Lamina propria replacement therapy with cultured autologous fibroblasts for vocal fold scars

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OBJECTIVES: **To develop a canine model of vocal fold scar and to evaluate its treatment with lamina propria replacement therapy using autologous cultured fibroblasts.**

MATERIALS AND METHODS: **Full thickness of the lamina propria layer in canine vocal folds was injured with a laser. Fibroblasts were cultured and expanded in the laboratory from a buccal mucosal biopsy. The scarred vocal folds were treated with 3 weekly injections of fourth, fifth, and sixth passage autologous fibroblasts. Mucosal waves and acoustic parameters were measured at baseline, after scarification, and several months after injection therapy. Histologic evaluation of the vocal folds for fibroblasts, collagen, elastin, reticulin, and hyaluronic acid was performed.**

RESULTS: **Nine beagle dogs were used, and 1 animal served as control. Vocal fold scarring resulted in absent or severely limited mucosal waves and significantly worse acoustic parameters. Significant improvements in mucosal waves and acoustic parameters were obtained after lamina propria replacement therapy. After therapy, mucosal waves became normal in 4 animals and near normal in the other 4. No statistical difference was found in mucosal waves between baseline and post-therapy. All animals tolerated therapy without complications. The treated vocal folds demonstrated an increased density of fibroblasts, collagen, and reticulin, a decreased density of elastin, and no change in hyaluronic acid.**

CONCLUSIONS AND SIGNIFICANCE: **Therapeutic options for vocal fold scars are limited. Lamina propria replacement therapy in the form of autologous cultured fibroblasts improves mucosal pliability and returns normal or near normal mucosal waves in experimentally scarred vocal folds. This novel therapeutic modality may hold new promise for treating vocal fold scars.** (Otolaryngol Head Neck Surg 2004;131:864-70.)

The vocal folds of the larynx can be anatomically divided into 3 tissue layers. The superficial layer is formed by the vocal fold epithelium, followed by the middle lamina propria layer and the deep muscular layer.[1] The lamina propria layer is of special interest because it is an amorphous, paucicellular layer composed of extracellular matrix molecules that provides the appropriate viscosity for proper vibration of the vocal folds.[2] Proper vibration of the vocal folds is mandatory for normal voice generation. Loss of the lamina propria layer leads to a decreased or absent mucosal pliability (mucosal waves) of the vocal fold surface. This is most easily observed upon videostroboscopic examination of the larynx.[3]

Surgery on the vocal folds for benign and malignant disorders can damage the lamina propria layer and this is the most frequent etiology of vocal fold scars. Damage to the lamina propria layer can also result from a variety of traumatic, neoplastic, iatrogenic, inflammatory, and miscellaneous etiologies.[4] The resulting voice is hoarse, of poor quality, and perceived by patients as a severe communication handicap. Vocal fold scar is a challenging problem for an otolaryngologist because effective therapy for this condition is currently lacking and rehabilitation of patients is difficult. Management of vocal fold scars with autologous fat implantation[5,6] and autologous fascia augmentation[7] have been reported but treatment results have been less than satisfactory.

The lamina propria layer consists mostly of extracellular matrix (ECM) molecules such as collagen, elastin, and proteoglycans.[2] It also contains a small population of cells composed primarily of fibroblasts and some myofibroblasts and macrophages.[8] Fibroblasts are homogeneously distributed throughout the lamina propria layer and presumably play the major role in production of ECM molecules. Theoretically, introduction of a fibroblast population into the subepithelial intermediate layer of scarred vocal folds could

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lead to reconstitution of lamina propria components and reestablishment of mucosal pliability. This would result in improved mucosal waves on the vocal fold surface and improved phonation.

The ability to take cells from an individual, expand those cells in the laboratory, and inject them into the same individual to repair symptomatic defects is a newly evolving therapeutic modality in medicine. Initial work in this area of "cultured autologous cellular" therapy was directed towards chondrocytes. A U.S. Food and Drug Administration-approved autologous cultured chondrocyte product (Carticel, Genzymebiosurgery, Cambridge, MA) has been available since 1997 for orthopedic use.[9] Autologous cellular therapy has also been applied clinically to include transplantation of autologous cultured melanocytes for treatment of segmental vitiligo,[10] autologous keratinocytes for treatment of ulcers[11] and burns,[12] and autologous fibroblasts for treatment of facial wrinkles.[13-15] No adverse effects such as malignant transformation of injected cells or significant tissue reaction have been reported so far with the use of autologous cellular therapy.

