The Laryngoscope

TRIOLOGICAL SOCIETY CANDIDATE THESIS

Injection of Cultured Autologous Fibroblasts for Human Vocal Fold Scars

Dinesh K. Chhetri, MD; Gerald S. Berke, MD

Objectives: This study evaluated the safety and effectiveness of injection of autologous fibroblasts into the lamina propria layer to treat vocal fold scarring.

Study Design: A prospective, open-label, single arm, pilot study at a single tertiary care center.

Methods: Autologous fibroblasts were expanded in cell culture from punch biopsies of the buccal mucosa of five human subjects with vocal fold scarring. Three doses of $1–2 \times 10^7$ cells/mL were injected into the superficial lamina propria layer of each scarred vocal fold at four-week intervals. The primary efficacy measure was an objective evaluation of the mucosal wave grade; secondary measures included acoustic analyses, a patient-completed voice handicap index (VHI) survey, and voice quality questionnaire. Safety assessments included clinical laboratory blood tests, vital signs, and monitoring for adverse events. Patients were followed for 12 months following the first treatment.

Results: Mucosal wave grade improved and the improvement was sustained through month 12. Sustained improvements through month 12 were also noted for the VHI and voice quality questionnaire. Multiple injections of autologous fibroblasts into the lamina propria were well tolerated. Temporary otalgia was noted following treatment in two subjects.

Conclusions: This study demonstrates that injection of autologous fibroblasts into the scarred vocal fold lamina propria layer is safe. Sustained trends for improved outcome were supported by 12-month data for mucosal wave grade, VHI, and voice quality questionnaire.

Key Words: Larynx, vocal scar, lamina propria, fibroblasts, clinical trial.

Level of Evidence: 2c.

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INTRODUCTION

The vocal folds of the human larynx are highly specialized structures that are capable of self-sustained oscillation for production of sound for speech, communication, and singing. The vocal folds are divided anatomically into three tissue layers.[1] The superficial layer is the vocal fold epithelium, followed by the middle lamina propria layer, and the deep muscular layer. The epithelial layer is very thin compared with the other layers and acts biomechanically as a functional unit with the lamina propria layer and thus these two layers are combined and called the "cover" layer in biomechanical studies of the vocal fold.[1] The lamina propria (LP) layer is an amorphous, paucicellular layer composed mostly of fibroblasts, macrophages, and extracellular matrix (ECM) molecules.[2] This layer provides the appropriate viscoelasticity (mucosal pliability) for normal oscillation of the vocal folds during phonation, which can be appreciated clinically as mucosal waves on the vocal fold surface upon videostroboscopic examination of the larynx. Vocal fold scarring is most often seen after surgery on the vocal folds but can also result from radiation therapy, vocal fold trauma, and idiopathic causes. In vocal fold scars, the lamina propria layer is lost or disorganized, which leads to glottic insufficiency and diminished or absent mucosal waves.[3,4] It is the most commonly encountered clinical finding causing dysphonia that currently has no effective therapy. Therefore, treatment of vocal fold scarring is of special interest in voice research.

Two treatment approaches have been pursued in treatment of vocal fold scars: 1) prevention or treatment of scars with biocompatible materials placed into the LP layer; and (2) cellular and growth factor-based lamina propria replacement therapy.[3–6] To date no biocompatible material exists that is also durable and matches the native viscoelastic properties of the LP layer. Treatment with autologous fat implantation and autologous fascia augmentation has been reported, but treatment results have been less than satisfactory.[7–9] Cellular-based approaches using tissue engineering and regenerative medicine techniques may be more promising in the treatment of vocal scars because patients' own cells are made to work to re-create the ECM of the LP layer. All the ECM components such as collagen, elastin, proteoglycans, and other interstitial molecules in the LP layer are produced by the fibroblast cell.[2] Thus, injection of

From the Division of Head and Neck Surgery, David Geffen School of Medicine at UCLA, Los Angeles, California, U.S.A.

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Send correspondence to Dr. Dinesh K. Chhetri, MD, 62-132 CHS, Division of Head and Neck Surgery, Los Angeles, CA 90095.
E-mail: dchhetri@mednet.ucla.edu

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autologous fibroblasts into the lamina propria layer could lead to reconstitution of normal lamina propria components and improve voice by reestablishing normal mucosal pliability of the vocal folds.

