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Insights Into the Role of Collagen in Vocal Fold Health and Disease

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Summary

As one of the key fibrous proteins in the extracellular matrix, collagen plays a significant role in the structural and biomechanical characteristics of the vocal fold. Anchored fibrils of collagen create secure structural regions within the vocal folds and are strong enough to sustain vibratory impact and stretch during phonation. This contributes tensile strength, density, and organization to the vocal folds and influences health and pathogenesis. This review offers a comprehensive summary for a current understanding of collagen within normal vocal fold tissues throughout the life span as well as vocal pathology and wound repair. Further, collagen's molecular structure and biosynthesis are discussed. Finally, collagen alterations in tissue injury and repair and the incorporation of collagen-based biomaterials as a method of treating voice disorders are reviewed.

Keywords

Collagen; Vocal fold; Biomaterial; Wound healing; Biomechanics

INTRODUCTION

Vibratory mechanics associated with phonation are possible due to the properties of the fibrous and interstitial proteins within the various layers of the vocal fold. Collagen is one of these fibrous proteins in the extracellular matrix (ECM) that contribute to the tissue's viscoelastic properties, which are necessary for voice production. At approximately 30% of the total body protein mass, collagen is one of the most abundant proteins in the human body and composes an even higher ratio of approximately 43% of the total proteins within the vocal fold lamina propria.^{1,2} Unlike elastin, collagen's partner protein in the vocal folds,

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collagen does not directly facilitate tissue stretch. Although collagen demonstrates some elastic properties, its high fracture toughness primarily provides connective strength to counter elastin's stretch-and-recoil properties.^{3,4} Considering the repetitive nature of the stretch-and-recoil action experienced in the vocal folds, collagen contributes structural support, establishes the elasticity threshold of the tissue during stretching, and ensures tissue integrity following repeated stress recoil incurred at high frequencies.³ The vocal folds are thus able to meet the demands of repetitive high-frequency impact from normal phonation.

Collagen's ability to provide tensile strength and maintain tissue integrity is largely due to its molecular and structural makeup. Unfortunately, there is little work that discusses collagen's molecular properties and production within the literature despite its integral role in vocal function. Knowledge of collagen's molecular composition provides unique insight to vocal fold health and physiology; in a similar fashion, it provides a deeper understanding of the basis for disease and tissue repair when injuries are sustained. This paper offers a comprehensive review on collagen in the vocal folds throughout the life span, its contributions to normal laryngeal anatomy and physiology, and its incorporation into novel bioengineering therapeutic approaches. Details of collagen's molecular structure and biosynthesis will be highlighted as it pertains to vocal fold health, wound healing, and disease.

COLLAGEN IN THE VOCAL FOLD

The vocal folds are thought to develop increasing complexity with differentiation toward a layered structure with maturation. During embryonic development, fetal vocal folds contain a monolayer of loose collagen fibers between the epithelium and the vocalis muscle.⁵⁻⁸ At this stage, the lamina propria remains largely homogenous as the collagen fibrils are uniformly configured in a wicker basket fashion.⁶ The first signs of differentiation within the vocal folds begin shortly after birth, with increasing collagen abundance noted throughout development until adulthood.⁷ At 2 months of age, a dual-layer structure begins to appear within the lamina propria with distinct cellular densities.⁸ This bilaminar structure emerges along with a hypocellular region adjacent to the vocalis muscle, and is present from 11 months to 5 years of age.⁸ By 7 years of age, middle and deep layers begin to form with varying cell population densities. A developed lamina propria defined by differential collagen and elastin fiber composition in layers is not present until after 10 years of age, with mature layers apparent by 17 years and remaining throughout adulthood.^{8,9} Although this understanding of vocal fold development is historically accepted, recent contrasting work by Nita et al suggests that a layered structure similar to that found in adults can be identified in fetal vocal folds.¹⁰

