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Autologous Fibroblasts for Vocal Scars and Age-Related Atrophy: A Randomized Clinical Trial

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Abstract

Objectives/Hypothesis: To assess the safety and efficacy of autologous cultured fibroblasts (ACFs) to treat dysphonia related to vocal fold scar and age-related vocal atrophy (ARVA).

Study Design: Randomized, double-blinded, placebo-controlled, multi-institutional, phase II trial.

Methods: ACFs were expanded from punch biopsies of the postauricular skin in each subject; randomization was 2:1 (treatment vs. placebo). Three injections of $1–2 \times 10^7$ cells or placebo saline was performed at 4-week intervals for each vocal fold. Follow-up was performed at 4, 8, and 12 months. The primary outcome was improved mucosal waves. Secondary outcomes included Voice Handicap Index (VHI)-30, patient reported voice quality outcomes, and perceptual analysis of voice.

Results: Fifteen subjects received ACF and six received saline injections. At 4, 8, and 12 months after ACF treatments, a significant improvement in mucosal wave grade relative to baseline was observed in both vocal scar and ARVA groups. Relative to control group, mucosal waves were significantly improved in the ARVA group at 4 and 8 months. Perceptual analysis significantly improved in the vocal scar group 12 months after ACF treatments compared to controls. Vocal scar group reported significantly improved vocal quality from baseline. VHI and expert rater voice grade improved in both groups, but did not achieve significance. No adverse events related to fibroblast injections were observed.

Conclusions: In this cohort, injection of ACFs into the vocal fold lamina propria (LP) was safe and significantly improved mucosal waves in patients with vocal scar and ARVA. ACF may hold promise to reconstruct the LP.

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Keywords

Randomized clinical trial; vocal folds scar; vocal fold; voice; presbyphonia; vocal fold atrophy; autologous fibroblasts; voice quality

INTRODUCTION

Dysphonia due to altered vocal fold lamina propria (LP) remains among the most recalcitrant of voice diagnoses. Vocal fold scarring and age-related vocal atrophy (ARVA) are examples of conditions that have an ineffectual LP.[1–4] Currently, they both pose significant clinical challenges, and therapeutic options are limited.[5] Cell-based therapies have been proposed as a novel way to accomplish vocal fold LP repair. Vocal fold cell injections have been studied widely in animals, but with very little experience in humans. This work presents a randomized, double-blinded, placebo-controlled, multi-institutional clinical trial of a cell-based therapy for vocal fold structural repair in humans.

Vocal fold vibration for voice production is facilitated by a healthy LP layer that allows propagation of the mucosal waves over the thyroarytenoid muscle body layer.[6] A healthy LP is a complex, three-dimensional structure consisting of cells and extracellular matrix (ECM) with both interstitial proteins (proteoglycans and glycoproteins) and fibrous proteins (collagen and elastic proteins) organized to promote mucosal pliability.[7] LP has been described as a trilaminar structure with superficial, intermediate, and deep layers. The superficial layer contains interstitial proteins with few collagen or elastin fibers, whereas the intermediate layer is characterized by an abundance of elastin fibers, and the deep layer is identified by its collagen fibers.[8–10]

Stroboscopic findings of vocal scar include decreased mucosal waves, vibratory aperiodicity, and glottal insufficiency. Histologically, the microstructure of the scarred LP can vary depending on the injury type, but generally demonstrates excessive and disorganized collagen deposition.[10] Current therapeutic approaches largely seek to address glottic insufficiency or to reduce cover stiffness through mucosa freeing techniques or superficial medial implants.[7] Current implant materials include esterified hyaluronic acid (HA),[11] autologous fascia,[12,13] and fat.[14] Limited data suggest modest efficacy of steroid injection into the LP to treat mild-to-moderate vocal fold scars.[15–17] This approach, however, may be associated with vocal fold atrophy.[16,18] Newer approaches using regenerative medicine techniques have attempted to restore function at the molecular and cellular level.[1,2,19–21]

The aged vocal folds are also characterized by reduced mucosal waves on stroboscopy and glottal insufficiency.[22] Histologically, atrophy of the epithelium, all three layers of the LP, and the vocalis muscle can be present in aged larynges.[23,24] In addition to the increased collagen as noted in scar, histologic studies in aging demonstrate decreased elastic fibers and decreased HA.[8,24,25] ARVA is typically treated with voice therapy, injection augmentation, or framework surgery to medialize the vocal folds and overcome a glottal gap.