A canine study has previously shown the potential for autologous fibroblast injection therapy for treatment of vocal fold scars.[10] Canine lamina propria replacement therapy was performed where autologous fibroblasts were harvested from buccal mucosal biopsies and expanded in the laboratory. Fourth or fifth passage fibroblasts were injected into previously scarified vocal folds. The scarred vocal folds had absent or severely limited mucosal waves and poor acoustic parameters. Significant improvements in mucosal waves and acoustic parameters were obtained following autologous fibroblast injection therapy. The purpose of this study was to conduct a human clinical trial to assess the safety and efficacy of autologous fibroblast injection therapy for vocal fold scars. The hypothesis was that autologous fibroblasts injected into scarred vocal folds is safe and will lead to improved mucosal waves and voice outcomes. This pilot study on the treatment of vocal fold scarring with autologous fibroblast injection demonstrates that the treatment was well tolerated and was associated with both objective measures of vocal fold functional improvement and subjective benefits reported by the patients.

MATERIALS AND METHODS

Subject Eligibility

Five adult subjects who were being evaluated in a voice clinic for dysphonia with unilateral or bilateral vocal fold scarring were enrolled in this study. They were required to be nonsmokers with grade 1 (absent) or grade 2 (limited to the most medial edge of the vocal folds) mucosal waves as determined by videostroboscopy, to have failed prior therapy for vocal fold scarring, and to be dissatisfied with their voice (see below for mucosal wave grade assessment). Prior to enrollment, the protocol was approved by the Institutional Review Board and subjects provided informed consent.

Autologous Fibroblasts (Azficel-T)

Punch biopsies of buccal mucosa were obtained from each subject and sent to Isolagen Technologies Inc. (current name Fibrocell Science Inc., Exton, PA) for expansion of the fibroblast cell population. Buccal mucosa rather than skin biopsy was chosen as the source for autologous fibroblasts because the collagen production profile of buccal mucosa parallels that of the vocal fold fibroblasts compared with dermal fibroblasts.[11,12] Following enzymatic dissociation, the fibroblasts were cultured for two to five passages in Isocove's Modified Dulbecco's Medium without phenol red (IMDM), supplemented with antibiotics and fetal bovine serum and cryopreserved in Profreeze™ (Lonza, Walkersville, MD) supplemented with dimethylsulfoxide (DMSO). Prior to use, the cells were thawed, washed free of DMSO and serum, resuspended in 1.2 mL Dulbecco's Modified Eagle's Medium at a concentration of 1.0–2.0 $\times$ 10^7 cells/mL, shipped in an insulated container at 2° to 8°C to the treatment center, and administered within 24 hours. The final cell product was demonstrated to be sterile, free of Mycoplasma and endotoxin contamination, and cell viability was assessed to be $\geq$85%. In addition, a gram stain was performed prior to release. Prior to use, the cell suspension was stored at 2° to 8°C. The vial was allowed to warm to room temperature for 15 to 30 minutes immediately before use. All manufacturing and release steps were conducted under an Investigational New Drug exemption and cGMP conditions.

Treatment

All five subjects received 1–2 $\times$ 10^7 cells/mL. All injections were performed in an office setting by an otolaryngologist (D.K.C.) skilled in injection laryngoplasty. The subject was seated comfortably on an examination chair with the neck slightly extended to expose laryngeal landmarks. The nasal cavity was decongested with neosynephrine, and the nasal cavity and larynx was anesthetized with topical 4% lidocaine spray. A distal chip flexible fiberoptic laryngoscope (VNL 1170K, KayPentax, Lincoln Park, NJ) connected to a video monitor was passed via the nostril into the nasal cavity and advanced to the hypopharynx until the vocal folds were visualized. While visualizing on the video monitor, autologous cells were layered in the vocal fold subepithelial layer (in the lamina propria compartment). The injection needle was advanced into the larynx using the trans-cricothyroid membrane technique. Appropriate placement of the autologous cells in the subepithelial compartment was confirmed visually in all cases as "ballooning" of the epithelial layer as the injection expanded this compartment. Subjects received treatment to one or both vocal folds, depending upon whether unilateral or bilateral scarring was present. Only one vocal fold was treated at each treatment session, alternating to the opposite vocal fold (if being treated bilaterally) at the next treatment session. Each scarred vocal fold was treated a total of three times (total of three treatment sessions for unilateral vocal fold scarring and six treatment sessions for bilateral vocal fold scarring). Subjects were observed in the clinic for one hour prior to discharge. Prophylactic antibiotics were not prescribed.

