Treatment Outcomes of Injection Laryngoplasty Using Cross-Linked Porcine Collagen and Hyaluronic Acid

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Sponsorships or competing interests that may be relevant to content are disclosed at the end of this article.

Abstract

Objective. To investigate the treatment outcomes and prognostic factors of injection laryngoplasty (IL) using cross-linked porcine collagen (PC) and hyaluronic acid (HA) in unilateral vocal fold paralysis (UVFP).

Study Design. Case series with chart review.

Setting. A tertiary teaching hospital.

Subjects and Methods. This study reviewed 60 consecutive patients with UVFP who underwent IL with PC (n = 33) or HA (n = 27). Objective evaluations included maximal phonation time (MPT) and 10-item voice handicap index (VHI-10). Kaplan-Meier method was applied to evaluate the subjective treatment outcomes according to the patients’ self-assessment of symptom recurrence via chart review for the follow-up period of 15 months. Log-rank tests were applied to evaluate the association between clinical factors and subjective treatment outcomes.

Results. Objective outcome measurements revealed significantly improved MPT and VHI-10 at 1, 3, and 6 months posttreatment, with nonsignificant differences between the PC and HA groups. Subjective treatment outcomes also revealed a nonsignificant difference between the 2 groups. The median symptom-free durations were 10.9 and 14.4 months for the PC and HA groups, respectively. Subsequent analyses failed to identify prognostic significance of sex, time to treatment, etiology, side, injection approaches, and the presence of aspiration. No significant adverse effects occurred during the follow-up period.

Conclusion. This study demonstrated comparable subjective and objective improvements following IL using PC or HA in patients with UVFP. No significant prognostic factor of IL was discovered in the present research. Porcine collagen and HA as medium duration materials might play a role in the future of IL.

Keywords

vocal fold, augmentation, medialization, hyaluronan, larynx

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treatment outcomes among patients receiving IL. Therefore, the objectives of this study were to (1) compare the treatment outcomes of IL using PC and HA and (2) investigate the potential prognostic factors of IL in patients with UVFP.

Materials and Methods

Patients

This study reviewed 60 consecutive patients with UVFP treated at a tertiary teaching hospital between January 2009 and December 2011. Clinical diagnosis of UVFP was based on immobility of the vocal process with evidence of an atrophic, bowing unilateral vocal fold upon videolaryngostroboscopic evaluation. The inclusion criteria for IL were breathy voice and/or aspiration due to laryngeal incompetence, which interfered with the patient’s daily life. The exclusion criteria included a medical history of collagen vascular disease, previous allergic reactions to animal/collagen products, and previous surgical procedures for UVFP. For subjects without a clearly identifiable cause, we performed contrast-enhanced computed tomography (CT) scans from the skull base to the chest to rule out occult neoplasms along the route of the recurrent laryngeal nerve. All patients provided signed informed consent, and the Research Ethics Review Committee of Far Eastern Memorial Hospital approved this study.

Injection Materials

Purified telopeptide-free, glutaraldehyde cross-linked PC was derived from specific pathogen-free porcine dermal tissue (Summam Collagen Implant I-Plus, Taipei, Taiwan). Each syringe contained 35 mg/mL purified cross-linked collagen, primarily composed of type I atelocollagen. For the subjects receiving HA, we used Perlane (Restylane; Q-Med, Uppsala, Sweden), which consists of 20 mg/dL bacterial fermented hyaluronic acid (particle size: 400 μm).

Injection Laryngoplasty

The procedure was conducted under local anesthesia in an office setting. First, we sprayed 10% Xylocaine solution on the pharynx, tonsils, vallecula, and epiglottis, followed by laryngeal gargling of a 2% Xylocaine solution. We randomly injected HA or PC onto vocal folds unless the patient had a specific preference. The procedure was performed via a transcutaneous (from the thyrohyoid notch) or transoral approach, and the injection locations were (1) lateral to the vocal process and (2) mid-third of the vocal fold at the depth of vocalis muscle. The required amount of augmentation was based on individual need and usually ranged from 0.5 to 1.0 mL. The entire procedure was completed within 20 minutes in cooperative patients. All patients underwent voice conservation for 3 days following the procedure.