Ideally, treatment of both vocal fold scar and ARVA should focus on recreating the complex normal LP architecture.[7,8,10,23–26] Fibroblasts produce and maintain ECM molecules and

architecture. Therefore, viable fibroblasts injected into the LP could act to reconstitute LP components and improve mucosal pliability. A phase I single arm clinical trial showed that autologous fibroblasts cultured and expanded in the laboratory and injected into the LP were safe and improved mucosal pliability and voice in some subjects with vocal fold scar.[19] The purpose of the current study was to expand on those findings via this phase II trial to evaluate the safety and efficacy of autologous cultured fibroblasts (ACF) for vocal fold scar and ARVA. We hypothesize that the cell-based treatment is safe and can improve mucosal waves and voice outcomes compared to saline injections.

MATERIALS AND METHODS

Subjects

The study protocol was approved by the institutional review boards of the participating institutions. Adults with dysphonia caused by idiopathic vocal fold scarring (subjects 18–60 years of age) or ARVA (subjects >60 years of age) who failed any one or more treatments. Exclusion criteria included: vocal fold scarring from previous vocal fold surgery or radiation therapy, pregnancy/lactating, active smokers, acute laryngitis, concurrent other vocal therapy, and other vocal cord pathologies that could be etiologic for their dysphonia (e.g., vocal polyps, cysts, cancer). Subjects were recruited at the University of California–Los Angeles, New York University, and Stanford University voice centers.

Study Design

A phase II, randomized, double-blind, placebo-controlled, multicenter study was designed to enroll a total of at least 20 subjects with dysphonia caused by either idiopathic vocal fold scarring or ARVA. Subjects were randomized into ACF or placebo (saline) treatment groups. Randomization was planned at an allocation ratio of 2:1 ACF to placebo subjects. Subjects with both unilateral and bilateral vocal fold scarring or ARVA were included, but only one vocal fold was treated at each treatment session; the treatment alternated between vocal folds at treatment visits. Each affected vocal fold received a total of three injections in the LP compartment 4 weeks apart. Subjects with bilateral disease received six injections 2 weeks apart and subjects with unilateral disease received three injections 4 weeks apart. Follow-up examinations were performed at 1, 4, 8, and 12 months after the final injection.

Autologous Cultured Fibroblasts and Controls

The cellular product (Azficel-T) received an Investigational New Drug designation by the Food and Drug Administration (FDA) specifically for use in the vocal folds. All subjects underwent a 3-mm postauricular dermal punch biopsy under local anesthesia using a sterile technique. Autologous fibroblasts were isolated from the tissue biopsies by Fibrocell Inc. (Exton, PA) using Good Manufacturing Processes. After expansion in culture, the cells were harvested and tested for cell identity, sterility, mycoplasma contamination, endotoxins, and concentration. Cells for injection were suspended in buffered saline in a sterile vial, delivered via overnight courier to each investigative site in an insulated container with a cold pack at 2° to 8°C, and administered within 24 hours. Control subjects also received sterile vials containing the saline vehicle for injection; vials were labeled, packaged, and shipped identically to the cell samples to maintain blinding of the clinical team. Immediately prior to

injection, the vial was warmed to room temperature for 15 to 30 minutes. Contents were resuspended by gentle inversion three times before aseptically drawing the contents into a sterile 1-mL syringe with a 21-gauge needle. The final cell injectates were sterile, free of mycoplasma and endotoxin contamination, with a concentration of 1.0 to 2.0 × 10^7 cells/mL and cell viability of more than 85%.

Vocal Fold Injections

Each injection consisted of 1.0 mL of ACF for each affected vocal fold.[19] Subjects randomized to the placebo group received 1.0 mL of buffered saline. Injections were performed by experienced laryngologists in the awake outpatient setting employing the thyrohyoid technique with a 25-gauge needle. Localization of the injection to the superficial LP was confirmed in real-time by visualization of ballooning in the sub-epithelial space by the laryngologist performing the injection.

Outcomes

Safety endpoint was incidence of adverse events. Primary efficacy endpoints were change from baseline in videostroboscopic mucosal wave grade, Voice Handicap Index (VHI)-30 score,[27] and expert perceptual analysis score. Subject ratings of voice quality were also obtained as a secondary endpoint.[28] The dependent variables were obtained at baseline and 4, 8, and 12 months posttreatment.