The mature adult vocal fold can be grossly divided into three layers: the epithelial layer, the lamina propria, and the deep muscle layer. The lamina propria can be further categorized into superficial, intermediate, and deep layers. The classification for these layers has been delineated roughly via differences in collagen and elastin ratios; collagen is most abundant and densely deep in the lamina propria, elastin is most abundant in the intermediate layer, and neither of these proteins make up a significant proportion of the superficial layer.³ In adulthood, differences in collagen abundance can be observed between men and women,

with higher collagen concentrations found in male vocal folds.⁹ Although decreases in collagen are noted in female samples in advanced age, no differences in collagen concentration were seen between adult and geriatric men.⁹

Immunofluorescence and picrosirius polarization studies demonstrate that collagen type III is predominant in the entire lamina propria, whereas collagen type I is distributed in the superficial and deep layers of the lamina propria.^{11,12} The deep layer of the lamina propria contains densely organized bands of collagen.⁵ These bands are demarcated via strong optical refraction known as birefringence in polarized light microscopy within the deep lamina propria.^{9,13} These collagen fibers penetrate the superficial muscle bundles of the vocal muscle and are arranged in a network of collagen I and III fibers.⁵ These fibers are oriented anteriorly to posteriorly and are aligned to the vocalis. In the intermediate layer, there is a less densely organized band of collagen type III, with weakly birefringent fibers. The superficial layer exhibits only a narrow band of collagen IV and VII fibers immediately below the epithelial basement membrane.⁵ These aptly named anchoring fibers attach the basement membrane zone to the deep lamina propria (SLP) (Table 1).

Increased collagen density along the deeper layers of the lamina propria contributes tissue strength, whereas decreased density along the superficial edge preserves elastic characteristics for small amplitude oscillation.¹⁴ To measure tensile or compressive stress to strain, the Young modulus is used. Sasaki and Odajima found the stress-strain curve of collagen molecules isolated from bovine tendon to be nearly linear with a modulus of roughly 2.9 ± 0.1 GPa.¹⁵ The linearity of the graph was hypothesized to result from the hydrogen bond network within the collagen molecule.¹⁵

COLLAGEN MOLECULE STRUCTURE

Collagen's contribution to vibratory biomechanics and shear resistance of the vocal folds can be attributed to its molecular and structural composition. The central structural motif of collagen is composed of three parallel polypeptide alpha (α)-chains that coil upon each other to form a triple helix (Figure 1).^{16–18} Each α -chain is composed of approximately 1000 amino acids and has a repeating Gly-XY triplet. Within the α -chain, amino acids glycine, proline, and 4-hydroxyproline act as building blocks for the triple helical structure. Hydrogen bonds between these chains hold them together for a tight assembly, which provides collagen's characteristic strength. Each α -chain has a left-handed helix and the three α -chains wrap around to form a right-handed helix measuring approximately 1.4 nm in diameter and 300 nm in length.^{16–18} Although collagens can be heterotrimeric, meaning that there are differences among the three α -chains, most collagens are homotrimeric and contain identical α -chains. Type I collagen is an example of a heterotrimer as it contains two identical α -chains and a differing third chain. Meanwhile, collagen II is an example of a homotrimer as it contains three identical chains. The composition of the polypeptide chains depends on the collagen type. For example, the $\alpha 1$ -chain in collagen I is different from that of the $\alpha 1$ -chain in collagen II.^{16–18}

Collagen gene

Although structural similarities exist among various types of collagen, each type of collagen is uniquely different and has tissue-specific functions.^{18,19} The umbrella category of genes termed *COL* genes provide instructions for making components of collagens. Each specific *COL* gene is located in a unique location within the human genome with a distinct exon coding region and encodes for an individual collagen type.^{18,19} For example, the *COL3A1* gene, also known as collagen, type III, alpha 1, produces pro- α 1-chains of type III collagen.¹¹ These unique genes and varying processes for biosynthesis are responsible for the production of 29 different types of collagen¹⁷; however, only a portion are pertinent to the vocal fold^{11,16,17} (Table 1). Transcription of these genes results in the formation of procollagen, which then undergoes the process of biosynthesis to produce mature collagen molecules in the form of long, thin fibrils.^{16,17}

