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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.

Keywords

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

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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 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.

Traditionally, injectable fillers such as Teflon, polydimethylsilicone, calcium hydroxypalpitate (or Radiesse Voice), or biological materials such as fat and bovine collagen have been used for treatment of vocal fold scarring to increase bulk of the tissue and improve glottal closure.[1,38,53] Although improvement in rheological properties and glottal closure are observed post injection, these therapies are more supportive rather than regenerative as they do not repair associated damage with scarring and may also result in

long-term complications such as chronic inflammation, implant migration, granuloma formation, and quick resorption times.[38,53] These complications necessitated the development of alternative tissue-engineered treatments for the restoration of normal vocal fold function. HA, because of its non-immunogenicity, non-antigenicity, innate biocompatibility, tunable viscoelastic properties, and ease of modification, is one of the most widely researched vocal fold tissue engineering materials.

HA in the vocal folds—HA is a naturally occurring glycosaminoglycan consisting of repeating units of D-glucuronic acid and N-acetyl D-glucosamine[28,54,55] (Figure 1). It is ubiquitously found in the ECM of all tissues, but is highly concentrated in mechanically active tissues such as the vocal folds, cartilage, and dermis. It is synthesized in the inner plasma membrane by a transmembrane protein family called hyaluronan synthases[57] and pushed out into the ECM, where it resides for 3–5 days before being degraded by a family of enzymes called hyaluronidases.[58] It is the most abundant glycosaminoglycan in the vocal folds, with roughly 6 μg of HA per mg of total protein present at a given time.[22] HA is negatively charged under physiological conditions and interacts with water to form extensive hydrogen bonds, which allow it to undergo deformation and resist trauma caused to the tissue by expanding up to 1000 times in weight. It thus acts as a space filler,[29] shock absorber,[30] and tissue damper,[54] which are especially important properties for the vocal folds, because constant vibration results in continuous stresses that need to be absorbed without causing permanent damage. At a physiological pH, the highly polarized HA reacts with ions and is the major modulator of tissue viscosity and osmosis, thus regulating hydration and vocal quality in the vocal folds.[30] Removal of HA from the vocal folds resulted in a 25%–40% increase in stiffness of the vocal folds.[55] Further, the shear thinning properties of HA reduce vocal tissue stiffness to enable vibration, creating optimal conditions for phonation.[55] Additionally, HA is bioactive and has been implicated in cell migration and wound healing responses. Cell surface receptors CD44[59] and receptor for hyaluronan-mediated motility[60] bind to HA and initiate cascades of events such as inflammation, cell motility, and cell growth, thus playing an important role in wound healing and aiding the progression to scarring.[28,61] This bioactivity of HA in promoting wound healing combined with its role in maintaining vocal fold hydration and biomechanics make HA an attractive building block for scaffolds of tissue-engineered vocal folds.

Given the promise of HA for its regenerative capacity, some focus is needed to increase its short half-life. To increase residence time, HA can be functionalized to provide sites for cross-linking, with typical substitutions to the carboxylic group or hydroxyl group on the HA backbone (Figure 1). Hylan-B and HYAFF are cross-linked HA gels that have been used for vocal fold augmentation.[62–65] However, these HA-based gels used harsher cross-linking chemistries leading to loss of bioactivity. Newer hydrogel combinations have tried to functionalize HA through minimal modifications and milder cross-linking chemistries to preserve biological activity. Functionalized HA contains cross-linking sites such as thiols, methacrylates, aldehyde, and dihydrazide groups.

Thiol functionalized HA—In this section, we will review hydrogels that involve modifications to the carboxylic group on the HA backbone (Figure 1) to provide thiols as

sites for cross-linking (Figure 2). Cross-linking agents include poly(ethylene glycol) diacrylate (PEGDA) (eg, Carbylan-SX) and thiolated gelatin (eg, Carbylan-GSX). Carbylan-SX and Carbylan-GSX can be tuned to have slower degradation rates and flexible viscoelastic properties by varying parameters such as degree of substitution, concentrations of starting reagents, ratio of thiols to acrylates, and molecular weights of the HA and PEGDA. Biocompatibility of the gels is unaffected while varying these parameters.[67–69]