Mucosal Wave Grade

Videostroboscopy was performed at the beginning of each visit prior to any injections during treatment visits using a rigid 70° scope or a flexible distal chip laryngoscope. Subjects were asked to sustain the vowel /e/ at a comfortable pitch and loudness, and vocal fold vibration was recorded digitally. Videostroboscopic recordings were reviewed using Kay-PENTAX KDS software (PENTAX Medical, Montvale, NJ) for frame-by-frame analysis by a laryngologist or speech–language pathologist blinded to study treatment, and per study design the mucosal wave grade assigned on the same day of recording. Mucosal waves were graded as follows using a previously published scale[21]: 1 = absent, 2 = limited to the most medial edge of the vocal folds, 3 = present laterally up to one-quarter of the width of the vocal folds, 4 = present up to but less than one-half the width of the vocal folds, 5 = present at more than one-half the width of the vocal folds (normal).

Vocal Handicap Index-30

A validated 30-item subject-completed questionnaire, VHI-30, with 10 items in each of the three subscales: emotional, physical, and functional.[27]

Perceptual Analysis

Voice was recorded prior to laryngoscopy using a Shure (Niles, IL) head-mounted microphone connected to the Kay-PENTAX stroboscopy system. Subjects were instructed to voice the sustained vowel /a/ at a comfortable pitch and volume. Five-second voice samples within the middle of the phonation were extracted and normalized using SoundForge software (Magix Software, Berlin, Germany). These extracted recordings were presented to

three expert laryngologist raters in a randomized and blinded fashion. Voice grade (overall severity of dysphonia) was rated for baseline, 4, 8, and 12 months samples on the following scale: 0 = normal, 1 = mild dysphonia, 2 = moderate dysphonia, and 3 = severe dysphonia. Interrater reliability was assessed. If strong agreement (M-κ > 0.6) was achieved, the average score across the three raters was used. Consensus was generated if interrater reliability was not strong (M-κ < 0.6).

Subject Report of Voice Quality Using a Questionnaire

A subject-reported voice quality assessment questionnaire, consisting of two questions, was used. Subjects were asked to select “improved,” “no change,” or “worsened” in response to the question: “How has your voice quality changed since baseline?” Subjects were also asked to respond “yes” or “no” in response to the question: “Do you consider the treatment a success?”

Subject Rating of Voice Quality Using a Visual Analog Scale

Subjects were asked to rate their voice improvement using a visual analog scale (VAS). The VAS ranged from “worst possible change from baseline” on the left side to “most possible improvement from baseline” on the right side. The VAS at each time point was measured and given a score from −50 to 50.

Assessment of Safety

Assessment of subject safety included the incidence of adverse events (AEs), analysis of changes from baseline in laboratory values (e.g., hematology, blood chemistry, liver function tests, urinalysis), and vital signs. Subjects were observed for 1 hour after each injection. Subjects were also contacted by telephone on days 2 through 4 after the first treatment to inquire about any potential adverse events. Concomitant medications/procedures and AEs were recorded throughout the study.

Statistical Analysis

Version 9.2 or later of the SAS statistical software package (SAS Institute, Cary, NC) was used to perform all statistical analyses. Statistical significance was compared using a Wilcoxon signed rank test, Wilcoxon rank sum test or Fisher exact test (Tables I–VIII). Wilcoxon signed rank *P* value was used to assess significance of change from baseline at various time points within the ACF or placebo group. Wilcoxon rank sum *P* value was used to assess significance of change from baseline between the ACF and placebo groups. M-κ analysis was performed to assess interrater reliability for perceptual voice ratings.

Because this was a safety/efficacy trial, the primary purpose was to provide proof of concept in designing future studies. Sample size is therefore small. The study does provide power to detect a meaningful difference in overall response, at a planned enrollment of 20 subjects and 2:1 randomization rate. Using a two-sided Fisher exact test and type I error rate of 0.05, the study has 80% power to detect a difference in binary response rate comparison if the response rate in active subjects is 80% versus 15% for the control group.