Collagen biosynthesis

The biosynthesis of collagen is carried out in a stepwise fashion similar to many other secreted proteins. This process includes transcription of the gene, translation to protein, posttranslational modification, and secretion into the extracellular space (Figure 2).²⁰ The type of collagen in each cell type varies based on the microenvironment and the requirements of the tissue and organ. The cell type is the main determining factor of the specific transcriptional activity; however, various growth factors and cytokines also play a role. Gene transcription is initiated in the cell nucleus. Once complete, translation follows as the collagen messenger RNA (mRNA) directs protein assembly to construct the α -chains. The newly formed procollagen peptide chains are then moved along the endoplasmic reticulum for further processing.¹⁶ Posttranslational modification of procollagen occurs in the Golgi complex and is crucial for the formation of mature collagen molecules into a fibril structure. The C-propeptides at the ends of the collagen α -chains play an essential role in assembling the three α -chains into one procollagen triple helix. Specifically, the precise alignment of the C-terminal domains of all the three α -chains fastens the chain ends and orients the chains from the C-terminus to the N-terminus to form the triple helix.^{16,21,22} Procollagen molecules are then packed into secretory vesicles and transported to the extracellular space. Here, proteases N-proteinase and C-proteinase remove the N- and C-propeptide ends from the procollagen helix to form tropocollagen. The resulting tropocollagen protein has a length of 300 nm and a diameter of 1–2 nm^{16,22} and maintains a triple-stranded helix structure; the removal of the terminal domains allows the collagen to form the fibril structure necessary for assembly into the large organized fiber bundle networks seen in the vocal folds. This assembly is achieved via cross-linking of fibril chains that overlap each other. The overlapping configuration leaves an approximately 67-nm space between adjacent collagen molecules and forms periodic regions known as D-periods^{21,22} (Figure 3). It is this intermolecular cross-linking configuration that confers the strength and stability necessary for vocal fold vibration. A study of collagen biosynthesis estimated that the rate of translation of mRNA for a collagen chain is about 209 amino acid residues per minute and that hydroxylation and helix formation occur either during translation or very rapidly thereafter.²³ Tropocollagens in the extracellular space polymerize spontaneously, and once tropocollagen is transported in the extracellular space, it polymerizes spontaneously into collagen fibers.¹⁶

Collagen in wound healing and scar formation

Rapid biosynthesis of collagen occurs following injury as tissue damage triggers a complex cascade of events for wound healing. In the vocal folds, similar to other tissue systems, this process can be simplified and described in three major phases: inflammation, ECM deposition and epithelialization, and remodeling.²⁴ Acute inflammation occurs shortly after injury, triggering vasoconstriction and dilation, edema, and immune cell recruitment to the wound.^{24,25}

Collagen deposition generally begins after inflammation subsides. During this proliferation stage, fibroblast and epithelial cells infiltrate into the wound milieu and initiate the reconstitution of the ECM through fibronectin and collagen production to form a provisional matrix within the injury site.²⁶ Fibrocytes migrate to the site from surrounding undamaged tissues through chemotactic signaling, and a separate subpopulation of fibrocytes specific to the vocal fold lamina propria originates from circulating peripheral blood.^{27,28} Once recruited to the injury site, the fibrocytes differentiate into fibroblasts for active repair and produce large amounts of collagen and elastin. Peak gene expression levels for precursors of collagen, procollagens I and III, have been demonstrated at 72 hours post injury in vocal fold tissue.²⁷ The release of new matrix materials leads to the formation of granulation tissue, which consists of new connective tissues that help fill in the wound bed.²⁵ Collagen and other ECM molecules concurrently initiate epithelialization and produce a functional epidermal barrier. Meanwhile, tissue repair and reorganization of matrix components continue after the functional barrier has been formed.²⁶