Mucosal Wave Grade Assessment

The primary efficacy assessment was the change from baseline in mucosal wave grade assessed four months after the first treatment. Mucosal wave grade was assessed through 12 months following the first injection treatment using videostroboscopy on each treated vocal fold. The same investigator performed all videostroboscopic procedures. Both a 70° rigid endoscope and a distal chip flexible fiberoptic endoscope were used initially for videostroboscopy. It was determined that the mucosal wave grade in each patient was the same for both endoscopic assessment techniques (rigid versus flexible) and therefore the rigid endoscope was used for most of the study period due to its superior optical quality. For videostroboscopy, the endoscope was attached to a miniature video camera and the larynx visualized under a stroboscopic light source (Model 9100, KayPentax). The subject was asked to phonate a sustained vowel *i* and the vocal fold vibration was recorded digitally. The recordings were reviewed later by a laryngologist and an experienced speech pathologist and a consensus was generated on the mucosal wave grade. Mucosal wave was graded as follows: 1 = absent; 2 = limited to the most medial edge of the vocal folds; 3 = present laterally up to 1/4 of the width of the vocal folds; 4 = present up to but less than ½ the width of the vocal folds; 5 = present at more than ½ the width of the vocal folds (normal). Statistically significant change from baseline was assessed with Wilcoxon signed rank test.

Vocal Handicap Index Assessment

Subjects completed a voice handicap index (VHI) survey, which is a 30-item subject-completed questionnaire developed

TABLE I.
Subjects' Demographic, Past Vocal History, and Videostroboscopic Findings.

Subject No./Age/Sex	Etiology or Associated Disorders	Scar Duration	Failed Prior Rx	MW Grade Right	MW Grade Left	Status of Epithelium	Glottic Gap
1/35/M	Tonsillectomy, GERD	5 years	PPI, voice Rx	2	2	Intact	Yes
2/47/M	Bowing, Atrophy	6 years	PPI, voice Rx, IL	2	1	Intact	Yes
3/66/F	SCCA/XRT Right VFP	33 years 13 years	PPI, voice Rx, IL, fat injection	1	1	Scarred	Yes
4/62/M	Cold MDL for nodule	30 years	PPI, voice Rx, IL, type 1 thyroplasty	1	4	Sulcus	Yes
5/68/F	Laser MDL for edema	4 years	PPI, voice Rx, IL, fat injection	1	1	Scarred	Yes

MW = mucosal wave; GERD = gastroesophageal reflux disease; PPI = proton pump inhibitor; voice Rx = voice therapy; IL = injection laryngoplasty; SCCA = squamous cell carcinoma; XRT = radiation therapy; VFP = vocal fold paralysis; MDL = microsuspension direct laryngoscopy with excision of lesion.

by Jacobson et al. with 10 items in each of the three subscales: emotional, physical, and functional.[13] Subjects answered each item by rating from "0", indicating the subject never felt this about a voice problem, to "4", where the subject always felt this to be the case. Results were reported individually for each subscale and for the entire survey. Each subscale has been found to be significantly different if it changes by eight points, whereas the total VHI score was found to be significantly different if it changes by 18 points.

Voice Quality Assessment

The voice quality assessment questionnaire was a subject-reported outcome measure consisting of two questions where the subject was to select the best response. Subjects were to select "improved," "no change," or "worsened" in response to the question: "How has your voice quality changed since baseline?" Subjects were to respond "yes" or "no" in response to the question: "Do you consider the treatment a success?"

Voice Acoustic Assessments

At each visit, a sample of the subject's voice (a continuous vowel sound *a*) was recorded and digitized to measure acoustic parameters of voice using custom-designed computer software for recording and acoustic analysis. Acoustic parameters evaluated included noise-to-harmonic ratio and maximum phonation time (length of time in seconds a subject was able to phonate a sustained vowel *a* in one single deep breath) as a measurement of respiratory and sound control. In each case, the results from three utterances were averaged to obtain these acoustic parameters.

Safety Assessments

Assessments of subject safety included the incidence of adverse events (AEs), and analysis of changes from baseline in laboratory values (hematology, blood chemistry, liver function tests, urinalysis, etc.) and vital signs. Subjects were observed for one hour after each treatment injection. Repeat endoscopy was performed the day after the first treatment. Subjects were contacted by telephone on days 2 through 4 after the first treatment to check for any adverse events.

Statistical Considerations

Version 8.0 of the SAS statistical software package (SAS Institute Inc., Cary, NC) was used to perform all statistical analyses. There was insufficient clinical experience on how this new procedure would affect vocal fold scarring to estimate sample size. The decision to limit sample size to five was selected after discussion with the Food and Drug Administration. The sample size is, however, typical of an exploratory, early phase study in which the objective is to investigate the feasibility of treatment administration.

RESULTS

Subjects and Treatment Injections

Five subjects who were evaluated in a voice clinic for dysphonia and met all eligibility criteria were enrolled and completed the study (Table I). There were three males and two females. The mean age was 56 years. By design, the subjects represented a spectrum of vocal fold scars from isolated lamina propria loss to full thickness cover defect (scarred lamina propria *and* epithelium). Prior failed treatments included anti-reflux medication (5 of 5, 100%), voice therapy (5 of 5, 100%), injection augmentation (nonfat) laryngoplasty (4 of 5, 80%), fat injection laryngoplasty (2 of 5, 40%), and Type 1 thyroplasty (1 of 5, 20%). All five subjects underwent buccal mucosal biopsy without complications, and all biopsy sites rapidly healed by secondary intention. An average of 122 days (range, 63–196 days) was required from biopsy to treatment. One patient required a second biopsy due to bacterial contamination detected late in the fibroblast expansion process. Cell viability at injection was higher than specification (>85%) in all subjects (actual range, 86%–96%).