Fibroblasts are readily obtained by punch biopsy of skin or mucosa and can be cultured free of other cell types. It is straightforward to obtain a pure fibroblast population free from keratinocytes or epithelial cells, the other major cell type in skin and mucosa respectively, because fibroblasts are so hardy in culture and outgrow other cell populations. Studies with human fibroblasts indicate they usually divide for up to 60 generations in culture then enter "senescence" where they stop growing and die.[16,17] Additionally, after injection into a body site fibroblasts are expected to remain more or less within the injection site.[17] These properties make fibroblast cellular therapy suitable for injection into the vocal cords.

Cultured autologous fibroblast therapy has so far been directed mainly in the cosmetic plastic surgery field for the treatment of facial wrinkles and scars.[13-15] Boss et al[14] reported treating 1,450 patients for facial rhytids with cultured autologous fibroblasts. They performed a total of 4,800 injections from 1995 through 1999. The follow-up period was 36 to 48 months. Of the patients, 92% were satisfied with the therapy. There were a total of 13 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 2 patients had moderate reactions with swelling and erythema for 7 to 10 days. Watson et al[15] reported a 6-month prospective pilot study in 10 adults to assess the efficacy of cultured autologous fibroblasts to treat facial rhytids. 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.

The specific objectives of this research was to develop an in vivo canine model of vocal fold scar and to apply autologous cultured fibroblast therapy as a form of lamina propria replacement therapy to treat this condition. We anticipated that the fibroblasts would reconstitute the ECM products that would then lead to an improvement in mucosal pliability that could be appreciated upon videostroboscopic examination of the larynx. Herein we report the results in a small group of animals.

MATERIALS AND METHODS

In Vivo Canine Model of Phonation

This study was performed in accordance with the U.S. Public Health Service Policy on Humane Care and Use of Laboratory Animals, the NIH Guide for the Care and Use of Laboratory Animals, and the Animal Welfare Act. Our institutional Animal Research Committee approved the research protocol.

The in vivo canine model of phonation has been established in the University of California, Los Angeles, Laryngeal Physiology Laboratory for almost 2 decades to study laryngeal physiology in situ.[18] Briefly, in this model, after general anesthesia is induced in the animal, upper and lower tracheotomies are made. The lower tracheotomy is used to assist ventilate the animal and the upper tracheotomy is used to provide rostral airflow via a cuffed endotracheal tube to drive phonation. During acute experiments, when the animal is to be sacrificed at the end of the experiment, a midline incision from the hyoid bone to the sternal notch is made. For survival surgery, small upper and lower transverse incisions are made on the neck. Neck exploration is performed to locate both recurrent laryngeal nerves and superior laryngeal nerves close to their entrance into the larynx. Custom designed monopolar electrodes with silicone insulation are applied to the isolated nerves. The electrodes are attached to a constant current nerve stimulator (WR Medical Electronics Co., Model 2SLH, St. Paul, MN). The nerves are typically stimulated at 80 Hz with 0-3.0 mA for 1.5 msec pulse duration to achieve complete adduction of the vocal folds.

Once vocal folds are adducted, airflow drives phonation and sound is generated. Videostroboscopic and acoustic parameters are then measured. Phonation is performed at 2 airflow settings: 550 cc/s and 350 cc/s. The proximal endotracheal tube is connected to a flowmeter (Edwards Datametrics Model 1600, Wilmington, MA) and the airflow rate is controlled using a wall-mounted control. Airflow is humidified and heated by

bubbling through 5 cm of heated water so that the temperature of the air is 37°C when measured at the glottal outlet.

Videostroboscopic Analysis

Videostroboscopy was performed using a rigid 0-degree endoscope attached to a CCD camera (Karl Storz, Telecam 20 2101 20, Culver City, CA) with illumination from a stroboscopic light source (Kay Elemetrics, Model RLS 9100, Lincoln Park, NJ). Recordings were performed on a three-fourths-inch videocassette recorder (Sony, Model VO9850, Park Ridge, NJ). The use of videostroboscopy allowed slow motion examination of the vibrating vocal folds and better delineation of the mucosal waves on the vocal fold surface. Mucosal waves were rated on a scale of 1 to 5: 1 = absent, 2 = limited to the most medial edge, 3 = present laterally up to one fourth of the width of the vocal folds, 4 = present up to but less than one half the width of the vocal folds, 5 = present more than one half the width of the vocal folds (normal).