It is during the final phase of wound healing where scar maturation and remodeling occur over a period up to 12 months. The increase in and rapid deposition of various collagen types within the wound milieu manifest in disorganized collagen bundling.²⁶ Decorin, fibromodulin, and fibronectin are proteoglycans and glycoproteins present in the vocal fold that contribute to regulation of collagen synthesis, bundle organization, and resulting scar formation.²⁹ Decorin and fibromodulin influence collagen fibril thickness and synthesis rate and are both notably decreased in scar. Whereas decorin regulates collagen assembly and the lateral alignment of fibrils, fibromodulin inhibits transforming growth factor-beta, which induces collagen synthesis. Meanwhile, fibronectin, which is hypothesized to induce cell migration and ECM synthesis, is elevated in fibrotic tissue.^{30,31}

The resulting scar subsequently changes the physical properties of the tissue and deleteriously affects the vocal tissue's ability to generate mucosal wave and to maintain small amplitude oscillation. Between 1 and 3 months after injury, increases in collagen III and decreases in collagen types I and IV are observed over the initial remodeling period.^{32,33} Not only do the elevated levels of collagen affect the tissue properties, but also increased cross-linking among the molecules leads to stronger fibrils with higher fracture thresholds and overall fibril bundle disorganization.^{4,34} This loss of normal collagen architecture contributes to increased tissue viscosity.^{4,34}

Approximately 21 days after injury, collagen deposition becomes relatively stable but continues for 12 months. Despite the gradual remodeling changes occurring over this time, the viscoelastic properties of repaired tissue never fully return to that of normal uninjured

tissue. Thus, vocal quality can be significantly affected following a vocal fold injury; as such, many efforts have targeted collagen for the potential treatment of scar. In the same vein, much work has focused on incorporating and adapting collagen into biomaterials to develop possible therapeutic avenues for voice disorders.

COLLAGEN IN BIOMATERIALS

Collagen is one of the most useful biomaterials due to its extensive distribution in the human body and is the favored choice of biomaterial when compared to natural polymers and their synthetic analogs.³⁵ The use of collagen-based biomaterials has been rising in the field of tissue engineering and regenerative medicine. The attractiveness of collagen as a biomaterial is expanding due to multiple advantages inherent to collagen's characteristics. Specifically, collagen is biodegradable, nontoxic, biocompatible, nonantigenic, hemostatic, and highly compatible with other synthetic polymers. The biodegradability of collagen is easily regulated by cross-linking, enhancing the mechanical and resistance properties.^{35,36} Collagen can easily form fibers with extra strength and stability through self-aggregation and cross-linking, and can be easily extracted from various tissue sources. Collagen-based biomaterials are built by two methods. In one approach, the decellularization of the collagen matrix is used by removing the cell and conserving the ECM structure and tissue shape. In the second approach, functional scaffolds are created via extraction and cross-linking of collagen.^{36,37} The ease of processing collagen into innumerable forms such as films, sheets, calcifiable matrix systems, tubes, sponges, powders, injectable gels, hydrogels, and nanospheres makes it exceedingly advantageous in its application as a carrier system in the delivery of drugs, proteins, and genes.^{35,36}

Collagen-based biomaterials have a wide range of experimental applications in *in vivo* and *in vitro* studies. Given the abundance of collagen in various tissues and organ systems, it is the most widely used tissue-derived natural biomaterial. Specific combinations of the amino acid sequences in the α -chain of the triple helix are recognized by cells; this facilitates cell attachment and migration as well as cell-based degradation by collagenase. These properties therefore make collagen a favorable tissue culture matrix for studying various cell behaviors such as migration, proliferation, and differentiation. The substantial growth ability of various cell types, including fibroblasts, chondrocytes, endothelial cells, neural cells, osteoblasts, and stem cells, has been demonstrated within collagen hydrogels.^{35,38,39} Collagen-based 3-D scaffolds are a cornerstone material for organ regeneration or building 3-D models,^{35,39} and given its innate bio-compatibility and low immunogenicity, collagen is considered the protein of choice for numerous biomaterial preparations.