One subject (subject 4) had unilateral vocal fold scarring and received a total of three injections. The other four subjects had bilateral vocal fold scarring and received a total of six injections each. Only one vocal fold was injected at each visit, thus patients with bilateral vocal fold scarring were seen at two-week intervals, with the injections alternating from one side to the other. Of the total 27 treatment injections performed, the full treatment dose of 1.0 mL was injected in 24 instances (89%). In the other three instances, 0.85 mL (subject 1, treatment 1), 0.5 mL (subject 5, treatment 6), and 0.3 mL (subject 3, treatment 6) were injected. In the first instance, less than 1.0 mL was injected because a small amount was lost during aspiration of cells from the treatment vial, whereas in the latter two instances, the treatment injections were terminated prematurely

TABLE II.
Mucosal Wave Grade at Various Intervals.

Subject	Scar Type	Vocal Fold	Day 0	Week 4	Week 8	Month 3	Month 4	Month 8	Month 12
1	Lamina propria only	Left	2	–	2	–	4	–	4
		Right	2	–	3	–	4	–	4
2	Lamina propria only	Left	1	1	3	5	4	–	5
		Right	2	2	4	5	5	–	5
3	Lamina propria and epithelium	Left	1	1	1	1	1	1	1
		Right	1	1	1	1	1	1	1
4	Sulcus	Right	1	2	4	4	4	4	4
5	Lamina propria and epithelium	Left	1	1	1	2	2	2	3
		Right	1	1	1	2	2	2	2
	Mean mucosal wave grade		1.3	1.3	2.2	2.9	3	2	3.22
	Wilcoxon signed rank *P* value			.32	.07	.04	.02	.10	.02

Mucosal wave grades prior to and following treatment with autologous fibroblasts. Subjects 1, 2, 3, and 5 received six treatments (three to each vocal fold) starting at Day 0 and completing at Week 10 as described in Materials and Methods. Subject 4 received three treatments starting at Day 0 and completing at Week 8. Mucosal wave grades were assigned by consensus between a laryngologist and an experienced speech pathologist. Missing grades (–) indicate that the test was not available for that visit date. Statistical significance of the change from baseline was assessed with Wilcoxon signed rank test. Scar type indicates the baseline assessment of scar (involvement of lamina propria only or lamina propria and epithelium).

due to an adverse event of severe otalgia reported during the injection.

Mucosal Wave Grade

The mucosal wave grade improved after autologous fibroblast injection treatment (Table II). Improvement was noted following the second injection (8 weeks after the first treatment) and the change from baseline grade reached statistical significance by month 3 ($P = .04$) that was maintained through month 12 ($P = .02$). The primary efficacy analysis (change from baseline in mucosal wave grade for all treated vocal folds) assessed at four months after the first treatment was statistically significant ($P = .02$). Subjects with scarring limited to the lamina propria (subjects 1 and 2; Fig. 1) or a sulcus (linear defect on the vocal fold surface; subject 4; Fig. 2) had the greatest improvement following treatment. Although the mucosal wave grade in subject 4 improved from a grade 1 (absent waves) at baseline to a grade 4 (waves present up to but less than half the width of the vocal folds), the physical appearance of the sulcus remained unchanged throughout the study (Fig. 2). The two subjects with full thickness cover scarring (subjects 3 and 5) had minimal to no improvement based on the mucosal wave grade; however, one subject (subject 5) noted improvement in voice quality (see voice quality assessment results; Fig. 3).

Acoustic Analysis

The change from baseline in harmonic-to-noise ratio was examined for all subjects at each assessment. Most subjects experienced little change from baseline in the voice recording for acoustic analysis of the harmonic-to-noise ratio after treatment (data not shown). A closer examination of the measurements suggests a random distribution of this measure, which likely means that this measure is not useful in this group of subjects.

Maximum Phonation Time

The change from baseline in maximum phonation time was examined for all subjects at each assessment. There was a trend for an increased duration in maximum phonation time at months 3–4 that was not sustained through month 12 (data not shown).

VHI

The mean subject VHI scores were calculated for each of the three subscales and the overall total (Table III). Higher VHI scores indicate a more severe perception of the impact of the voice problem. The overall VHI score and functional, physical, and emotional VHI subscale scores improved from baseline through month 3. Note that 4 of the 5 subjects were receiving treatment through month 2, suggesting that the initial treatment resulted in improved VHI scores, which improved with additional treatments. The improvement in VHI scores was sustained through month 12.