Acoustic Analysis

A 1-second sample of phonation was recorded with a Bruel and Kjaer condenser microphone, sampled at 20 kHz, low-pass filtered at 3 kHz, and saved on the computer hard drive. A 0.5-second portion of this signal was analyzed using speech analysis software (CSpeech version 3.1, Paul Milenkovic, Madison, WI) to obtain the acoustic parameters of jitter, shimmer, and signal-to-noise ratio (SNR).

Vocal Fold Scarification

Vocal fold scarification was performed using a carbon-dioxide laser set at 4 watts continuous mode and controlled using a foot pedal. Previous experience showed that injury down to the muscular layer was necessary to consistently obtain vocal fold scar devoid of mucosal waves. Therefore a deep burn was made to the muscular layer and the entire lamina propria layer was thus ablated. The superior surface of the entire length of the vocal fold was injured. The vocal fold surface was expected to re-epithelialize but not regenerate the lamina propria layer. Scarification was documented by severely decreased or absent mucosal waves after healing by re-epithelialization.

Autologous Fibroblast Culture

After baseline phonation, an approximately 5×5 mm^2 biopsy of the buccal mucosa was obtained. The biopsy site was allowed to heal secondarily. The harvested mucosa was immediately placed in a sterile tube with culture medium and transported to the tissue culture laboratory. The culture medium used throughout was Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum and 1% mixture of penicillin, aminoglycoside, and amphotericin B. The mucosal biopsy was washed, minced, and incubated in a culture dish with medium. Incubation conditions were 37°C and 5% CO_2. Fibroblasts grew radially in the culture dish and were harvested by trypsinization when an adequate cellular monolayer was established. Harvested cells were propagated in culture flasks with medium. Cells were further passaged when 90% to 100% confluence was reached. Fourth, fifth, and sixth passage cells were harvested for vocal fold injection. Cells were centrifuged to separate from medium, washed with phosphate buffered saline, and suspended in about a milliliter of buffered saline for injection. Cell density and viability were counted using a hemocytometer.

Vocal Fold Injection

The larynx was visualized under direct laryngoscopy. Phonation and videostroboscopy were performed prior to the first injection. Approximately 0.8 to 1 mL of autologous cultured fibroblasts was injected transorally into the scarred vocal fold. A 25-gauze spinal needle was used and the injection was performed in the subepithelial plane. The distal 1.5 cm of the needle tip was bent at an angle so it could be advanced posteriorly to anteriorly in the subepithelial plane of the vocal fold. The cells were then "layered" into this plane as the needle was slowly pulled back. Injection was performed slowly to allow dissection into the plane between the epithelium and the muscular layer.

Histologic Analysis

Animals were humanely sacrificed according to established protocols and laryngectomy performed. The larynges were fixed in formaldehyde, decalcified using EDTA, and coronally cut at 0.5-cm slices from the anterior commissure to the vocal process of the arytenoid and embedded in paraffin blocks. Microscopic sections 3-μm thick were then cut from the anterior and middle vocal fold areas and stained for histologic analysis using routine procedures used daily at the UCLA histopathology laboratory. The densities of fibroblasts, collagen, reticulin, elastin, and hyaluronic acid were evaluated by staining each histologic section with hematoxylin and eosin (H&E), Masson trichrome, reticulin, elastica Verhoeff-van Gieson (EVG), and Alcian blue with and without pretreatment with hyaluronidase, respectively.

A senior attending head and neck pathologist and a resident physician pathologist blinded to the physiologic findings reviewed the histologic slides. The reviewers were not only blinded to the results of videostroboscopic and acoustic analysis but also to

Table 1. Mucosal wave rating* at baseline, scar, and post-treatment stages

Animal no.	Rating baseline	Interval post-scar	Rating scar	Interval post-tx	Rating post-tx
Control group:					
1	5	7 weeks	1	23 weeks	1
Treatment group:					
2	5	6 weeks	2	39 weeks	4
3	5	6 weeks	2	39 weeks	4
4	N/A	N/A	2	31 weeks	5
5	1	8 weeks	1	31 weeks	5
6	4	15 weeks	1	22 weeks	5
7	1	15 weeks	1	22 weeks	3
8	3	6 weeks	1	27 weeks	3
9	5	6 weeks	1	27 weeks	5
Average	3.63	8.63 weeks	1.33	29 weeks	4.25†

*Mucosal waves were rated as follows: 1, absent, 2, limited to the most medial edge; 3, present laterally up to 1/4 of the width of the vocal folds; 4, present up to but less than 1/2 the width of the vocal folds; 5, present at more than 1/2 the width of the vocal folds (normal).

