scarring on vocal fold vibration.

Some studies have used molecular work to prevent vocal fold scarring. Hirano *et al*.[25] used the previously described HGF injections in an acute rabbit model and measured its effects on vocal fold vibration and histological characteristics. In this study, they found that samples treated with HGF at the time of injury maintained a well-organized layer structure in the vocal fold with reduced collagen deposition as compared to the control group. In addition, the HGF treated samples did not exhibit tissue contraction of the lamina propria whereas the control did. Finally, results from the study indicated that HGF-treated samples were markedly less stiff, had less viscosity, and had better vibratory function than those in the control group. Results from this molecular study supported HGF therapy as a valid preventative technique in vocal fold wound healing.

A study by Chhetri *et al*.[26] used lamina propria replacement therapy with autologous fibroblasts as a technique for vocal fold scar treatment. They used a laser to create scar tissue on the vocal folds of canines and harvested tissue from the buccal cavities of the animals for tissue culture. Next, fourth passage cultured fibroblasts were injected into the scarred vocal folds of the canines.

Analysis revealed significant improvements in the mucosal waves and acoustic parameters following replacement therapy, showing promise for this method in future research.

Other studies have investigated the implementation of biomaterials in vocal fold rehabilitation. Jia *et al*.[27] developed HA-based soft microgels and cross-linked microgel networks for use in scarred vocal fold tissue in a canine model. The study determined that HA-based microgel networks can be constructed to have similar viscoelasticity to canine vocal fold tissue, indicating that these biomaterials would be useful in aiding in tissue regeneration.

Similarly, Hahn *et al*.[28] developed collagen-based microgel networks to examine their use in lamina propria regeneration. Scaffolds were comprised of collagen and HA or collagen and alginate, and pig vocal fold fibroblasts were added to the separate mixtures. The collagen–alginate hydrogels did not demonstrate scaffold compaction or loss of mass as compared to the collegen–HA hydrogels. In addition, the collagen–alginate hydrogels demonstrated ECM synthesis unlike the collagen–HA hydrogels in the study.

Xu *et al*.[29] developed a three-dimensional, biodegradable xenogeneic scaffold for the regeneration of vocal fold fibroblasts in the lamina propria using a decellularized bovine lamina propria. Results indicated that human vocal fold fibroblasts easily attached to the engineered acellular scaffold. High levels of decorin were noted as well as normal levels of viscoelasticity, which would potentially support vocal fold vibration in tissue generated from the scaffold.

Duflo *et al*.[30] engineered an HA–gelatin hydrogel to determine the appropriate amount of synthetic ECM necessary for wound repair in a rabbit model. HA hydrogels used in the study consisted of various gelatin concentrations. Analysis of gene expression revealed that all HA derived hydrogels, including the hydrogel without gelatin, resulted in increased tissue elasticity and viscosity. However, the HA hydrogel consisting of 5% gelatin showed the most improvement in all measured biomechanics in the study when injected immediately after injury.

More recent studies have examined the impact of architecture and other characteristics of proposed scaffolds on tissue regeneration and gene expression. Hughes and colleagues[31], for example, investigated the effects of aligned and unaligned electrospun scaffolds on vocal fold fibroblast behavior. Because vocal fold tissue is highly disorganized after scarring, it is important that treatment methods aim to reorganize this tissue during regeneration. Alignment of the ECM scaffold may play a role in the tissue's ability to reorganize. In this study,

electrospinning was used to produce aligned and unaligned nanofibers for each of the scaffolds, and human vocal fold fibroblasts were seeded onto the scaffolds. Both aligned and unaligned scaffolds maintained a population of cells, but human vocal fibroblasts only oriented along the aligned scaffold. In addition, cell layers were arranged and confluent on the aligned scaffolds, but disorganized on the unaligned scaffolds.

Vocal fold scar results in numerous changes to the delicate anatomy of the vocal mechanism. These effects include physiological differences as well as changes in gene expression at molecular level of the lamina propria. Prevention and treatment must target where these changes occur in order to impact the structure and function of the vocal folds. Future research is necessary to determine viable treatment approaches for vocal fold scarring at the molecular level.