COLLAGEN-BASED BIOMATERIALS FOR THERAPEUTIC APPROACHES IN THE VOCAL FOLD

The use of biomaterials for the treatment of scars stems from the current limited and inconsistent success for certain voice disorders, particularly those associated with tissue and structural defects. Collagen has been considered a plausible vocal fold filler or biomaterial because of its resemblance to host tissue in the lamina propria. Collagen allows the ingrowth of host tissue and can eventually become replaced by host collagen. Additionally, collagen

has been demonstrated to soften scar tissue.³⁷ These attributes make collagen an ideal candidate for biomaterial-based therapy in the vocal fold.

In the last decade, there has been a vast increase in the number of injectable materials with enhanced biomechanical profiles. These recent advances have focused on matching the biomechanical and viscoelastic properties of the SLP and have eliminated any immune response and inflammatory reaction caused by early chemically derived injectable materials.⁴⁰ Collagen is the predominant component of the ECM of many tissues including skin, ligament, cartilage, tendon, and bone, and hence remains the most widely used tissue-derived natural polymer. The historical use of collagen to treat voice disorders resulting from glottal incompetence has focused on an injectable format. Injections of collagen preparation have been used for augmenting vocal folds and improving glottic function. In one study with cadaveric human vocal folds, a homologous collagen compound was reliably injected in multiple positions in the vocal fold tissue. These positions include the SLP, the medial portion of the thyroarytenoid (TA) muscle, and the lateral portion of the TA muscle.⁴¹

Materials used for vocal fold injections (Table 2) have included the soluble bovine collagen products Zyderm Collagen Implants I and II, and more recently, cross-linked bovine preparations Zyplast and Phonagel (Collagen Corporation, Palo Alto, CA). In a comparison of soluble bovine collagen preparations differing only in the amounts of chemically induced cross-linkage, fibroblast invasion on the implant progressed more rapidly in materials with greater cross-linking, such as Zyplast and Phonagel.⁴² However, concerns associated with bovine collagen including autoimmune responses such as airway complications, immunologic destruction of the implant, and material rejection rendered bovine-based collagen materials unfit for laryngeal applications.⁴³ This finding led to the use of new types of collagen: autologous,⁴³ homologous,⁴² and micronized AlloDerm.⁴⁴ Autologous collagen preparations, such as Autologen, are able to keep their *in vivo* architecture and resist protease degradation. The amount of collagen required is a limiting factor, because the patient's own skin is the starting donor material.⁴³ Due to inconvenience in processing and donor site morbidity,⁴² autologous preparations were succeeded by the more feasible homologous forms. Homologous collagen comes from cadaveric human tissue. This homologous form had favorable changes in TA muscle contraction and vocal fold vibratory patterns when injected into the superficial layer of the lamina propria (SLLP) and the medial portion of the TA muscle.⁴² Injections of homologous collagen preparations into the SLLP do not appear beneficial, as these injections have resulted in poor vibratory patterns of the vocal folds.⁴¹ Micronized AlloDerm made from human collagen and elastin particles is a currently available injectable substance. Injection laryngoplasty with micronized AlloDerm is available as an efficient treatment for incomplete glottal closure or vocal fold contour defects due to vocal fold immobility, sulcus, scar, presbyphonia, and Parkinson's disease.⁴⁵