RESULTS

Subjects and Demographics

A total of 22 subjects were recruited, and 21 subjects received the full course of injections. Fibroblasts from one subject did not grow to the standard concentration for injection and the subject was excluded. Demographic details are provided in Table I.

Mucosal Wave Grade

In subjects with ARVA, the mucosal waves improved at 4 ($P < .001$), 8 ($P < .001$), and 12 ($P = .008$) months in the ACF group, but not in the control group (Table II). A statistically significant improvement in mucosal wave grade was found in the ACF group when compared to the control group at 4 ($P = .006$) and 8 ($P = .004$) months. In subjects with scar, the mucosal wave grade improved at 4 ($P = .008$), 8 ($P = .008$), and 12 ($P = .008$) months in the ACF group, but not the control group (Table III). When compared to the control group, no statistically significant improvement in mucosal wave grade was found.

VHI-30 Assessment

In subjects with ARVA (Table IV) and vocal scar (Table V), the median VHI consistently decreased; however, it did not achieve statistical significance.

Perceptual Voice Assessments

M-κ analysis demonstrated “fair” (0.21–0.40) agreement between the three raters. Thus, a consensus dysphonia grade was generated among the three voice raters and used for analysis. In subjects with ARVA and vocal scar (Table VI), the median voice grade consistently shifted from moderate to severe dysphonia to normal to mild dysphonia at 4, 8, and 12 months for the ACF group, but not for the control group. In subjects with vocal scar, ACF was associated with significantly better voice grade compared to controls ($P = .036$) at 12 months.

Questionnaire Voice Quality Assessment

In subjects with ARVA and vocal scar (Table VII), difference between ACF and control groups did not achieve significance.

VAS Assessment of Voice Quality

In subjects with scar, significant improvement in voice quality was noted at 4 ($P = .016$), 8 ($P = .016$), and 12 ($P = .016$) months in the ACF group, but not in the control group (Table VIII).

Assessment of Safety

One subject reported cough for 1 week after the first injection. One subject developed a hemorrhage at the vocal fold injection site, which resolved in 1 month. Two subjects reported significant ear pain after an injection that resolved within a few hours. Three subjects reported sore throat and vocal fatigue for 1 week after injections. One subject developed a skin infection at the postauricular fibroblast harvest site that resolved with

antibiotics. Of note, this subject was on chronic high-dose steroids for autoimmune disease. No other significant adverse events were observed.

DISCUSSION

Disruption of the LP is associated with vocal fold scar and ARVA; this disruption is inherently challenging, as current treatments fail to restore the morphology and ultrastructure of the LP. Currently available treatments largely aim to treat glottic insufficiency, but few improve mucosal pliability. Current treatment outcomes for vocal scar and ARVA are unsatisfactory.

Principles of regenerative medicine likely hold promising therapeutic options to recreate the LP. Broadly, regenerative medicine aims to reconstruct tissue and its functions by administering cells, scaffolds (in situ tissue engineering), or growth factors.[1,28,29] Cell-based therapy includes transplantation of pluripotent stem cells, mesenchymal stromal cells, or mature fibroblasts. Recent literature reviews[30–32] identified more than 30 in vitro and in vivo studies on cell therapy for vocal fold scarring alone. Translating these promising concepts to human trials has been much slower, as significant assurances of feasibility and safety are prerequisites before human administration.

Development of a fibroblast therapy for vocal fold scarring and ARVA has progressed from preclinical animal study[33] to phase I clinical trial,[19] and now to this multicenter phase II safety and efficacy trial with randomization to treatment or control arms. Fibroblasts were selected because they are most prevalent in the LP and thought to be primarily associated with ECM metabolism and remodeling. Unlike pluripotent stem cells, fibroblasts are easily isolated from an office biopsy and expand rapidly in culture. Fibroblasts carry little risk of uncontrolled growth after injection and are unlikely to differentiate into undesired tissue types. For these reasons, Azficel-T received FDA approval in 2011 to treat moderate to severe nasolabial fold wrinkles in adults with autologous fibroblasts. In multiple randomized controlled trials, ACFs were safe and effectively improved rhytids, acne scars, and other dermal defects. Effect persisted for at least 12 months after injection.[34–36]

The current study applied ACF concepts underlying the treatment of rhytids to regeneration of the vocal fold LP. In a canine model, mucosal waves and acoustic parameters significantly improved after LP injections with ACFs.[33] In a phase I clinical trial, the safety of ACF injections were confirmed in five human subjects, and the study found that four of the five patients exhibited both objective and subjective improvements in voice quality and in mucosal waves.[19]

In this study, ACFs obtained from postauricular skin were injected into patients with vocal fold scar and ARVA. A control group received saline vocal fold injections. No serious adverse events were observed within the 1-year follow up. Significantly improved mucosal waves were observed at 4, 8, and 12 months in subjects with ARVA and vocal fold scar versus baseline. Improved mucosal waves in the ARVA group were statistically significant when compared to the control group at 4 and 8 months.

