Hyaluronic acid for the treatment of vocal fold scars

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Purpose of review

In vocal fold scars the lamina propria layer is lost or deficient. Lamina propria replacement therapy remains a clinical challenge because this layer has a highly specialized three-dimensional organization of extracellular matrix molecules and unique viscoelastic properties. Use of a polymer such as hyaluronic acid appears most promising for replacement therapy because it has the optimal viscoelasticity and also plays a role in the maturation and maintenance of vocal fold lamina propria.

Recent findings

A variety of cross-linked hyaluronic acid formulations and growth factor therapies targeted to increase hyaluronic acid production have been used in the treatment of both acute and established vocal fold scars. Therapeutic strategies have focused on prevention of scar at the time of initial injury, and rejuvenation of lamina propria layer in established scars. Both strategies show improved histologic, viscoelastic, acoustic, and aerodynamic measures.

Summary

Cross-linked hyaluronic acid formulations appear useful in the treatment of vocal fold scarring. Their use at the time of acute injury especially appears to lessen the degree of long-term scar formation and appears promising. While animal studies have demonstrated the safety profile of many hyaluronic acid formulations, further improvement in these materials and well designed and controlled human trials are needed to further establish the safety and efficacy of these materials and therapeutic approaches.

Keywords

growth factors, hyaluronic acid, vocal fold scarring

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Introduction

Hyaluronic acid is an important component of the extracellular matrix (ECM) in a number of human tissues such as vocal folds, synovial fluid, skin, and cartilage. Also known as hyaluronan it is a glycosaminoglycan polymer of disaccharides composed of repeating units of glucuronic acid and acetyl-glucosamine [1]. In the vocal fold, it is found in the lamina propria layer and has gained attention from voice scientists due to its potential role in modulating vocal fold vibratory biomechanics and prevention of scars. It is a highly charged polymer at physiologic pH which allows it to bind water extensively and thus determine the viscoelasticity of the lamina propria layer [2]. Although it has no protein component, hyaluronic acid is considered part of a group of large-chain proteoglycans that include aggregan and versican, and is found in large quantities in tissues concerned with viscosity and fluid movements (e.g. synovial fluid) or space filling (e.g. vitreous humor of the eye) [2]. Moreover, it has a simple molecular structure with no structural differences across species from human to bacteria, and the lack of amino acids renders it nonimmunogenic.

Hyaluronic acid in the normal vocal fold

The vocal fold lamina propria compartment is a paucicellular layer that mostly contains ECM. The ECM components mainly consist of fibrous proteins such as collagen and elastin, small interstitial matrix proteoglycans such as decorin and fibromodulin, the large aggregating proteoglycan versican, glycoproteins such as fibronectin, and the glycosaminoglycan hyaluronic acid [2–4]. It is likely that the fibrous proteins provide the tensile strength while the interstitial proteins provide the viscoelastic characteristics of the vocal fold. The distribution of hyaluronic acid in vocal folds has been studied through immunohistological staining of excised animal and human larynges and intraoperative biopsies. Quick-freezing and dehydration of the tissue as well the technique used to detect and quantify hyaluronic acid impact the findings [5,6]. With these caveats, hyaluronic acid has been reported to constitute about 0.82% of the human lamina propria [7]. There may be gender and interlaryngeal differences although this has not been firmly established [6,8]. Age-related hyaluronic acid loss has

not been seen [8]. Hyaluronic acid appears to be more abundant in the intermediate lamina propria layer [2,9]. There is some evidence for higher concentration of hyaluronic acid in the inferior medial surface of the vocal fold where mucosal upheaval begins during phonation [5,9].