Hydrogels have been used extensively in tissue engineering and soft tissue augmentation with the purpose of vocal fold restoration. Collagen hydrogels facilitate cell attachment and migration,^{38,39} and are biodegradable synthetic or natural polymers whose mechanical and biological properties can be varied. The flexibility for tailoring the mechanical and biological properties of the polymer has paved the way toward novel treatment opportunities. One advantage of using hydrogels is their ability to mimic the microenvironment, and hence

hydrogels can modulate the desired cell function. Natural hydrogels are biocompatible and less prone to immune rejection due to the structural similarity with the physiological ECM.⁴⁶

Collagen composite hydrogels have also been evaluated for their ability to support ECM and vocal fold fibroblast (VFF) synthesis with limited hydrogel compaction and resorption. Collagen-hyaluronic acid (HA) and collagen-alginate were tested for possible vocal fold SLP regeneration. In assessing the collagen-alginate vs the collagen-HA hydrogels for potential as vocal fold SLP regeneration scaffolds,⁴⁷ new reticular collagen fibers were noted for the collagen-alginate material only. This is not surprising as HA is known to reduce collagen synthesis by human dermal fibroblasts.⁴⁷ The collagen-alginate proved to stimulate ECM synthesis and accumulations of VFFs in 3-D culture.⁴⁷ Moreover, the collagen alginate hydrogel demonstrated significant potential in ECM synthesis by VFF without causing matrix compaction known to be detrimental to vocal fold SLP regeneration. Composite collagen-alginate hydrogels could potentially make it possible to adapt specific properties to individual vocal folds. Finally, recent work incorporating collagen into a gelatin sponge scaffold system to sustain the release of basic fibroblast growth factor offers a promising treatment approach for vocal scar.⁴⁸ Due to the major impact of contour defects on vocal function, novel integration of collagen into sponge delivery systems and composite alginate hydrogels demonstrate potential for various voice disorders.

CONCLUSION

Collagen is a critical protein within the vocal folds that contributes not only to the structural composition of the tissue but also to the vibratory mechanics that allow for phonation. Further, collagen plays a major role in tissue injury-induced voice disorders as it is intimately involved in the wound healing paradigm. Although advancements have been made in understanding collagen's molecular properties, biosynthesis, and role within the wound healing process, restoration of normal vocal fold tissue characteristics following injury remains a challenge. Recent therapeutic approaches target disorganized collagen bundling associated with scar via biomaterial-based treatments to improve tissue viscosity. Integration of collagen into biomaterials has also offered alternatives for treatment of scar as well as other voice disorders stemming from glottal incompetence. As biomaterial-based therapies continue to evolve along with our understanding of the complexities of vocal fold repair, there remains potential for new therapeutic developments and improvements in the treatment of voice disorders.

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FIGURE 1.

Schematic representation of the collagen structure. Nuclear transcription of collagen gene produces three α -chains (here, two α_1 -chains and one α_2 -chain are representative of type I collagen). The three α -chains are intracellularly assembled into the triple helix molecule called procollagen. Procollagen is converted into tropocollagen by the removal of the N- and C-propeptides in the extracellular space via metalloproteinase enzymes. Self-assembly and covalent cross-linking form collagen fibrils ranging from 10 to 300 nm. Collagen fiber formed by the aggregation of collagen fibrils ranges from 0.5 to 3.0 μm in diameter. Adapted from Alberts et al.¹⁶

FIGURE 2.

Schematic representation of collagen biosynthesis starting from the nuclear transcription of the collagen genes, followed by mRNA processing, ribosomal protein synthesis (translation) and posttranslational modifications, secretion, and the final steps of fibril formation and fiber assembly. ER, endoplasmic reticulum; mRNA, messenger RNA. Adapted from Von der Mark.²⁰

FIGURE 3.

