A Review of Hyaluronic Acid and Hyaluronic Acid-based Hydrogels for Vocal Fold Tissue Engineering

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Summary: Vocal fold scarring is a common cause of dysphonia. Current treatments involving vocal fold augmentation do not yield satisfactory outcomes in the long term. Tissue engineering and regenerative medicine offer an attractive treatment option for vocal fold scarring, with the aim to restore the native extracellular matrix microenvironment and biomechanical properties of the vocal folds by inhibiting progression of scarring and thus leading to restoration of normal vocal function. Hyaluronic acid is a bioactive glycosaminoglycan responsible for maintaining optimum viscoelastic properties of the vocal folds and hence is widely targeted in tissue engineering applications. This review covers advances in hyaluronic acid-based vocal fold tissue engineering and regeneration strategies.

Key Words: Hyaluronic acid–Vocal fold–Tissue engineering–Scarring–Extracellular matrix.

INTRODUCTION

The vocal folds are mechanically active soft tissues that can self-sustain oscillations ranging from 100 Hz to 1000 Hz in response to airflow to produce sound.[1,2] Of the US population, 24.49% consider voice an integral part of their jobs.[3] Up to 9% of Americans experience a voice disorder at some stage in life.[4] Annual direct healthcare costs for voice disorders exceed $200 million,[5] leading to reduced occupational performance[6] and inferior quality of life.[7] Damage to the vocal folds and ensuing voice disorders can result from a variety of factors including intubation,[8] phonotrauma,[9] chemical irritants in the environment,[1] and laryngopharyngeal reflux.[9] Chronic, detrimental exposures combined with the high mechanical stresses during phonation can cause permanent changes to vocal fold tissue composition and biomechanics, which manifest as scarring.[10] Scarred vocal folds suffer from incomplete or compromised mucosal wave formation because of the elevated viscous properties of the tissue and excess collagen deposition,[11,12] leading to an unsustainable phonation quality.

Tissue engineering and regenerative medicine aim to restore the native extracellular matrix (ECM) composition that governs the biomechanics of vocal folds, while also supporting the pliability and viscoelastic properties of the tissue by inhibiting the excess wound healing that leads to scarring. Hyaluronic acid (HA), a naturally occurring glycosaminoglycan responsible for regulating viscoelastic properties of the vocal folds,[13] is a promising building block for tissue engineering of the vocal folds because of its innate biocompatibility and bioactivity.[14,15] This review will cover the application of HA in vocal fold tissue engineering. This review is organized into the following sections: vocal fold composition, vocal fold biomechanics, pathophysiology of vocal fold scarring with an emphasis on changes in HA, and current HA-based tissue engineering solutions for scarring. The readers are directed to comprehensive reviews by Fishman et al[16] for recent advances in stem cell-based regeneration, and by Li et al[17] for broader perspectives in vocal fold tissue engineering.

Vocal fold composition

The ability to sustain small-amplitude, high-frequency oscillations can be attributed to the anisotropic, layered structure of vocal fold tissue. True vocal folds consist of five layers: a stratified squamous epithelium that overlies the heterogeneous, three-layered lamina propria, and the thyroarytenoid muscle.[18] The epithelial-lamina propria interface contains a basement membrane zone with anchoring fibers that attach the basal cells of the epithelium to collagen IV and laminar proteins.[10] The lamina propria is an ECM-rich, loose, non-muscular tissue of the vocal folds that is subdivided into three layers known as the superficial (SLP), intermediate (ILP), and deep (DLP) layers.[19]

Collagen and elastin are the most abundant fibrous proteins in the lamina propria.[10,20] Collagen (predominantly type I and type III[21]) constitutes 43% by weight of the total protein in the ECM and modulates the tensile strength of vocal folds, whereas elastin constitutes 8.5% by weight of the total protein in the ECM and contributes to elasticity and elongation of the vocal folds.[10,22–24] Histologic staining for collagen fibers shows an increase in thickness and density of fibers from the SLP to the DLP.[24–26] Histologic staining for elastin reveals that mature, dense, longitudinally aligned elastin fibers are present in the ILP and only minor elastin staining is seen in the DLP.[13,23]

Apart from these fibrous proteins, the vocal fold ECM also consists of interstitial glycosaminoglycans and proteoglycans such as HA, decorin, fibromodulin, and versican.[10,27] HA is found dispersed throughout the lamina propria, but is slightly more concentrated in the ILP.[13,22] It acts as the major modulator of viscoelasticity and osmosis in the vocal folds. It is also involved in migration and wound healing.[28] Other proteoglycans like decorin found mostly in the SLP, and fibromodulin found mostly in the ILP and the DLP, help in modulating collagen fibrils in the vocal folds by thinning the fibrils and delaying their formation into thicker fibrils, thus supporting the layered structure of the lamina propria.[29,30] Variations in the lamina propria

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composition because of gender and age have also been noted with male vocal folds containing higher concentrations of HA and collagen than female vocal folds do.[26,31]

The cellular composition of the lamina propria consists of sparsely dispersed cells such as fibroblasts, myofibroblasts, and macrophages.[32] Fibroblasts make up the bulk of the cells in the vocal folds and are essential to generating and maintaining ECM composition. Myofibroblasts are differentiated fibroblasts that stain positively for muscle-specific actin, and are instrumental in injured vocal fold repair.[10] Macrophages are confined to the SLP and are sparsely distributed. Given that macrophages are mostly associated with wound healing, they may help regulate microscopic damage present in healthy vocal folds because of constant vibration.[32] The regenerative capacity of the vocal folds, however, is limited, leaving them susceptible to permanent irreversible damages, affecting the quality of voice because of altered tissue biomechanics.