Mechanism for mucosal wave improvement was not assessed in this study. Being a human study, it is impossible to noninvasively monitor the injected cell viability and any ECM remodeling. However, animal studies from the preclinical product development did address these questions. Through a radioactive cell labeling technique, Zhao et al. demonstrated implanted skin cultured autologous rabbit fibroblasts could survive in vivo for at least 5 months and actively secrete new collagen.[37] Zeng et al. showed genomic stability of the skin-cultured fibroblasts in vivo and confirmed proliferation, viability, and function after injection into nude mice.[38]

Although encouraging, the current study is not without limitations. Multiple independent variables were included, consistent with the hypothesis-generating nature of this current work. Inclusion/exclusion criteria were drafted for greatest inclusivity (such as including patients with either unilateral or bilateral disease and including both scarring and ARVA). Similarly, the lack of a definitive accepted voice outcome measure prompted us to assess multiple dependent variables. A multivariate analysis with corrections for multiple comparisons would more accurately assess statistical significance in this situation, but does require larger sample sizes than were practical for this early work. The relatively small control group is also a concern. The decision to allocate twice as many patients to the treatment arm as to controls was intended to reduce unnecessary risk to patients receiving placebo injections. Additionally, the 2:1 chance of receiving fibroblast treatment was an effort to improve patient recruitment. Future trials could consider a crossover design, whereby control subjects would receive the therapy at a delayed time point.

CONCLUSION

Vocal fold scar and ARVA are difficult laryngeal conditions to treat due to the limited therapeutic options to replace and reconstruct the human LP. In the current cohort, LP replacement therapy via ACFs was safe. Compared to control saline injections, ACFs improved mucosal pliability in patients with vocal scar and ARVA. A larger phase II trial with greater power to detect outcomes of clinical relevance is now warranted.

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Table I.

Subject Demographics in ACF and Control Groups.

	ACF (Vocal Scar)	ACF (ARVA)	Control	All Subjects
No. of subjects	8	7	6	21
Age, yr				
Mean	53	72	67	64
Median	57	73	70	63
Minimum, maximum	33, 65	63, 83	36, 90	33, 90
Gender, n (%)				
Male	5 (62%)	5 (71%)	5 (83%)	15 (71%)
Female	3 (48%)	2 (29%)	1 (17%)	6 (29%)
Treatment laterality				
Unilateral, n	3	1	1	7
Bilateral, n	5	6	5	14

ACF = autologous cultured fibroblast; ARVA = age-related vocal atrophy.

Table II.

MW Grade for ACF Treatment Group and Control Group in Subjects With Age-Related Vocal Atrophy.

	ACF					Control				
	Vocal Fold	**Day 0**	**4 Months**	**8 Months**	**12 Months**	**Vocal Fold**	**Day 0**	**4 Months**	**8 Months**	**12 Months**
N					N					
1	Left	1	5	3	5	1 Left	1	2	2	5
	Right	2	4	3	4	Right	2	1	1	4
2	Left	2	4		3	2 Left	2	3		
	Right					Right	3	4		
3	Left	1	2	2	2	3 Left	2	2	2	2
	Right	2	3	4	2	Right	3	4	3	4
4	Left	2	5	4						
	Right	3	5	5						
5	Left	3	4	4						
	Right	3	4	4						
6	Left	2	3	3	3					
	Right	2	4	3	4					
7	Left	2	4	4	4					
	Right	2	4	4	4					
Mean MW grade		2.1	3.9	3.6	3.4		2.2	2.7	2	3.8
Median MW grade		2	4	4	4		2	2.5	2	4
Mean MW grade change from baseline			1.8	1.5	1.7			0.5	0	1.8
Median MW grade change from baseline			2	1.5	2			1	0	1.5
Wilcoxon signed rank *P* value			<.001	<.001	.008			.375	1	.25
Wilcoxon rank sum *P* value			.006	.004	.999					

ACF = autologous cultured fibroblast; MW = mucosal wave.