Subject's Impression of Voice Quality Questionnaire

Beginning at week 8, four of five (80%) subjects indicated their voice quality had "improved" since baseline. The improvement was sustained through month 12. One subject (subject 3) reported no change since baseline. No subjects felt their voice had become worse since baseline. Twelve months after treatment, four of five (80%) subjects indicated that they considered the treatment a success. Subject 3, who presented with both lamina propria and epithelial scarring after external beam radiation therapy for squamous cell carcinoma, as well as unilateral vocal fold paralysis from a carotid endarterectomy (medialized), neither noted improvement in voice quality nor felt the treatment was a success. This was corroborated by the lack of improvement in objective mucosal wave grade following treatment (Table II).

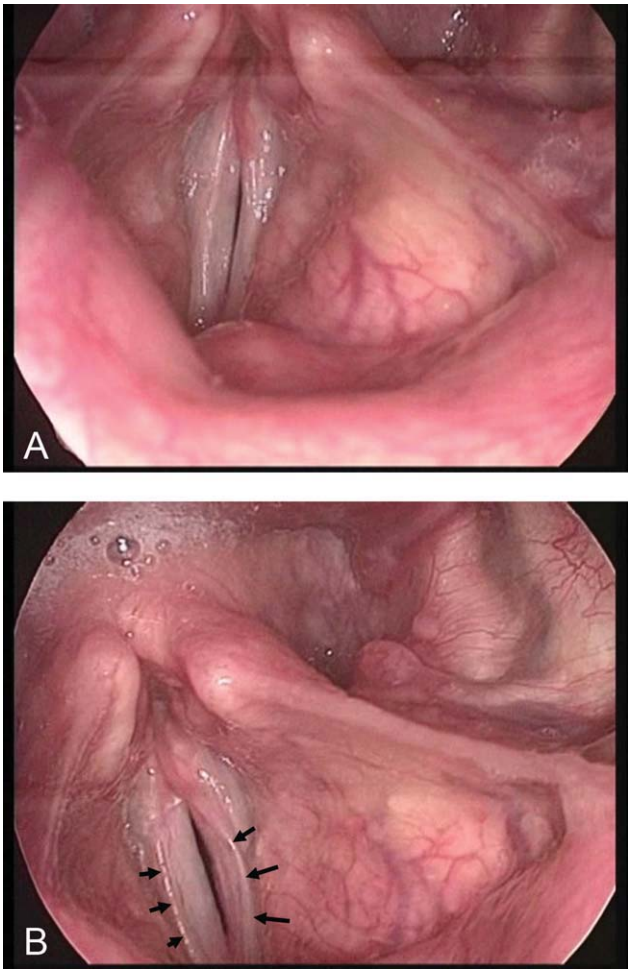

Fig. 1. Vocal fold mucosal waves in subject 2 (A) at baseline and (B) at 4 months after injection therapy. Although mucosal waves are absent or limited to the most medial edges prior to therapy, robust mucosal waves are seen after injection therapy bilaterally (arrows).

Safety

Three (60%) of the five subjects treated reported at least one adverse event (AE) during the study period. These three subjects reported a total of 16 AEs. The adverse events were neck pain, vaginal moniliasis, skin abrasion, ear pain (12 Occurrences), and voice alteration. No serious adverse events were reported, and no subjects withdrew from the study due to an adverse event. Two subjects experienced a total of 12 adverse events considered related to treatment. These were mild to severe otalgia suffered during treatment in two subjects. Both subjects (nos. 3 and 5) were categorized as having extensive full thickness vocal fold scars of the cover layer, involving scarring of the epithelial layer as well as lamina propria loss. The otalgia was considered definitely related to study treatment as it not only occurred during the injection, but also the pain was experienced at the moment the cells being injected in the lamina propria compartment started dissecting the epithelial layer off of the muscle layer. The otalgia was considered of mild to moderate severity in 10 instances and severe in two instances. In the latter two instances, the treatment was terminated prematurely prior to injection of the full 1.0 mL dose. The otalgia resolved rapidly after the injection was terminated and was absent by the time of discharge from the clinic.

There were no clinically significant changes in laboratory parameters. All the laboratory parameters were within normal limits in all subjects at all assessment time periods. There were no clinically significant changes in vital signs (respiration rate, heart rate, temperature, and blood pressure) from baseline during the course of the study (data not shown).