Outcome Evaluation

Objective treatment outcomes, including maximal phonation time (MPT) and 10-item voice handicap index (VHI-10), were recorded before and 1, 3, and 6 months after the treatment. Subjective treatment outcomes were evaluated according to the patients’ self-assessment of the return of presenting symptoms via chart review for the follow-up period of 15 months.

Statistical Analysis

The χ² test and Fisher’s exact test were used to compare the categorical data. The MPT and VHI-10 were compared using Student and paired t tests. Subjective treatment outcomes were evaluated by time-to-event analysis, which is frequently applied in survival analysis. Pragmatically, “event” denotes treatment failure (ie, recurrence of initial symptoms). For patients lost or dead, we recorded whether the symptoms had recurred at the last clinical visit and censored the case from subsequent analyses. “Time” represents the duration of clinical follow-up until an event has occurred or until the case is censored. The Kaplan-Meier method was applied to evaluate the symptom-free rates after IL with PC or HA. Log-rank tests were performed to examine the association between clinical factors and subjective treatment outcomes. All statistical analyses were conducted using Stata software, version 10.0 (Stata Corporation, College Station, Texas) and SAS software, version 9.1 (SAS Institute, Cary, North Carolina). Statistical significance was defined as P < .05.

Results

Among the recruited patients with UVFP, left vocal fold was involved in 41 cases, while right vocal fold was involved in the other 19 cases. The age distribution was 27 to 90 years (median, 58 years). In the 16 patients with recent-onset UVFP without an obvious cause at presentation, CT scans from the skull base to the chest were performed, revealing that 7 patients (44%) had an undiagnosed neoplasm, including 5 lung cancers, 1 esophageal cancer, and 1 jugular foramen neurilemmoma. Overall, the etiologies of UVFP included idiopathic (n = 17), iatrogenic (n = 28), neoplastic (n = 11), and miscellaneous causes (n = 4). The distribution of characteristics between the PC and HA groups is illustrated in Table 1, which shows nonsignificant differences in age, sex, etiology, time to treatment, site, injection techniques, and the presence of aspiration.

Thirty-three patients received PC and 27 patients received HA for IL (Figures 1 and 2). Objective treatment outcomes showed that MPT improved significantly at 1, 3, and 6 months posttreatment in both PC and HA groups (Table 2, P < .01, compared with baseline, paired t test). Similarly, VHI-10 also improved significantly at 1, 3, and 6 months after the IL using HA and PC (Table 3, P < .01, paired t test). Further comparisons of MPT and VHI-10 measured before treatment and at 1, 3, or 6 months after IL using PC or HA all revealed nonsignificant differences (Tables 2 and 3, P > .05, Student t test). No significant adverse effects developed during the follow-up period. Among the 28 patients who suffered from aspiration due to laryngeal incompetence, 16 (57%) and 11 (39%) patients...
reported significant or partial improvement after IL, respectively, regardless of using PC or HA ($P = .87$, Fisher’s exact test).

The mean (standard deviation) follow-up periods for patients receiving HA and PC were 9.1 (5.4) and 9.0 (4.8) months, respectively, and the difference was nonsignificant ($P = .94$, Student $t$ test). During the study period, 8 patients died while another 6 patients were lost to follow-up. Ancillary analysis showed similar dropout rates between the PC and HA groups ($P = .45$, Fisher’s exact test). Subjective treatment outcome demonstrated a nonsignificant difference of the symptom-free rates between PC and HA ($P = .22$, log-rank test). The median symptom-free durations after IL were 10.9 and 14.4 months for the PC and HA groups, respectively ($P = .22$, log-rank test). Subsequent evaluation of the prognostic factors, including sex, etiology, time to treatment, side, injection techniques, and the presence of aspiration, revealed nonsignificant correlations with the subjective treatment outcomes ($P > .05$, log-rank tests).