Table III.

MW Grade for ACF Treatment Group and Control Group in Subjects With Vocal Fold Scar.

	ACF					Control				
	Vocal Fold	Day 0	4 Months	8 Months	12 Months	Vocal Fold	Day 0	4 Months	8 Months	12 Months
N						N				
1	Left					1 Left	1	1	1	2
	Right	1	1	3	3	Right	1	1	1	2
2	Left	1	1	2	1	2 Left				
	Right	1	2	3	2	Right	2	4	5	5
3	Left					3 Left	2	3	3	3
	Right	3	4	3	4	Right	3	3	4	4
4	Left									
	Right	2	4	4	4					
5	Left	2	4	3	4					
	Right									
6	Left	3	4	5	5					
	Right									
7	Left	1	3							
	Right	2	2							
8	Left	2	3	4	3					
	Right	2	4	4	4					
Mean MW grade		1.8	2.9	3.4	3.3		1.8	2.4	2.8	3.2
Median MW grade		2.0	3.0	3.0	4.0		2.0	3.0	3.0	3.0
Mean MW grade change from baseline			1.1	1.6	1.4			0.6	1.0	1.4
Median MW grade change from baseline			1.0	2.0	2.0			0.0	1.0	1.0
Wilcoxon signed rank *P* value			.008*	.008*	.008*			0.5	0.25	0.063
Wilcoxon rank sum *P* value			.323	.286	.650					

*Statistically significant.

ACF = autologous cultured fibroblast; MW = mucosal wave.

Table IV.

VHI-30 Scores for the ACF Treatment Group and Control Group in Subjects With Age-Related Vocal Atrophy.

	ACF					Control			
	Day 0	**4 Months**	**8 Months**	**12 Months**		**Day 0**	**4 Months**	**8 Months**	**12 Months**
N					N				
1	65	41	25	43	1	41	56	53	47
2	40	24		40	2	118	1		
3	99	85	83	78	3	71	68	60	73
4	18	35	16						
5	19	25	23						
6	74	62	55	64					
7	52	6	8	10					
Mean VHI	52.4	39.7	35.0	47.0		76.7	41.7	56.5	60.0
Median VHI	52.0	35.0	24.0	43.0		71.0	56.0	56.5	60.0
Mean VHI change from baseline		−12.7	−19.5	−19.0			−35.0	0.5	4.0
Median VHI change from baseline		−14.0	−17.5	−21.0			−3.0	0.5	4.0
Wilcoxon signed rank *P* value		.219	.094	.125			.75	1.0	.500
Wilcoxon rank sum *P* value		1.000	.286	.095					

ACF = autologous cultured fibroblast; VHI = Voice Handicap Index.

Table V.

VHI-30 Scores for the ACF Treatment Group and Control Group in Subjects With Vocal Fold Scar.

	ACF					Control			
	Day 0	**4 Months**	**8 Months**	**12 Months**		**Day 0**	**4 Months**	**8 Months**	**12 Months**
N					N				
1	28	52	60	46	1	46	51	64	41
2	52	36	35	26	2	90	26	19	4
3	61	49	44	50	3	39	21		
4	83	25	5	7					
5	19	29	27	19					
6	67	60	55	32					
7	22	24							
8	67	2	24	16					
Mean VHI	49.9	34.6	35.7	28		58.3	32.7	41.5	22.5
Median VHI	56.5	32.5	35	26		46.0	26.0	41.5	22.5
Mean VHI change from baseline		−15.3	−18.1	−25.9			−25.7	−26.5	−45.5
Median VHI change from baseline		−9.5	−17.0	−26.0			−18.0	−26.5	−45.5
Wilcoxon signed rank *P* value		.313	.203	.094			0.5	1.0	0.5
Wilcoxon rank sum *P* value		.630	1.000	.667					

ACF = autologous cultured fibroblast; VHI = Voice Handicap Index.

Table VI.

Perceptual Voice Grade (Overall Severity) for ACF Treatment Group and Control Group in Subjects With ARVA and Vocal Fold Scar.