Vocal fold biomechanics

An understanding of vocal fold cover (epithelium and SLP)[10] biomechanics provides the foundation required for designing a tissue-engineered model that closely mimics vocal fold dynamics.[33] Viscoelastic properties of the vocal folds are quantified by a complex shear modulus, which is an additive measure of the elastic modulus and the dynamic viscosity.[34] Chan and Titze conducted experiments on human larynges using a parallel plate rotational rheometer with frequencies ranging from 0.01 to 15 Hz and found that the elastic modulus of the mucosa varied from 10 to 1000 Pa, and the dynamic viscosity decreased monotonically as frequency increased.[34] A follow-up study using a controlled strain rheometer allowed for frequency measurements up to 50 Hz,[35] and results were comparable with lower frequency data. To measure viscoelasticity at physiologically relevant frequencies, alternative strategies have used simple linear, rather than rotational rheometry, allowing for measurements between frequencies of 1 and 200 Hz. Consistent with prior results, elastic moduli were between 20 and 1000 Pa, and dynamic viscosity decreased with increasing frequency.[36] Torsional wave analysis, which accounts for anisotropic variations in soft tissue at phonation frequencies,[37] shows that the elastic modulus of excised human larynges (age 60–90 years) lies between 160 and 1600 Pa. Ideally, an elastic modulus within this range should be targeted for tissue-engineered scaffolds.

Vocal fold scarring

Vocal fold lesions that disrupt ECM organization can alter the viscoelastic properties of the vocal folds and result in a hoarse, unsustainable phonation quality.[11] Local macrophages and myofibroblasts are able to repair minuscule damage due to vocal fold edema and inflammation caused by acute phonotrauma.[10,32] However, when damage surpasses a threshold, due to either direct injury or external trauma to the vocal folds, permanent pathologic changes can occur. Scarring is the downstream manifestation of these injuries, and leads to incomplete mucosal wave formation and eventual dysphonia.[12,38]

Pathophysiology of vocal fold scarring

A large number of animal models have been studied to understand the biochemistry of scarring.[39–42] Changes in the ECM microstructure and loss of homeostasis are implicated in scarring. Disruption in collagen I deposition is the most common feature of scarring, with studies showing an increase in collagen I and procollagen I levels.[39,43,44] Histologically, collagen I loses its longitudinal organization and is instead seen dispersed in disorganized, thick bundles throughout the vocal folds.[39–41,45,46] Elastin production is decreased, and a loss of organization of fibers in the ILP could explain the decreased pliability of the tissue.[39,42]

Reduced levels of decorin, which inhibits collagen fibrillogenesis,[12,40] combined with lower expression levels of fibromodulin, which delays collagen synthesis,[12,47] result in elevated collagen fibril formation, thus decreasing vocal fold flexibility. Fibronectin, which acts as a modulator of inflammation and cell migration during wound healing, is elevated for as long as 6 months post injury, enhancing migration of fibroblasts and dysregulating collagen morphogenesis.[39,43,48] Cellular response includes high density of myofibroblasts as seen through staining for muscle-specific actin in scarred tissue. These cells produce collagen continuously, thus adding to the increased tissue stiffness[42] and making phonation difficult.

Optimum levels of HA are responsible, in part, for wound healing processes and scarless wound healing in fetuses.[1] Significant reduction of HA reported in rabbit and pig models[49,50] could explain the formation of excessive scar tissue and increased stiffness. At the same time, no changes in HA content have been reported in other models.[39,42] In a recent study, elevated levels of hyaluronan synthase, which synthesizes HA, were reported during the early stages of scarring in rats, whereas elevated levels of hyaluronidase, which digests HA, were reported 2 months post injury. Combined, these findings could explain advancement to scarring due to loss of HA in later stages of wound healing.[51] Reduction in the shock-absorbing properties of the vocal folds because of changes in HA composition could also be responsible for altered biomechanics and poor healing of the tissue.

Because scarring is a macroscopic manifestation of multiple diseases and is known to vary depending on extent of injury and wound healing, treatment is challenging, with methods varying from medical to surgical intervention. But as of yet, no gold standard for treatment has emerged. Tissue engineering provides an attractive alternative to surgery as it tries to promote wound healing to aid in restoring ECM homeostasis and normal vocal outcomes.

Tissue engineering for the vocal folds

Tissue engineering can be defined as the application of scientific and engineering principles to the construction, development, and maintenance of biological substitutes for living tissues using a structure-function relationship.[52] The aim of tissue-engineered vocal fold therapy is to restore native ECM and biomechanical properties that are lost in scarring as well as to suppress progression of scarring using a combination of scaffolds, regulatory signals, and cells.