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2

Diagnosis of vocal fold scar

Hayley Herbert and John Rubin

Introduction

The accurate diagnosis of vocal fold scar requires a multidisciplinary, systematic approach. Scarring of the vocal fold presents with a diverse range of symptoms and signs and should be considered in the differential diagnosis of any dysphonic patient. Diagnosing scar requires a high index of suspicion as the absence of normal vibratory tissue is often more difficult to detect than other pathology.

Vocal fold scar occurs when the lamina propria of the vocal fold becomes damaged. This is often associated with loss of critical extracellular matrix components, volume deficiency and reduced pliability[1] that interferes with the mobility and/or integrity of the layers of the vocal fold, impairing the mucosal wave and resulting in dysphonia. In accordance with source-filter theory described by Fant[2], alterations to vocal fold vibration affect the acoustic signal. The vast majority of these changes that affect the acoustic signal occur in the cover of the vocal fold. Scarring may also result in incomplete glottic closure thereby increasing vocal symptoms.

Causes of scarring

Acquired injury is the most common cause of vocal fold scarring. Congenital causes such as sulcus vocalis should also be considered, in particular in cases such as monocorditis, or long-standing hoarseness with secondary evidence of muscle tension.

Possible causes include those listed in Table 2.1.

Table 2.1 Causes of vocal fold scarring

Mechanism	Examples
Phonotrauma	Overuse, misuse
Chemical	Smoking, laryngopharyngeal reflux
Thermal	Laser, diathermy, inhalational
Trauma	Blunt and penetrating
Surgery	All vocal fold surgery, intubation, radiotherapy, injection laryngoplasty
Inflammatory	Systemic Lupus Erythematosus (SLE), Sjogrens, Rheumatoid Arthritis, scleroderma
Infective	Papilloma, bacterial laryngitis
Cancer	Squamous cell cancer, adenocarcinoma, adenoid cystic cancer
Vascular	Arteriovenous malformations, varices, telangiectasia
Congenital	Sulcus vocalis, congenital cyst

Phonotrauma

Phonotrauma typically impacts the basement membrane of the epithelium and the superficial layer of the lamina propria (SLLP). Dikkers *et al*[3] have studied benign lesions with electron microscopy and demonstrated deposition of electron-dense matter with loss of normal hemidesmosomes and anchoring fibers. They associated this with vibratory stress.

Gray and colleagues[4] have posited patterns of injury on the basis of immuno-histochemistry as follows:

1. Basement Membrane Zone (BMZ) and SLLP disruption with disorganization of the anchoring fibers and increased fibronectin, which they cited as being almost unique to the vocal fold and suggestive of a repetitive injury.
2. BMZ relatively intact, but a paucity of structural glycoproteins and interstitial proteins in the SLLP, as seen in certain cases of Reinke's edema and polyps.

Rubin and Yanagisawa[5] reported that the majority of phonotrauma occurs at the mid-membranous vocal fold, as this is the locus of greatest stress during normal phonation. Vocal overuse or more specifically misuse may result in vocal scarring in this region.

Chemical

Cigarette smoke and laryngopharyngeal reflux are irritants that can cause injury to the vocal folds (including the formation of Reinke's edema) whereby the normal structures of the epithelium and SLLP are altered. This may later form a vocal fold scar with fibrosis of the layers of the fold.

Thermal

Thermal injury to the vocal folds can be inflicted on surrounding normal tissues as the energy is transmitted into the subepithelial layers. Thermal damage also may occur beyond the edge of the cut, which is usually regarded as the margin of excision. The spot size, tissue relaxation time and energy delivered should all be carefully adjusted to achieve the desired function (e.g. incision or coagulation) with minimal damage to the normal tissues of the vocal fold. Diathermy similarly needs to be appropriately adjusted to reduce collateral damage and potential scar. The size and anatomic position of the larynx make it susceptible to inhalational injury. Smoke and steam burns can cause immediate and delayed damage to the larynx. Life-threatening edema can result from inhalational burns – especially steam, which has a heat capacity 4000 times that of air. Delayed scarring may result from this damage.

Trauma

Blunt and penetrating injuries to the larynx may lead to disruption to the laryngeal framework, webbing within the glottis, and injuries to the vocal folds as a result of the initial trauma, associated hemorrhage or infection.

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