The exact role of vocal fold hyaluronic acid remains to be studied in detail. However, due to its ability to attract water and form a gel it is thought to affect tissue viscosity, osmosis, and flow resistance and therefore determine the biomechanics of vocal fold oscillation [3]. When hyaluronic acid was removed from the cover layer, dynamic viscosity increased by up to 70% but the elastic shear modulus decreased between 25% and 40% [2,5]. Thus, a dual role for hyaluronic acid has been proposed: presence of hyaluronic acid leads to lower tissue viscosity and lower phonation onset pressure, but it also contributes to the maintenance of passive tissue elasticity to optimize the fundamental frequency of voice [5]. The viscous and shear properties of hyaluronic acid have been shown to be concentration-dependent [10]. However, dynamic (phonatory) investigations correlating hyaluronic acid concentration with vocal fold mucosal waves and aerodynamic parameters such as phonation onset pressure have not been performed.

Hyaluronic acid may also play an important role in the maintenance of the vocal fold ECM. ECM proteoglycans are known to have important roles in the maintenance of matrix components by assembling and holding together large proteins and promoting cellular differentiation and proliferation [1]. Cells such as fibroblasts can sense their extracellular matrix stiffness via cell adhesion complexes and change their mechanical response [11,12]. The hyaluronic acid cellular receptor, CD44, was found most concentrated in the vocal fold epithelium in the midmembranous area, suggesting that hyaluronic acid is active in the region of greatest vibratory amplitude [13]. In addition, whereas less than 10% cells within the macula flava stain CD44-positive in vocal folds that have never phonated, nearly 95% of the cells stain CD44-positive in normal phonated larynges [14••]. Moreover, in never-phonated vocal folds the qualitative amount of hyaluronic acid was considerably less and the laminar structure of the lamina propria layer was not seen compared with normal phonated vocal folds. These studies support the role for hyaluronic acid as a cellular signal in the maintenance and maturation of the lamina propria layer in response to vocal fold stress.

Hyaluronic acid in the scarred vocal fold

In vocal fold scarring there is loss of the three-dimensional organization of fibrous proteins such as collagen and elastin and alterations in additional ECM components [15,16]. Although hyaluronic acid has been known to play a critical role in the development, maturation, and maintenance of the normal lamina propria in many organ systems, our understanding of its role in vocal fold healing and scarring after injury still remains rudimentary. In the rat, significantly elevated levels of hyaluronic acid-synthase-1 and hyaluronic acid-synthase-2 activity were seen as early as 1 and 4 h postinjury, respectively [17]. In a rabbit model of vocal fold scar, hyaluronic acid levels were elevated on day 5, compared to days 3, 10, and 15 after injury [18]. However, the overall levels of hyaluronic acid in scarred vocal folds remained significantly diminished compared with normal controls. Tateya *et al.* [19] examined rat vocal folds between 2 and 12 weeks after scarring and also found reduced levels of hyaluronic acid at all time periods. However, no differences in hyaluronic acid levels were seen in rabbits 60 days after scarring and in canines at 2 and 6 months after scarring [16,20]. In humans undergoing a second-look biopsy 3–13 months after endoscopic laser treatment for early-stage glottic cancer, diminished or absent hyaluronic acid levels were seen, although results were quite variable, with deeper resections leading to increased loss of hyaluronic acid [21••]. These studies support the notion that hyaluronic acid plays a more active role in the acute phase of vocal fold scarring and wound repair.

Hyaluronic acid in the treatment of vocal fold scar

Hyaluronic acid has received considerable attention as an optimal agent to augment the lamina propria compartment as well as to prevent vocal fold scarring because it is nonimmunogenic, nontoxic, noninflammatory, and easily injectable [22]. Hyaluronic acid also appears to play a critical role in fetal scarless healing: whereas hyaluronic acid level initially rises then falls significantly in adult wound healing, it remains elevated until completion of scarless healing in fetal wounds [23]. Moreover, as mentioned earlier, hyaluronic acid plays a major role in determination of vocal fold viscoelasticity and in ECM maintenance and maturation. However, the simple composition of hyaluronic acid also allows rapid degradation by hyaluronidase enzymes and free radicals. Its loose, hydrophilic structure facilitates easy access for rapid cleavage, and even large molecular weight hyaluronic acid can be degraded in less than a week. Hyaluronic acid injected into rabbit vocal folds was absorbed within a week [24]. Therefore, cross-linked hyaluronic acid has been utilized in investigations of the role of hyaluronic acid in vocal fold scarring.