DISCUSSION

This report describes the first human clinical trial for treatment of a voice disorder using a cell-based approach. The results show that treatment with autologous fibroblasts significantly improves both objective

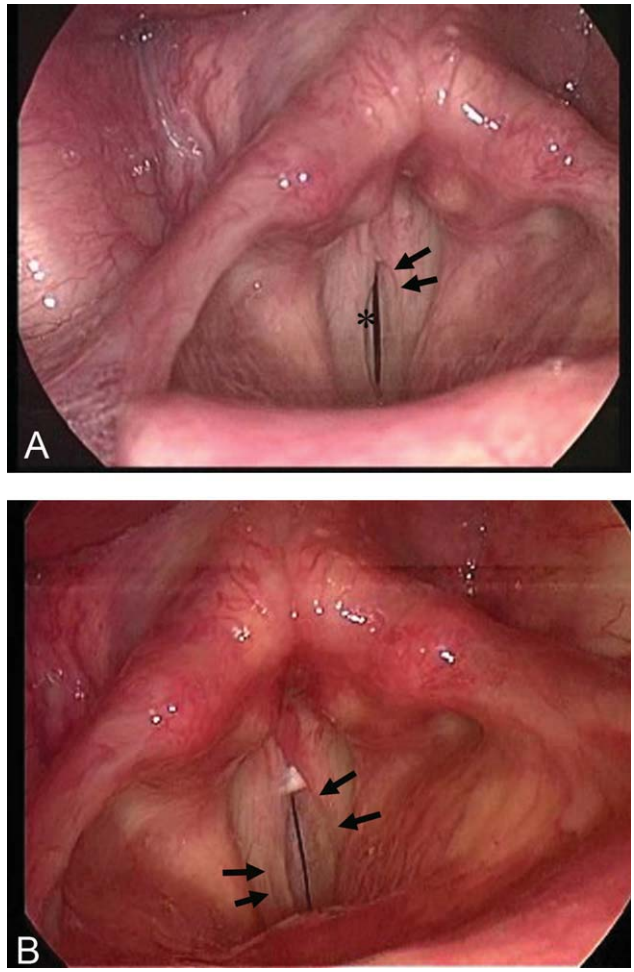

Fig. 2. Vocal fold mucosal waves in subject 4 (A) at baseline and (B) at 4 months after injection therapy. Although mucosal waves are absent on the right vocal fold, which has a sulcus (*) prior to therapy, mucosal waves with near normal excursion are visible after injection therapy (arrows). The appearance of sulcus remains unchanged. Mucosal waves on the normal left vocal fold are also more robust after injection therapy due to improved vocal fold entrainment (arrows).

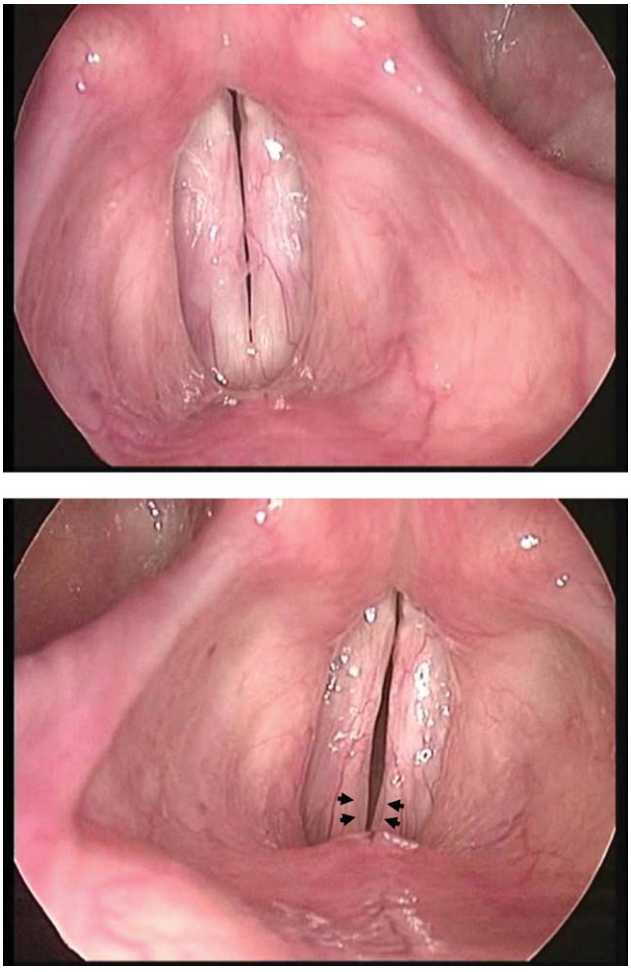

Fig. 3. Vocal fold mucosal waves in subject 5 (A) at baseline and (B) at 4 months after injection therapy. Mucosal waves are absent and the vocal folds are mildly edematous at baseline. After injection therapy, mucosal waves are seen limited to the medial edges of the anterior portions of the vocal folds bilaterally (arrows) and the vocal fold edema has improved.