Time intervals were as follows: "Interval post-scar", time from vocal fold scarification (at this interval second phonation and first injection therapy were performed). "Interval post-tx", time from first injection therapy to final phonation (for control animal this is time from "post-scar interval").

†Average post-tx rating is for treatment group only.

the scarred versus control side in each animal. The relative density of cells or staining on 1 vocal fold was then semiquantitatively rated increased, decreased, or equal compared to the opposite vocal fold. Two slides per parameter measured per vocal fold side were used; 1 from anterior portion of the vocal fold and the second from the middle. When the raters completed their individual ratings, they generated a consensus together.

Statistical Analysis

Statistical evaluation was performed using the JMP4 software (SAS Institute, Cary, NC). Two-tailed Student's *t*-test for paired observations was performed to compare means. Spearman's ρ (r_s) was calculated for correlation analysis. Level of significance was set at $P = 0.05$.

RESULTS

Nine beagle dogs were used for this study. Phonation was performed at baseline ("Baseline"), several weeks after unilateral vocal fold scarification ("Scar"), and several months after treatment of the vocal fold scar with autologous cultured fibroblasts ("Post-Tx"). Acoustic parameters were measured and mucosal waves rated during phonation at each experimental stage. After baseline phonation ("Baseline") the left vocal fold in all animals except animal No. 4 was scarified using the CO_2 laser. A preexisting scar (sulcus) was present in the right vocal fold in animal No. 4. Therefore baseline phonation in this animal was treated as "Post-scar" for data analysis and the right vocal fold received injection therapy. Buccal mucosa was harvested after baseline phonation for culture of autologous fibroblasts in all animals except No. 1, which was used as control and did not undergo injection therapy.

The first vocal fold injection was performed when fourth passage cultured fibroblasts reached 95% to 100% confluence. Phonation measures were recorded just prior to first injection therapy. In most animals, adequate expansion of cells was achieved between 6 to 8 weeks after harvest of buccal mucosa. In 2 animals (Nos. 6 and 7), the initial cell culture failed and buccal biopsy and cell culture were repeated. These two animals received their first injection at 15 weeks post-scarification (Table 1). As mentioned above, animal No. 1 was used as control and received no injection. In the treated animals, injection therapy was repeated twice at weekly intervals for a total of 3 injections. The animals were then followed for 22 to 39 weeks (average, 29 weeks) at which time final phonation parameters were measured and the animal humanely sacrificed for histologic examination of the larynx.

Each injection contained approximately 25 million fibroblasts. Cell viability was greater than 95% at final harvest and prior to transport to the laboratory for injection. Transport time to the surgical suite from cell culture laboratory was less then 15 minutes. There were no apparent complications from vocal fold injections such as respiratory distress, fever, or other adverse reactions. However, no interval laryngoscopic exam was performed after the third vocal fold injection until final phonation.

Videostroboscopic Analysis

Normal mucosal waves could not be generated in all animals at baseline (Table 1). Normal mucosal waves

Table 2. Acoustic analysis data

	Jitter %			Shimmer %			SNR %		
Animal No.	Baseline	Scar	Post-tx	Baseline	Scar	Post-tx	Baseline	Scar	Post-tx
Control group:									
1	2.48	5.97	2.38	9.29	39.30	14.51	19.90	7.36	14.01
Treatment group:									
2	0.73	0.90	0.75	2.81	3.89	4.51	20.25	17.56	18.24
3	0.34	1.03	0.90	1.81	4.45	3.35	25.23	18.95	17.72
4	N/a	1.29	1.64	N/a	8.72	8.10	N/a	16.11	10.93
5	0.62	6.15	1.69	2.31	19.53	6.61	23.59	6.10	16.06
6	0.69	2.91	0.24	3.00	32.23	2.51	21.05	5.97	25.89
7	0.94	5.92	0.87	6.22	34.04	10.05	16.82	5.13	13.66
8	0.61	2.29	0.55	4.33	13.55	3.29	15.89	14.46	18.70
9	0.47	2.43	1.27	2.55	14.01	6.12	21.65	12.78	16.88
Average	0.86	3.21	1.14	4.04	18.86	6.56	20.55	11.60	16.90

were generated in 4 animals, intermediate waves in 2, and waves were absent in 2. Animal No. 4, which had a preexisting scar, also had minimal mucosal waves.