The process of cross-linking binds multiple hyaluronic acid polymer chains together, forming a tight molecular structure which is less penetrable by enzymes, with a

subsequent longer half-life. Two most commonly used cross-linking agents are butanediol diglycidal ether (BDDE) and divinyl sulfone (DVS). For instance, Restylane (Q-med, Uppsala, Sweden) is bacterial hyaluronic acid cross-linked with BDDE at a concentration of 20 mg/ml. The degree of cross-linking can also be varied; a typical value is around 4–5%. A more highly cross-linked hyaluronic acid will have a more limited surface area to volume ratio, will not be as hydrophilic, and will be more viscous and more difficult to inject. Additionally, when choosing hyaluronic acid for a bioimplant, these variables (molecular weight, concentration, cross-link density, and chain entanglement) affect their physical and biologic behavior and should be considered. Furthermore, unlinked cross-linking agents can cause adverse toxic reactions, so only hyaluronic acid preparations that have undergone strict quality controls during production must be utilized.

The biologic behavior of hyaluronic acid has facilitated investigations into two separate possible therapeutic roles for hyaluronic acid in vocal fold scarring. First, in light of its role in scarless wound healing and lamina propria maturation, it may have a role in preventing or minimizing the development of vocal fold scars at the time of acute injury. Secondly, due to its viscoelastic attributes and its active role in the maintenance of ECM components, it may have a role in chronic established scars to rejuvenate the lamina propria layer.

Hyaluronic acid in the prevention of vocal fold scar

Hansen *et al.* [25] investigated the results of two different hyaluronic acid hydrogels [Carbylan-SX and hyaluronic acid cross-linked with polyethylene glycol diacrylate (HA-DPTH-PEGDA)] injected into rabbit vocal folds immediately after vocal fold biopsy. About 3 weeks later histologic analysis showed mild, moderate, and severe fibrosis in vocal folds treated with Carbylan-SX, HA-DPTH-PEGDA, and saline, respectively. The same group has recently developed a new variant of hyaluronic acid gel called Extracel, a thiolated derivative of gelatin covalently co-cross-linked with Carbylan-S. The potential role of this compound in prevention of vocal fold scarring was investigated similarly to their prior report, but vocal folds were evaluated 6 months after initial surgery [26••]. A significantly diminished fibrotic response was seen in the Extracel-treated scarred vocal folds, as evidenced by diminished expression of fibronectin, fibromodulin, and procollagen, compared with the saline-treated scarred vocal folds. Tissue stiffness was also lower in the treated group compared with the saline-injected control group. The results were not compared with normal vocal folds. However, these results support the notion that use of hyaluronic acid or its derivatives at the time of vocal fold scarring reduces the extent of scarring in the short and long-term period.

Finck and Lefebvre [27] implanted a few fibers of an esterified preparation of hyaluronic acid (MeroGel; Medtronic Xomed, Jacksonville, Florida, USA) in the Reinke's space after microflap surgery of the vocal folds for benign lesions such as cysts, nodules, and intracordal hemorrhages. Postoperative videostroboscopy in this noncontrolled study revealed maintenance of normal mucosal waves. The same group recently reported a prospective study with longer-term results and compared the treated group with a cohort of patients who had microflap surgery but not the hyaluronic acid implants [28•]. The early postoperative results (1–6 weeks postoperative) were similar between the two groups. However, longer-term follow-up videostroboscopic and acoustic evaluations demonstrated continuing significant improvements in glottic closure, noise to harmonic ratio, and perceptual voice evaluation in the implanted group only. These results provide preliminary support for the clinical use of esterified hyaluronic acid to prevent scar formation, but the heterogeneity of the treatment and control groups and the lack of randomization limit the utility of these findings.

As opposed to injecting formulations of hyaluronic acid into the vocal folds to prevent scarring, an alternate strategy has been proposed to increase the natural production of healthy lamina propria including hyaluronic acid by treatment with growth factors. Hepatocyte growth factor (HGF) plays a significant role in vocal fold wound healing and has been shown to prevent scarring and fibrosis in other organ systems. Based on their prior work showing that HGF *in vitro* increases hyaluronic acid production by fibroblasts, Hirano *et al.* [29] stripped the rabbit vocal fold mucosal cover layer and treated immediately with HGF. Rheological and dynamic phonatory evaluation 6 months after the procedure showed improved pliability compared with sham-treated vocal folds.