**Discussion**

Injection laryngoplasty is a common treatment modality for glottic gap restoration among patients with UVFP. The key to success lies in choosing materials that are easy to inject, minimize the local inflammatory response, maintain within the injected sites, and are commercially available at an acceptable price. Autologous fat represents the best choice for biocompatibility and unlimited resources; however, precise injection of fat globules usually requires a large-bore needle under general anesthesia. Furthermore, the survival rates of autologous fat can be quite unpredictable, leading to its reduced use by laryngologists. Autologous collagen is another ideal injectable for vocal fold insufficiency because of its biocompatibility and natural occurrence within the lamina propria of the vocal folds; however, time-consuming preparation and increased donor site morbidity have limited its clinical popularity.

Currently, various artificial materials have been effectively applied to augment atrophic vocal folds. The most widely reported materials for IL are likely Radiesse Voice and Radiesse Voice Gel (Merz Aesthetics, San Mateo, California). Radiesse Voice Gel primarily consists of carboxymethylcellulose, which lasts for 2 to 3 months, and it is

**Table 1.** Characteristics of the 60 patients with unilateral vocal fold paralysis receiving injection laryngoplasty using porcine collagen and hyaluronic acid.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Porcine Collagen</th>
<th>Hyaluronic Acid</th>
<th>$P$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean ± SD, y</td>
<td>58 ± 16</td>
<td>60 ± 17</td>
<td>.55</td>
</tr>
<tr>
<td>Sex, male/female, No.</td>
<td>20/13</td>
<td>14/13</td>
<td>.50</td>
</tr>
<tr>
<td>Etiology, No.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Idiopathic</td>
<td>8</td>
<td>9</td>
<td>.44</td>
</tr>
<tr>
<td>Iatrogenic</td>
<td>14</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Neoplastic</td>
<td>8</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>3</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Time to treatment, mean ± SD, mo</td>
<td>17 ± 23</td>
<td>18 ± 30</td>
<td>.83</td>
</tr>
<tr>
<td>Side, right/left, No.</td>
<td>10/23</td>
<td>9/18</td>
<td>.80</td>
</tr>
<tr>
<td>Injection technique, transoral/transcutaneous, No.</td>
<td>11/22</td>
<td>10/17</td>
<td>.76</td>
</tr>
<tr>
<td>Aspiration, presence/absence, No.</td>
<td>14/19</td>
<td>14/13</td>
<td>.64</td>
</tr>
</tbody>
</table>

*Figure 1. A 45-year-old man with left unilateral vocal fold paralysis (A). Follow-up videolaryngostroboscopic examination revealing an augmented vocal fold after injection laryngoplasty using porcine collagen (B).*

*Figure 2. A 42-year-old woman with left unilateral vocal fold paralysis. Videolaryngostroboscopic examination revealing a prominent glottic gap (A). After injection laryngoplasty using hyaluronic acid under local anesthesia, the glottic gap was completely corrected (B).*
usually applied as a trial material for short-term applications. Radiesse Voice contains calcium hydroxylapatite and has been reported to be effective for as long as 18 months, which is suitable to restore glottic insufficiency for a longer duration. However, the lower viscosity of Radiesse makes it prone to flow through tissue planes, and once it reaches the Reinke’s space, surgical removal may be required to restore the normal pliability of the lamina propria.

Based on the process of neuronal regeneration, a waiting period of 6 to 12 months is generally suggested before permanent vocal fold medialization. According to published literature, collagen-based products are suitable to fit this “window” between the onset of clinical symptoms and spontaneous recovery, adequate compensation, or the need for definite procedures. Bovine collagen has been clinically used to treat vocal disorders for decades. However, the hypersensitivity and potential transmission of bovine spongiform encephalopathy have lowered its clinical preference as an injectable material. Micronized homologous dermis has been proposed as a safe and efficacious alternative in IL, but this dry powder material requires additional rehydration time before usage. In addition, PC may be another suitable material for tissue augmentation because it is genetically more similar to humans and better tolerated by the host, with lower rates of allergic reactions. Despite the frequent usage of PC in aesthetic procedures, the first clinical series of IL using PC was recently published, demonstrating subjective and objective improvements.