The post-scarification ("Scar") phonation parameters were measured between 6 and 15 weeks after scarification. All scarified vocal folds had healed by re-epithelialization by this time. However, slightly diminished vocal fold bulk commensurate with the amount of scarification was grossly appreciated in all animals. In all animals, vocal fold scarification resulted in either absent mucosal waves (6 animals) or waves limited to the most medial edge (2 animals). Both animals (Nos. 6 and 7) receiving delayed injection therapy at 15 weeks after scarification had absent mucosal waves.

Injection therapy ("Post-Tx") led to improved mucosal waves in all animals. Mucosal waves were rated normal in 4 animals and the other 4 had high-intermediate ratings (Table 1). Two animals that had absent mucosal waves at baseline had improved mucosal waves to normal (animal No. 5, rating 5) and intermediate (animal No. 7, rating 3) values after treatment. Mucosal waves in the control animal were still absent 30 weeks after scarification. Mucosal wave excursions were similar between the experimental side and the normal side in all animals at all experimental stages.

Average mucosal wave rating was 3.6 at baseline (N = 8), 1.3 after scarification (N = 9), and 4.25 after injection therapy (N = 8). Mean mucosal wave ratings were significantly decreased after scarification and significantly improved after therapy. No statistical difference was found between mean mucosal wave rating at baseline and post-treatment.

Acoustic Analysis

Jitter, shimmer, and SNR measurements for each animal are listed in Table 2. Table 3 lists statistical results of the comparison of these acoustic parameters as well as mucosal wave ratings. Overall, mean jitter and shimmer increased and SNR decreased after scarification, and these values reversed after therapy. Average jitter and shimmer values were significantly different between baseline and scar, scar and post-therapy, but not between baseline and post-therapy. Average SNR was significantly different between baseline and scar. Improvements in acoustic parameters were also present in the control animal. However, the single control animal precluded meaningful statistical comparison between control and treated animals. Correlation analysis between acoustic parameters and mucosal waves was performed and although significant correlations were found within the acoustic parameters, no significant correlations were found between acoustic parameters and mucosal waves at baseline and post-therapy.

Histologic Analysis

The results of histologic analysis are listed in Table 4. Differences in fibroblast and collagen density were the easiest to distinguish between the experimental and the opposite normal vocal folds. All scarred vocal folds clearly had increased fibroblast cell density and collagen staining. Reticulin was also increased in 7 of the 8 treated animals. Animal No. 4, which was treated with autologous fibroblasts but was not scarred because of preexisting sulcus, had decreased reticulin. Elastin tended to be reduced on the scarred side of all treated animals, especially in the more superficial lamina propria layer. Hyaluronic acid was the most difficult to interpret due to subtle differences and was inconclusive, with overall no definite differences observed. The scarred vocal fold of the control animal also demonstrated increased fibroblasts and collagen but no differences in reticulin, elastin, or hyaluronic acid were seen.

Table 3. Statistical results of acoustic and stroboscopic analysis (*P* values*)

Comparison	Jitter	Shimmer	SNR	Mucosal waves
Baseline vs. scar (N = 9)	0.007	0.006	0.001	0.004
Scar vs. post-tx (N = 8)	0.035	0.023	0.061	<0.001
Baseline vs. post-tx (N = 8)	0.270	0.065	0.222	0.334

*Student's *t*-test, 2-tailed, for paired observations.

Table 4. Relative densities of vocal fold fibroblasts and extracellular matrix components on experimental versus normal side

Animal No.	Fibroblasts	Collagen	Reticulin	Elastin	Hyaluronic acid
Control group:					
1	Increased	Increased	Equal	Equal	Equal
Treatment group:					
2	Increased	Increased	Increased	Decreased	Equal
3	Increased	Increased	Increased	Decreased	Equal
4	Increased	Increased	Decreased	Decreased	Equal
5	Increased	Increased	Increased	Decreased	Equal
6	Increased	Increased	Increased	Decreased	Decreased
7	Increased	Increased	Increased	Decreased	Equal
8	Increased	Increased	Increased	Decreased	Equal
9	Increased	Increased	Increased	Decreased	Equal

DISCUSSION

Mucosal pliability of the vocal fold is directly related to the quality of its lamina propria layer. Injury to the lamina propria primarily leads to inhibited or absent mucosal waves across the vibratory margin of the vocal fold. We demonstrated this by deeply injuring the vocal fold in a small group of canines and measuring mucosal waves via videostroboscopy in an in vivo canine model of phonation. The full thickness of the lamina propria layer was injured using a CO_2 laser. Mucosal waves were absent or severely diminished in all scarified larynges.