ARVA Group										Vocal Fold Scar Group									
	ACF				Control						ACF				Control				
	Day 0	4Months	8Months	12Months		Day 0	4 Months	8 Months	12 Months		Day 0	4Months	8Months	12 Months		Day 0	4 Months	8 Months	12 Months
N					N					N					N				
1	2	1	1	2	1	1	2	1	1	1	2	2	1	2	1	1	2	3	2
2	1	0		1	2					2	1	1	1	0	2				
3	2	2	2	2	3	1	1	1	1	3	2	1	1	1	3	0	0	0	1
4	2	1	1							4	2	2	2	1					
5										5	1	0	1	1					
6	1	0	0	0						6	0	0	0	0					
7	2	0	1	1						7									
										8	1	0	0						
Mean grade	1.7	0.7	1	1.2		1	1.5	1	1	Mean grade	1.3	0.9	0.9	0.8		0.5	1	1.5	1.5
Median grade	2	0.5	1	1		1	1.5	1	1	Median grade	1	1	1	1		0.5	1	1.5	1.5
Mean grade change from baseline		−1	−0.8	−0.4			0.5	0	0	Mean grade change from baseline		−0.4	−0.4	−0.5			0.5	1	1
Median grade change from baseline		−1	−1	0			0.5	0	0	Median grade change from baseline		0	0	−0.5			0.5	1	1
Wilcoxon signed rank *P* value		.063	.125	.5			1	NA	NA	Wilcoxon signed rank *P* value		.25	.25	.25			1	1	0.5
Wilcoxon rank sum *P* value		.071	.143	.524						Wilcoxon rank sum *P* value		.222	.222	.036*					

*Statistically significant.

ACF = autologous cultured fibroblast; ARVA = age-related vocal atrophy.

Table VII.

Patient-Reported Voice Quality Assessment for the ACF Treatment Group and Control Group in Subjects With ARVA and Vocal Fold Scar.

	ARVA Group					Vocal Fold Scar Group				
	ACF		Control		Fisher Exact Test, *P* Value	ACF		Control		Fisher Exact Test, *P* Value
	N	%	N	%		N	%	N	%	
4 months										
Improved	3	42.9	2	66.7	1	7	87.5	2	66.7	.491
No change	4	57.1	1	33.3		0		1	33.3	
Worsened	0		0			1	12.5	0		
Total	7		3			8		3		
8 months										
Improved	3	50	1	50	1	7	100	1	50	.222
No change	3	50	1	50		0		0		
Worsened	0		0			0		1	50	
Total	6		2			7		2		
12 months										
Improved	3	60	1	50	1	7	100	1	50	.222
No change	2	40	1	50		0		1	50	
Worsened	0		0			0		0		
Total	5		2			7		2		

ACF = autologous cultured fibroblast; ARVA = age-related vocal atrophy.

Table VIII.

VAS Assessment of Voice Quality for ACF Treatment Group and Control Group in Subjects With ARVA and Vocal Fold Scar.

		ARVA Group					Vocal Fold Scar Group				
Study Group	**Follow-up Month**	**N**	**Mean (SD)**	**Median**	**Wilcoxon Signed Rank *P* Value**	**Wilcoxon Rank Sum *P* Value**	**N**	**Mean (SD)**	**Median**	**Wilcoxon Signed Rank *P* Value**	**Wilcoxon Rank Sum *P* Value**
ACF	4 Months	7	18.7 (18.1)	15	.063	1	8	22.4 (11.9)	25	.016*	.6
Control	4 Months	3	21.7 (20.2)	15	.5		3	23.0 (29.1)	34	.5	
ACF	8 Months	6	15.3 (17.5)	12.5	.25	.714	7	29.4 (16.3)	35	.016*	1
Control	8 Months	2	4.0 (5.7)	4	1		2	11.0 (50.9)	11	1	
ACF	12 Months	5	17.8 (15.3)	15	.063	.857	7	34.3 (12.1)	38	.016*	1
Control	12 Months	2	16.0 (22.6)	16	1		2	24.0 (33.9)	24	1	

The VAS at each assessment was measured on a score from −50 (worst possible change from baseline) to +50 (most possible improvement from baseline).

*Statistically significant.

ACF = autologous cultured fibroblast; ARVA = age-related vocal atrophy; SD = statistically significant; VAS = visual analog scale.