Hyaluronic acid, which is a nonsulfated glycosaminoglycan that is naturally present in the lamina propria of vocal folds as a principal component of the extracellular matrix, represents another suitable material for restoring glottic insufficiency. The excellent biocompatibility of HA eliminates the need for routine preinjection skin tests for allergic reaction. Previous reports have also demonstrated the favorable rheologic features of HA, which has similar viscoelastic properties as cadaveric vocal folds. Histological studies have demonstrated that HA is durable for 9 months, and both animal and nonanimal derivatives of HA have been applied successfully in IL. Although augmenting atrophic vocal folds can correct glottic gaps and, therefore, reduce phonation thresholds and air leakage postoperatively, treatment effectiveness cannot simply be determined by the sustainability of the injected materials. Other factors, including neuronal regeneration, compensation from the healthy side of the laryngeal musculature, and local inflammation with the recruitment of fibroblasts and deposition of connective tissue over the injection sites, all contribute to the overall functional outcome.

Although augmenting atrophic vocal folds can correct glottic gaps and, therefore, reduce phonation thresholds and air leakage postoperatively, treatment effectiveness cannot simply be determined by the sustainability of the injected materials. Other factors, including neuronal regeneration, compensation from the healthy side of the laryngeal musculature, and local inflammation with the recruitment of fibroblasts and deposition of connective tissue over the injection sites, all contribute to the overall functional outcome. For example, although HA remains stable for up to 6 months, clinical improvement can be observed for as long as 24 months. Therefore, except for objective measurements (Table 2 and 3), we investigated the subjective outcomes based on the patients’ perspectives of symptom recurrence (Figure 3). Compared with a recent report of IL using another HA product, this study revealed less durable treatment.

### Table 2. Measurements of maximal phonation time (MPT) before and after injection laryngoplasty using porcine collagen and hyaluronic acid.

<table>
<thead>
<tr>
<th></th>
<th>Before</th>
<th>1 Month</th>
<th>3 Months</th>
<th>6 Months</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPT, s</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Porcine collagen</td>
<td>33 5.2 (4.4-5.9)</td>
<td>32 10.4 (9.0-11.8)</td>
<td>29 10.4 (8.9-11.9)</td>
<td>27 9.7 (8.3-11.2)</td>
</tr>
<tr>
<td>Hyaluronic acid</td>
<td>27 5.0 (4.0-6.0)</td>
<td>27 9.1 (8.1-10.1)</td>
<td>23 10.4 (8.7-12.0)</td>
<td>20 10.9 (9.2-12.5)</td>
</tr>
<tr>
<td>P value b</td>
<td>.79</td>
<td>.16</td>
<td>.99</td>
<td>.40</td>
</tr>
</tbody>
</table>

Abbreviation: CI, confidence interval.

a P < .01, paired t test, compared with measurement before injection laryngoplasty.
b Student t test, comparison between porcine collagen and hyaluronic acid.

### Table 3. Measurements of 10-item voice handicap index (VHI-10) before and after injection laryngoplasty using porcine collagen and hyaluronic acid.

<table>
<thead>
<tr>
<th></th>
<th>Before</th>
<th>1 Month</th>
<th>3 Months</th>
<th>6 Months</th>
</tr>
</thead>
<tbody>
<tr>
<td>VHI-10</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Porcine collagen</td>
<td>33 23.9 (20.8-27.0)</td>
<td>32 17.6 (13.9-21.3)</td>
<td>29 16.0 (12.7-19.3)</td>
<td>27 16.6 (14.1-19.0)</td>
</tr>
<tr>
<td>Hyaluronic acid</td>
<td>27 27.0 (23.6-30.3)</td>
<td>27 17.2 (14.3-20.0)</td>
<td>23 15.4 (13.9-16.9)</td>
<td>20 12.5 (9.9-15.0)</td>
</tr>
<tr>
<td>P value b</td>
<td>.19</td>
<td>.85</td>
<td>.77</td>
<td>.06</td>
</tr>
</tbody>
</table>