Initial *in vitro* testing of Carbylan-GSX[70] for biocompatibility and non-immunogenicity using both fibroblasts[15] and mesenchymal stem cells showed promising results with lower expression of inflammatory cytokines and higher expression of ECM proteins (Table 1). Improved cell adhesion because of Carbylan-GSX caused matrix remodeling guided by cell-ECM interactions.[78] Further, short-term and long-term studies using Carbylan-GSX in rabbits[79] (Table 2) have indicated pro-healing responses early during injury, with approach to normal vocal fold viscoelasticity 6 months following treatment.[82–85] This suggests that prophylactic administration of Carbylan-GSX early on during injury can guide improved healing and remodeling processes to restore normal vocal function. Similarly synthesized thiolated HA-gelatin hydrogels indicate a pro-healing response because of ECM remodeling in a rat model.[86]

Alternative strategies have also used thiolated HA to synthesize microgels to harness cell adhesion properties of these gels. Microgels made with thiolated HA-gelatin and reinforced to a composite hydrogel[76] show better adhesion and migration of vocal fold fibroblasts on these scaffolds in comparison with HA-gelatin scaffolds without the reinforced microgels. Carbylan-GSX, along with carboxymethylated HA (Extracel), has also been used as a delivery vehicle for combined therapy with bone marrow-mesenchymal stem cells embedded in the hydrogel. Significant improvements in rheological properties and reduction alpha smooth muscle actin expression in rats[87] (Table 2) show that cell-hydrogel combination therapy might work well in reducing myofibroblast differentiation, and thus help restore vocal fold ECM. These promising *in vivo* and supporting *in vitro* studies have paved the way for Extracel to enter planned preclinical trial stages.[88]

Methacrylate functionalized HA—In this section, we will review hydrogels that involve modifications to the hydroxyl group on the HA backbone (Figure 1) to provide polymerizable methacrylate residues (Figure 2). The advantage of this cross-linking site is that is allows for photopolymerization giving spatial control over gel geometry. HA hydrogels created by reacting photo cross-linkable methacrylate with oxidized HA or oxidized HA with a functional acrylamide[72] resulted in physiologically relevant viscoelastic gels with high degrees of tunability and biocompatibility as shown by their encapsulation of NIH/3T3 cells. HA hydrogels made out of methacrylated HA[77,89] (Figure 2) promote cell spreading and proliferation in three-dimensional networks, showing their ability to support cell adhesion. Further, applying vibration to methacrylated HA that is photopolymerized with PEGDA resulted in a significant decrease in collagen production by human dermal fibroblasts in comparison with static controls. This suggests that vibration can guide ECM changes along with scaffold properties, and hence, restoring native viscoelastic properties may be key to optimizing vocal function.

Hydrazide and aldehyde functionalized HA—In this section, we will review microgels that involve modifications to HA to provide aldehyde and hydrazide functional groups as sites for cross-linking (Figure 3). The resultant microgel characteristics allow for controlled degradation profiles and tunable degrees of functionalization, leading to rapid recovery from mechanical stress. Residual functional groups serve as sites to bind to bulk macromolecules. This is possible because the starting ratios of the HA aldehyde (HAALD) and HA adipic dihydrazide (HAADH) can be independently regulated.[73] These functionalized HA, when used to covalently cross-link with mature or immature collagen fibrils, reduce resorption time of HA significantly. Fibroblasts encapsulated in this gel combination with mature collagen showed proliferation over 28 days and retained their morphology and ability to synthesize ECM. Histologic staining at the end of culture showed much similarity between the scaffolds and the native vocal folds, thus showing promise for regeneration.[74] Sahiner et al also used these doubly cross-linked networks consisting of soft HA hydrogel particles modified to contain aldehyde groups in the backbone cross-linked with HAADH,[71] which showed good potential for regeneration owing to tailored viscoelastic properties as tested by torsional wave analysis, low gelation time, and high surface area of the networks to improve tissue integration. These hydrazone cross-linked HA gels with dextran when transplanted into ferret vocal folds for 21 days have shown highly tunable cross-linking and viscoelastic properties based on hydrogel compositions, and only mild adverse reactions.[80] Long-term investigations *in vivo* remain to be conducted.

Other HA functionalized gels—Other HA-based biocomposite hydrogels include HA-collagen and collagen-alginate gels investigated by Hahn et al., who reported 50% loss of mass in the collagen HA gels over 28 days, whereas collagen-alginate gels were stable for at least 42 days *in vitro*, suggesting that collagen-alginate gels are more promising for *in vivo* implantation.[75] Adipose-derived stem cells cultured in cogels[81] of HA or collagen with fibrin showed enhanced potential for differentiation and proliferation in comparison with gels with only fibrin or HA, with elongated cell morphology similar to that of fibroblasts. HA-alginate hydrogels combined with adipose-derived stem cells[81] implanted into rabbits showed improved macroscopic morphologies in comparison with saline controls, showing promise for promoting a healing response (Table 2).