Schematic representation collagen fibril assembly in the characteristic quarter-staggered form to form a collagen fiber. The cross-linking pattern between collagen molecules creates repeating overlap and gap regions along the fibrils that form D-periods. Periodic striation is seen with characteristic alternation of dark and light bands along the fiber. Adapted from Von der Mark.²⁰

TABLE 1

Summary of Collagen Types Found in the Vocal Fold

Collagen Type	Vocal Fold Location	Class	Alpha (α)-Polypeptides	Composition	Gene Symbol	Genome Location Human
I	Two layers, one immediately below the epithelium and another dense layer in the deep lamina propria superficial to the vocal muscle, penetrating between muscle fibers	Fibrillar	α1(I)	[α1(I)] ₂ [α2(I)]	<i>COL1A1</i>	17q21.34-q22
			α2(I)		<i>COL1A2</i>	7q22.1
III	Below the epithelium intertwined with collagen type I, and the intermediate lamina propria (SLP)	Fibrillar	α1(III)	[α1(III)] ₃	<i>COL3A1</i>	2q32
IV	BMZ	Network	α1(IV)	α1[IV] ₂ α2[IV]	<i>COL4A1 C</i>	13q34
			α2(IV)	α3[IV]α4[IV]α5[IV]	<i>COL4A2</i>	13q34
			α3(IV)	α5[IV] ₂ α6[IV]	<i>COL4A3</i>	2q36-q37
			α4(IV)		<i>OL4A4</i>	2q35-q37
			α5(IV)		<i>COL4A5</i>	Xq22
VII	Transition between BMZ and SLP	Anchoring fibrils	α6(IV)		<i>COL4A6</i>	Xq22
			α1(VII)	[α1(VII)] ₃	<i>COL7A1</i>	3p21.1

BMZ, basement membrane zone; SLP, superficial lamina propria.

TABLE 2**Summary of Collagen-based Biomaterials for Vocal Fold**

Biomaterial	Format	Composition	Complications/Disadvantages	Clinical Trial
Zyderm Collagen Implant I and II	Injectable	Soluble bovine collagen; 0.3% lidocaine; Zyderm II contains 2× (collagen) than Zyderm I. ³⁵ Zyderm I contains 95%–98% type I collagen and the remainder is type III collagen. Zyderm II is identical to the chemical makeup of Zyderm I.	Lack of efficient fibroblast integration; autoimmune response; breakdown of natural collagen; requires overinjection	Normal canine vocal folds ³⁵ Humans with glottic insufficiency
Zyplast	Injectable	Cross-linked soluble bovine collagen; glutaraldehyde ³⁶ Zyplast is cross-linked to 3.5% bovine dermal collagen I.	Autoimmune response, material rejection, immunologic destruction, breakdown of natural collagen	Humans with unilateral vocal cord paralysis, presbyphonia, cricoarytenoid ankylosis, postlaryngeal surgery
Phonagel	Injectable, specific for vocal cord or pharynx	Glutaraldehyde cross-linked collagen, cross-linked soluble bovine collagen, 0.0075% glutaraldehyde. ³⁶	Material rejection, anaphylaxis, respiratory issues, breakdown of natural collagen	Humans with unilateral vocal cord paralysis, presbyphonia, cricoarytenoid ankylosis, postlaryngeal surgery
Autologen	Injectable	Collagen extracted from autologous human skin, naturally cross-linked. It is a dispersion of dermal matrix shown to contain intact collagen of types I, III, and VI; elastic fibers; fibronectin; and glycosaminoglycans. ^{35,36,43}	Inconvenience in processing, donor site morbidity	Humans with sulcus vocalis, atrophy, secondary scarring
Micronized Alloderm	Injectable, also scaffold or tissue expander	Homologous form, derived from cadaveric human skin. AlloDerm is formed from allograft skin harvested from cadaveric tissue donors, which is processed into an immunologically inert dermal graft by removing the epidermal and dermal cells. ^{37,38,44,45}	Inconvenience in processing	Human patients with unilateral vocal cord paralysis

Notes: Two layers of thick, strongly birefringent collagen fibers (collagen type I), one immediately below the epithelium and another denser layer in the deep region superficial to the vocal muscle, penetrating between muscle fibers.