Hyaluronic acid in the treatment of established scars

Hertegård *et al.* [30] studied the degree of scarring and the viscoelasticity of rabbit vocal folds injected with either Hylan B gel (Genzyme Biosurgery, Ridgefield, Massachusetts, USA) or cross-linked hyaluronic acid (Restylane, Q-Med Inc., Uppsala, Sweden) 8 weeks after surgical scarring. Eleven weeks after the injection the vocal folds underwent histologic and viscoelastic measurements. The scarred and treated vocal folds both had significantly thicker lamina propria layer. Viscoelastic measurements showed no differences in treated or control scarred vocal folds but there was a large variation in measured parameters.

Hylan B Gel was used by Jahan-Parwar *et al.* [31] in a canine model. One month following a unilateral full thickness scarring, the vocal fold was injected submucosally with Hylan B gel in four canines, and a control

animal received saline injection. Three months after injection, hyaluronic acid-treated folds had a return of mucosal waves and regeneration of the lamina propria layer with associated mild chronic inflammation, while the control animal continued to demonstrate dense submucosal scar and lacked mucosal waves. Some residual foci of Hylan B were observed even at the 3-month histologic evaluation, demonstrating the lasting nature of cross-linked hyaluronic acid. These results reaffirm the notion that hyaluronic acid preparations do result in improved lamina propria reconstitution after injury.

One human study reviewed their 2-year experience injecting auto-crosslinked hyaluronic acid (ACP-HA, Fidia Advanced Biopolymers, Abano Terme, Padova, Italy) into the submucosal lamina propria layer in patients with sulcus vocalis and scars [32•]. While patients with scarred folds comprised the majority of patients, a few with vocal fold atrophy were also included, where the injection was made deep to the cover layer for medialization. Preoperative and postoperative perceptual voice evaluations were performed, and multivariate analysis revealed that patients had significantly improved outcomes at up to 12 months follow-up compared with the preoperative measures. The results support the safety of the hyaluronic acid preparation in human studies, but without the use of control groups the efficacy of the product in vocal fold scarring remains to be systematically investigated.

Growth factor therapy has also been applied in established vocal fold scars. In a recent study by Kishimoto *et al.* [33•], HGF was embedded in a cross-linked gelatin hydrogel and injected into the lamina propria layer 6 months after scarring in a canine model. Treated vocal fold revealed improved pliability, thus demonstrating the potential for HGF therapy in chronic scars as well. Other studies have evaluated the role of basic fibroblast growth factor (bFGF). bFGF is thought to accelerate healing by increasing the number and expression of reactive fibroblasts. In another recent study, Suehiro *et al.* [34•] delivered two injections of bFGF 1 month following canine vocal fold stripping. bFGF-treated vocal folds demonstrated significant lamina propria regeneration and improved glottal gap, normalized mucosal wave appearance, and lower phonation threshold pressures. These preliminary results support further studies into both bFGF and HGF as a potential treatment modality in vocal fold scars.

Conclusion

Hyaluronic acid is a glycosaminoglycan polymer that has significant roles in the maturation, maintenance, and biomechanical properties of the lamina propria layer of the vocal fold. The heterogeneity of hyaluronic acid formulations has been investigated to prevent scars at the time of acute injury as well as to treat established scars. The clinician must pay attention to factors such as hyaluronic acid concentration and degree of cross-linking, which greatly affect the stiffness and physiologic behavior. Although many investigations to date are uncontrolled animal studies, nearly all have demonstrated the safety and some efficacy of hyaluronic acid in both acute and chronic scars. Growth factor therapy has shown promise as well. Future studies should focus on further refinement of hyaluronic acid formulations, randomized controlled trials, and comparison of treatment outcomes with normative data.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

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Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 578).

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