Abbreviation: CI, confidence interval.
a P < .01, paired t test, compared with measurement before injection laryngoplasty.
b Student t test, comparison between porcine collagen and hyaluronic acid.
outcomes. However, the particle sizes, hydration status, material used, and extent of cross-linking were all different between these 2 products (Perlane vs Hylaform [Allergan, Irvine, California]). Furthermore, in our study, we usually injected materials deep into the vocalis muscles to avoid accidently misplacing HA into the Reinke’s space, while other surgeons may prefer to inject HA into a more superficial level between the vocalis muscle and the vocal ligament. This process may cause varying degradations of HA, which would be similar to the reported differential degradation of bovine collagen injected at various depths. In addition, a longer duration of clinical effects may have resulted from more precise placement of the injectable and less material leakage in procedures under general anesthesia, whereas in our study, all subjects received office-based IL under local anesthesia.

In this study, we noticed a longer symptom-free duration for HA than PC, which was similar to a previous study that showed the reabsorption rate of HA was lower than cross-linked bovine collagen. However, a randomized dermatological study had demonstrated similar effectiveness for PC and HA. Since no direct comparison of viscoelasticity, types and strengths of cross-linking materials, and clinical treatment outcomes between bovine and porcine collagen has been conducted previously, it may be hard to interpret such discrepancy and conclude whether hyaluronan- or collagen-based products are superior to the other.

Figure 3. Subjective treatment outcomes and the details of follow-up status among the 60 patients receiving injection laryngoplasty, which demonstrated a nonsignificant difference of the symptom-free rates between porcine collagen (PC) and hyaluronic acid (HA) \( (P = .22, \) log-rank test).
Previous studies have demonstrated a trend toward less surgical medialization laryngoplasty when patients with UVFP received IL within 6 months.\textsuperscript{15,45} Similarly, compared with conservative treatment strategies, patients receiving temporary vocal fold injections for UVFP are less likely to receive thyroplasty.\textsuperscript{16} However, our analytical results had failed to reveal similar benefits of early injection (\textbf{Table 4}). Such nonsignificant results of timing and other potential prognostic factors may be limited by the small case numbers and biased by subjective outcome measurements as in this and previous studies.\textsuperscript{15,16,45} Therefore, further studies incorporating more objective parameters over a longer follow-up period may help to delineate the prognostic factors in the patients with UVFP who receive IL.

To the best of our knowledge, this is the first study to compare the treatment outcomes of IL using PC or HA. However, this study has several limitations. Because of the lack of complete randomization in allocating injection materials, we cannot exclude subtle heterogeneity between the HA and PC groups, despite the nonsignificant difference among baseline characteristics (\textbf{Table 1}). Another concern is that if the patients were lost to follow-up, then subjective outcome measurements via chart review may imply that they are still having effective control of their presenting symptoms. Since we have censored the lost subjects at the date of last clinical visit (ie, simultaneously removing the case from the numerator [event or treatment failure] and denominator [number of patients who did not yet experience an event and hence remained at risk for developing events in the future], \textbf{Figure 3}), the potential influence of lost subjects on the estimation of symptom-free rates following IL should be minimal. However, it has been mentioned that for cohort studies with dropout rates higher than 20% (23% in the present study), the interpretation of treatment outcome may be compromised.\textsuperscript{46} Further prospective randomized studies with comprehensive and adherent data collection may help to clarify the effectiveness of IL using PC or HA. Last, although the safety and efficacy of applying dermal filler in IL are well documented in the literature, clinicians should consult specific legal regulations regarding the off-label use of these artificial materials.

\textbf{Conclusion}

This study demonstrated comparable subjective and objective improvements following IL using PC or HA in patients with UVFP. No significant prognostic factor of IL was discovered in the present research. Porcine collagen and HA as medium duration materials might play a role in the future of IL.

\textbf{Author Contributions}

Ming-Hsun Wen, acquisition and analysis of data, drafting and revising the article, final approval; Po-Wen Cheng, conception and design, revising the article, final approval; Li-Jen Liao, analysis and interpretation of data, revising the article, final approval; Hsu-Wen Chou, analysis and interpretation of data, revising the article, final approval; Chi-Te Wang, conception and design, acquisition and analysis of data, drafting and revising the article, final approval.

\textbf{Disclosures}

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\textbf{References}