In summary, biomimetic HA-based hydrogels show potential in promoting wound healing and restoring normal vocal function because of their excellent biocompatibility, ability to enhance viscoelastic properties, and ability to regulate ECM production. Many *in vivo* studies using HA hydrogels have further supplemented the gels with stem cells or growth factors to induce continuous release and restoration. Using HA as a drug delivery system for bioactive growth factors and stem cell therapy has shown potential for recovery from scarring, thus showing that a combination approach to healing might be needed for complete regeneration.

CONCLUSIONS

Investigations into the complex architecture of the vocal folds show that cell-cell, cell-ECM, and ECM-ECM interactions are essential for normal functioning of the tissue. To reconstruct this physiological environment *in vitro*, it is necessary to consider the factors that influence

the *in vivo* microenvironment. HA-based biomaterials have great potential for treatment of vocal fold scar because of the important role of hyaluronan in vocal fold biomechanics, cell migration, signaling, and wound healing. Innately present in the vocal folds, HA is bioactive, biocompatible, and non-immunogenic. Because of the ease of modification, cross-linking sites can be provided on the native HA molecule, which offers excellent tunability of viscoelastic and biological properties, based on degree of cross-linking and concentrations used. Multiple *in vitro* and *in vivo* studies have provided promising evidence that HA scaffolds can support healthy vocal fold tissue regeneration. Future studies should move from *in vitro* studies to controlled randomized animal trials and further refinement of viscoelastic properties based on patient needs to support full and sustained tissue regeneration.

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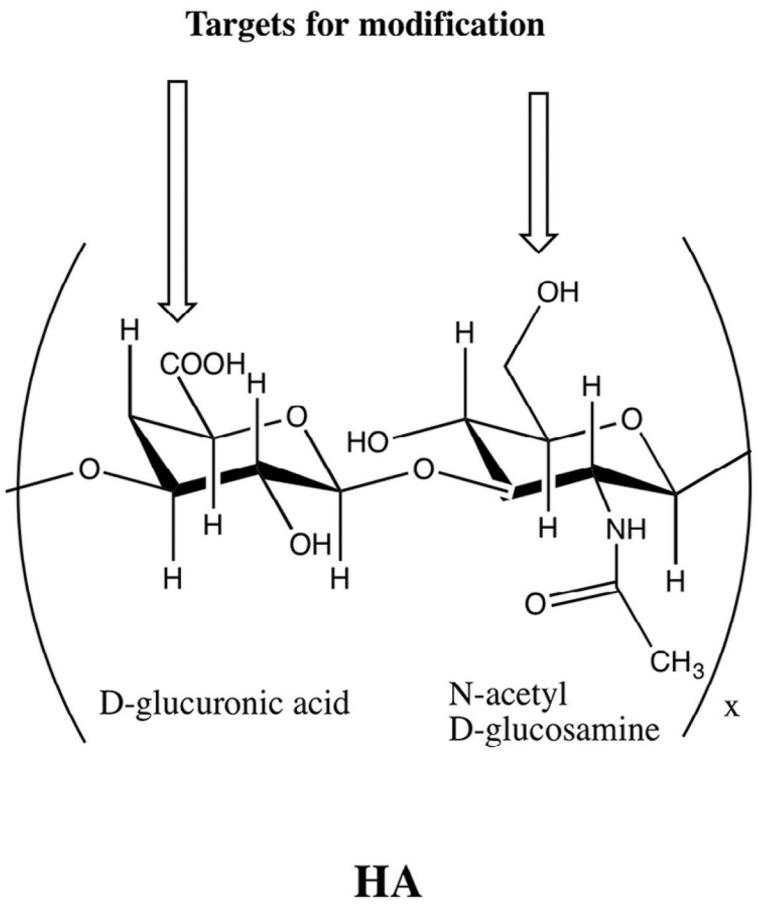

FIGURE 1.
Unmodified HA backbone. Substitutions can be made to the carboxylic (—COOH) or the hydroxyl (—OH) group on the backbone. Adapted from Burdick and Prestwich.[56]