measures of vocal fold function (mucosal waves) and subjective subject-reported outcomes. Assessment of the primary efficacy endpoint, the mucosal wave grade, showed an improvement starting at the week 8 visit that was sustained until the end of the study (month 12). Although the small number of subjects in this study limited statistical evaluation, nonetheless statistically significant improvement in mucosal wave grade was achieved (Table II). Acoustic analysis did not reach statistical significance in this group of patients, which is likely due to the small number and heterogeneity of subjects. The results for analysis of the voice handicap index (all categories) showed sustained improvements from baseline, and the magnitude of the improvements on the overall and subscales were felt to be clinically meaningful (Table III). A change of 8 points on each subscale is considered clinically significant different, whereas differences of 18 points on the total VHI score is considered clinically significant.[13] The voice quality questionnaire showed that the majority of subjects considered that their voice quality had improved, and that this improvement was sustained throughout month 12. Multiple treatments with autologous fibroblasts were well tolerated in an outpatient setting, with temporary otalgia being the only treatment-related adverse event. It appears that subjects with full thickness scars are more susceptible to otalgia and that in most instances the pain was tolerable enough so that the procedure could be completed and the full treatment dose given.

Another important finding in this study is that autologous fibroblast treatment was associated with the greatest benefit in subjects with vocal fold scarring limited to the lamina propria or sulcus. Subjects with full thickness cover defects (subjects 3 and 5) had less of an improvement in their mucosal wave grade after treatment, although improvements in other measures were noted. It is quite likely that the stroboscopic measure of mucosal wave grade does not represent all measures of voice production and perception. For example, subjects may have perceived improved efficiency or ease of phonation despite lack of improvement in mucosal waves on videostroboscopy, or there may have been improvement in other factors that were not measured in this study that led to subjects' improved perception of voice. Finally, one must also consider the placebo effect of repeated injections. Given the positive results from this pilot study, future randomized, double-blind, placebo-controlled studies will be necessary to establish the benefit of autologous fibroblast injection treatment for vocal fold scars. As might be expected given the nature of this treatment, when vocal fold scars are extensive and the regenerated epithelium is also scarred, treatment of the lamina propria layer alone is unlikely to improve the mucosal wave, which requires a pliable epithelial layer

TABLE III.
Total Voice Handicap Index (VHI) Scores at Various Time Intervals by Subject.

Subject	Day 0	Week 4	Week 8	Month 3	Month 4	Month 8	Month 12
1	101	68 (−33)	75 (−26)		56 (−45)		56 (−45)
2	82	79 (−3)	84 (+2)	66 (−16)	69 (−13)	67 (−15)	55 (−27)
3	79	75 (−4)	52 (−27)	55 (−24)	50 (−29)	51 (−28)	50 (−29)
4	68	62 (−6)	41 (−27)	36 (−32)	41 (−27)	34 (−34)	30 (−38)
5	89	98 (+9)	77 (−12)	79 (−10)	63 (−26)	58 (−31)	72 (−17)
Total with significant change		1/5	3/5	2/4	4/5	3/4	4/5

Lower scores indicate improved perception of the impact of the voice disorder by the subject. The total score is given followed by the change (in parentheses) compared with baseline VHI score at day 0. A change of 18 points from Day 0 is considered significantly different (bolded).

as well. These defects may be better treated with full thickness cover replacement. Tissue engineering techniques may ultimately hold promise for this therapeutic approach as well.

Vocal fold scars continue to be a challenging laryngeal pathology to treat for the otolaryngologist because currently there is no durable replacement material that can mimic the viscoelastic properties of the lamina propria constituents. In addition, lamina propria replacement therapy must also consider the complex three-dimensional organization of this layer that develops and matures over a significant time period. At birth, the lamina propria layer is a hyper-cellular monolayer that matures over the next 7 to 13 years into the trilaminar layer with the differential fiber and proteoglycan composition seen in adults.[14] This three-dimensional organization is complex with not only differences in the relative composition of the ECM molecules within the layer but also in the three-dimensional orientation of collagen and elastic fibers. Lamina propria replacement therapy would therefore need to address not only the viscoelasticity of the replacement material but its three-dimensional geometry and composition as well. Production of such lamina propria with its normal components in proper concentration and configuration remains a daunting task with currently available tissue-engineering techniques. However, one logical approach to lamina propria replacement therapy is to employ the fibroblast cell, the type of cell that originally produced and laid down the ECM components.

Fibroblasts are readily obtained from skin or buccal mucosa by punch biopsy and can be cultured free of other cell types. Human fibroblasts do not spontaneously become immortal in culture, a property that has significant implications when their reinjection into a human being is considered. Human fibroblasts divide for up to 60 generations in culture then enter "senescence" where they stop growing and die off.[11,12] These properties make fibroblast cellular therapy suitable for injection into the vocal folds. Cultured autologous fibroblast therapy in humans has so far been directed mainly in the cosmetic plastic surgery field for the treatment of facial wrinkles and scars.[15,16] Boss et al. treated 1,000 subjects with cultured autologous fibroblasts.[15] They performed approximately 4,000 injections from 1995 through 1999 with a follow-up period of 36 to 48 months. Ninety-two percent of the subjects were satisfied with the therapy. There were a total of 13 reported reactions (0.27%) to the injections, of which 11 were mild reactions with redness and swelling that resolved within 48 to 72 hours. The other two reactions were moderate with swelling and erythema for 7 to 10 days. Watson et al. reported a six-month prospective pilot study in 10 adults to assess the efficacy of cultured autologous fibroblasts to treat skin wrinkles and dermal depressions.[16] Microscopic examination of the injection site was also performed and demonstrated a denser and thicker layer of collagen in the dermal region, absence of any inflammatory reaction, and viable fibroblasts throughout. No adverse reactions were noted clinically or microscopically.

Several previous reports on autologous cellular therapy for vocal fold scars have involved animal models. In one report, autologous mesenchymal stem cells were first injected into canine vocal folds, which were then scarred experimentally four days later.[17] Two months later, less atrophy was observed in the treated vocal fold compared with the control vocal fold. Histologically, the injected cells appeared viable. However, phonation studies were not performed, and the mucosal wave grade or the acoustic quality of voice was not provided. In another study, human embryonic stem cells were injected into scarred rabbit vocal folds and histologic assessment performed one month later.[18] Persistence of the embryonic stem cells was observed and the injected vocal fold demonstrated decreased dynamic viscosity and elastic stiffness (as measured by parallel plate rheometry) compared with the untreated but scarred side. Another study performed injection of autologous fibroblasts from skin into scarred rabbit vocal folds.[19] This study "primed" the fibroblasts by adding various growth factors such as epidermal growth factor, hepatocyte growth factor (HGF), and decorin to the cell culture.[19] They reported that HGF-treated cells demonstrated increased synthesis of hyaluronic acid, and the HGF- and decorin-treated cells demonstrated diminished collagen synthesis in vivo. Although demonstration of appropriate changes to the ECM components is important, ultimately the resulting vibratory behavior of the vocal folds is the most important determinant of phonatory quality. Specifically, the return of vocal fold mucosal pliability as improved mucosal waves upon phonation should be one of the ultimate criteria for success. The current study was based on the one previous animal study that performed autologous fibroblast injection therapy into scarred canine vocal folds and demonstrated return of mucosal waves and improved acoustic parameters.[10]

A developing concept in research and treatment for vocal fold scarring is that an appropriate combination of scaffolds, cells, and regulatory factors may be required to adequately address the scar.[4,17] This study lays the groundwork for bridging the gap between basic science studies and future clinical trials, which will be required to treat vocal fold scars and related conditions. For example, vocal fold fibroblasts from geriatric subjects have been shown to be less productive of ECM components than fibroblasts from younger subjects.[20] Thus, autologous fibroblast cellular therapy may hold promise for the aging voice as well.[21] Future work could involve strategies to induce fibroblasts to become more productive of the ECM constituents that facilitate improved mucosal pliability. Active research is being undertaken by many groups looking for the ideal milieu of ECM molecules and growth factors that lead to proper healing and regeneration of lamina propria layer after vocal fold injury.[20–22] If a particular ECM protein requires upregulating, for example, autologous fibroblasts could be primed with appropriate growth factors, or altered by gene therapy to produce that ECM component in increased quantities.[19] Although cellular modifications prior to reinjection will require more oversight by appropriate regulatory agencies, such an approach may ultimately be the most productive in maintaining long-

term in vivo production of desired ECM molecules. For all these future remedies, appropriately designed and executed human clinical trials will be necessary. This report is the first small step in that direction.

CONCLUSIONS

This phase I clinical study showed that autologous fibroblasts cultured and expanded in the laboratory and injected into the lamina propria layer of the vocal folds is safe and appears to produce both objective and subjective improvements in voice quality that were sustained up to 12 months after treatment. Sustained trends for improvement are supported by data for mucosal wave grade assessment, VHI, and voice quality questionnaire. Subjects with vocal fold scarring limited to the lamina propria appeared to have the best response to treatment. Future approaches to treatment of vocal fold scars should include a focus on optimizing ECM production such as by treatment with growth factors or gene therapy, and development of strategies to replace the entire cover layer in those with full thickness scars.

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