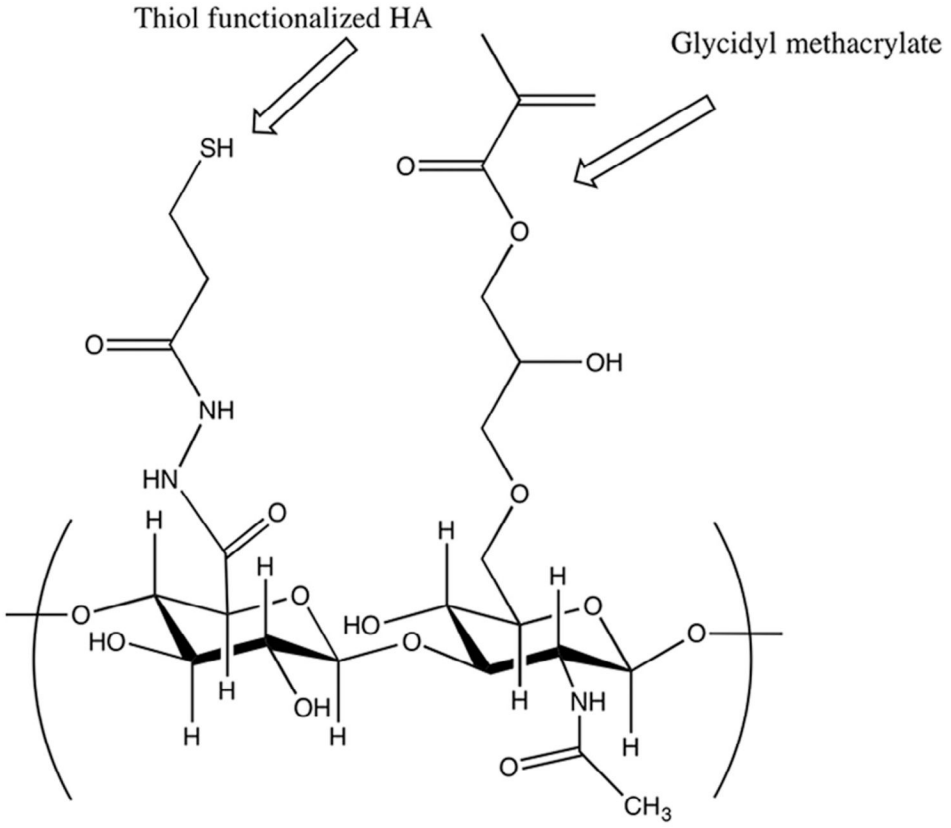

FIGURE 2.
Thiol functionalized and methacrylate functionalized HA. Adapted from Burdick and Prestwich.[56]

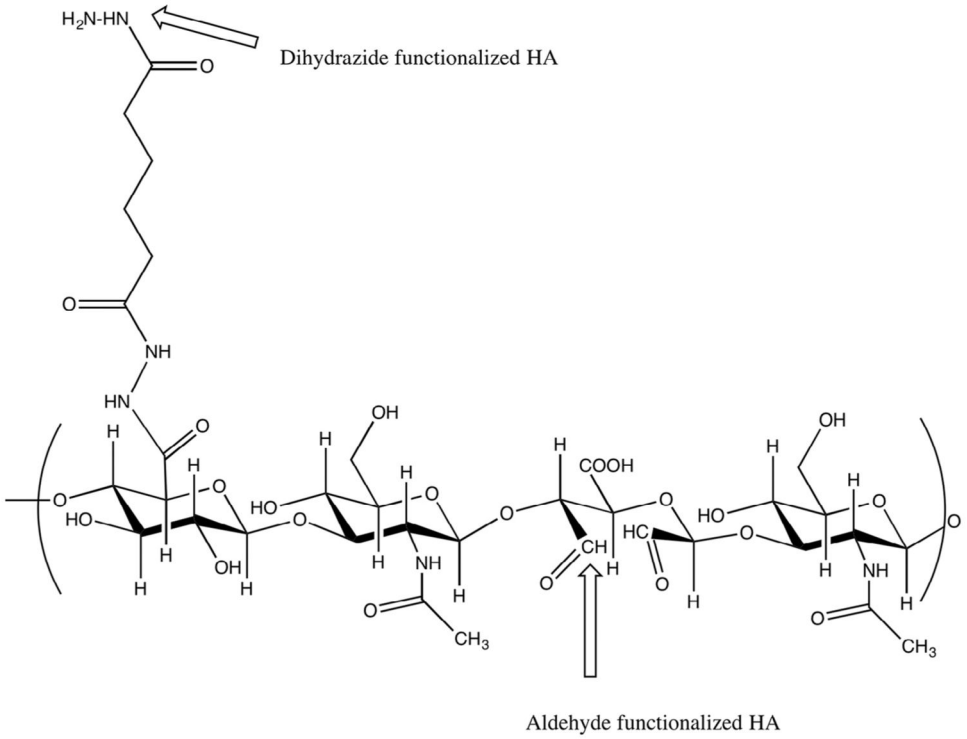

FIGURE 3.
Aldehyde and hydrazide functionalized HA. Adapted from Burdick and Prestwich.[56]

TABLE 1

In vitro HA-based Tissue Engineering

Scaffold	Cell Source	Results (Compared With Untreated Controls)
Thiolated HA + PEGDA + thiolated gelatin hydrogel (Carbylan-GSX)[15]	Human vocal fold fibroblasts in 2D and 3D	• Decrease in IL-6, IL-8, and HAS-3 expression • Increase in fibronectin, HYAL, and cyclooxygenase II
Carbylan GSX vs Matrigel[70]		• Decreased Col I, Col III, MMP-I, and fibronectin expression • Less alpha SMA expression on gel
Hystem-C (Carbylan GSX + carboxymethylated HA)[66]	MSCs and fibroblasts in coculture	• Increase in Col I, MMP-1 • Increased HGF expression • Reduced IL-6, VEGF expression
Doubly cross-linked network of soft HA hydrogels with dihydrazide- and aldehyde-functionalized HA[71] (HAADH, HAALD)	Fibroblasts	• Resisted 200%–300% strain • Compatible biomechanical properties with vocal folds • Biocompatible
Glycidyl methacrylate (GMHA) reacted with oxidized HA or acrylamide functionalized HA[72]	NIH/3T3	• Highly tunable biomechanical properties • Biocompatible
Hydrazide or aldehyde modified HA microgels[73] (HAADH, HAALD)	Porcine fibroblasts	• Total HA content decreased over time • Cell spreading and proliferation on both matrices
HA microgels with collagen[74]		• Elastic modulus stabilized over time in culture
HA-collagen vs collagen-alginate[75]	Porcine fibroblasts	• Mass loss of around 55% in HA-collagen gels • Increased proliferation on HA-collagen gels • Reticular fibers only seen in collagen alginate gels
HA-gelatin (HA-Ge) microgel-reinforced composite hydrogel[76]	Human vocal fold fibroblasts	• Increased motility on microgel-reinforced hydrogel • Promote cell adhesion and migration
Methacrylated HA photopolymerized with PEGDA[77]	Human dermal fibroblasts	• Increased HAS II, fibromodulin, decorin, and MMP-1 • Increase in sGAGs • Decrease in collagen

G′ is the elastic component and G″ is the viscous component of the viscoelastic moduli.

Abbreviations: HAADH, HA adipic dihydrazide; HAALD, HA aldehyde; HAS, hyaluronan synthases; HGF, hepatocyte growth factor; HYAL, hyaluronidases; IL, interleukin; MMP-1, matrix metalloproteinase-1; MSC, mesenchymal stem cells; sGAGs, sulfated glycosaminoglycans; SMA, smooth muscle actin; VEGF, vascular endothelial growth factor.

TABLE 2

In vivo HA-based Tissue Engineering

Scaffold	Duration	Animal Model	Results (Compared With Injured Untreated Controls)
Hydrazone (HAADH + HAALD) cross-linked HA-dextran microgel[80]	21 days	Ferret	• Compatible mechanical properties • Mild inflammation • Tunable *in vivo* residence time
Entrapped HA in $CaCl_2$ cross-linked alginate with adipose MSCs[81]	3 months	Rabbit	• Reduced collagen I deposition • Reduced G′ and G″ • Less disorganized elastin
Carbylan-SX (thiolated HA + PEGDA hydrogel)	21 days 6 months	Rabbit	• Reduced fibrosis • Reduced G′ and G″
Extracel[79,82]			• Reduced procollagen 1, fibronectin, TGF-β
Carbylan-GSX	21 days	Rabbit	• Reduced G′ and G″
Carbylan-SX[83]			• Increased hyaluronidase II
Carbylan GSX[84,85]	1, 3, 5, 10 days	Rabbit	• Increased procollagen I, fibronectin • Increased TNF-α, IL-1β, IL-6
HA-alginate hydrogel with adipose derived stem cells[81]	1 month, 3 months	Rabbit	• Reduced collagen I at 3 months • Increased HGF activity
Cross-linked HA-gelatin microgels[86]	3, 14, 28 days	Rat	• Reduced macrophages • Increased collagen I • Increased elastin
Extracel with MSCs[87]	1 month	Rat	• Increased procollagen III, fibronectin

Abbreviations: HAADH, HA adipic dihydrazide; HAALD, HA aldehyde; HGF, hepatocyte growth factor; IL, interleukin; MSC, mesenchymal stem cells; TGF, transforming growth factor; TNF, tumor necrosis factor.