The primary goal in the treatment of vocal fold scars is to return mucosal pliability. Secondary goals such as treatment of glottic insufficiency may exist as well. However, return of mucosal waves is the crucial measure of treatment success. We treated laser induced vocal fold scars with autologous cultured fibroblasts and found that mucosal waves improved after treatment in all animals. One control animal that was not treated continued to have absent mucosal waves 30 weeks after scarification.

After unilateral vocal fold scarring, mucosal waves were absent or severely diminished in *both* vocal folds. Injection of fibroblasts resulted in return of mucosal waves to both vocal folds. This is explained by the phenomenon of vocal fold entrainment[19] in which one vocal fold vibration affects the vibration of the other. For example, after partial laryngectomy in which reconstruction of resected vocal fold is performed using soft tissue or muscle of the neck, normal mucosal waves cannot be generated in the remaining normal vocal fold. On the other hand, mucosal waves of both vocal folds improve dramatically after a medialization procedure for unilateral vocal fold paralysis.[20] The loss of mucosal waves after unilateral vocal fold scarring is due to lack of entrainment from the scarred vocal fold that is devoid of lamina propria and cannot generate mucosal waves. Treatment of scarred vocal fold with autologous fibroblasts replenishes the lamina propria layer and results in return of mucosal waves to both vocal folds. The histologic data indicate that normal lamina propria components are present after replacement therapy. Although the significance of the changes in relative densities of the various ECM components are being further investigated, it is clear that the lamina propria generated by the injected fibroblasts is able to provide adequate viscoelasticity to the scarred vocal fold.

Surgical treatment strategies offered so far for vocal fold scars have mostly attempted to augment the vocal fold using autologous fat or fascia. Hsiung et al[7] reported injecting autologous fat in 33 patients with glottic insufficiency. Of the 13 patients with vocal fold scars, 7 reported excellent subjective results. They noted poorer results in patients with vocal insufficiency due to scars and vocal fold paralysis compared to patients with vocal fold atrophy. Neuenschwander et al[5] performed a retrospective analysis of 8 patients treated with autologous fat implantation for vocal fold scars. They reported significant improvement in muco-

sal waves but quantitative data was not presented. Duke et al[6] treated 2 patients with vocal fold scars with fascia augmentation and reported no improvement. Both fat and fascia grafts invariably undergo atrophy with time and delayed failures are seen.[5] It is quite plausible that cell-based therapy such as autologous cultured fibroblast injection for vocal fold scars could circumvent the failures seen due to absorption of injectable agents because ECM components are continuously replenished by the viable injected fibroblasts.

It is not altogether surprising that acoustic parameters do not correlate with mucosal wave ratings. When phonation parameters such as subglottic pressure, nerve stimulation thresholds, and extraneous vibrations of tissue and mucus are not controlled, acoustic parameters are often not helpful in evaluating the quality of speech signal. This further emphasizes that videostroboscopic measurements of mucosal waves is the most pertinent parameter in assessing treatment efficacy for vocal fold scars. The usefulness and importance of measuring the mucosal waves for treatment success is demonstrated by the results of acoustic parameters versus mucosal waves in the control animal. Although acoustic parameters were improved at 30 weeks, mucosal waves were still absent. However, this study does suffer from having only one control animal.

Further studies are needed to evaluate the role of cellular therapy for the treatment of vocal fold scars. At this point we do not know the optimal dose density of fibroblasts, nor do we know the optimal number of doses needed for treatment efficacy or the length of treatment efficacy. A quantitative assessment of the ECM protein components replenished by autologous fibroblasts also awaits further study. Finally, although we can demonstrate a return of mucosal waves after autologous fibroblast therapy we cannot adequately test the perceptual improvement in voice quality in an animal model. Reliable objective measures of voice quality do not yet exist. An individual's perception of one's own voice, and perhaps as well that of an objective listener, remains the final arbiter of treatment efficacy. The role of lamina propria replacement therapy for vocal fold scars awaits further study in clinical trials.

CONCLUSIONS

There is currently no effective therapy for vocal fold scars. Lamina propria replacement therapy addresses the underlying physiologic disorder in vocal fold scars, a deficient or injured lamina propria. Lamina propria replacement therapy in the form of autologous cultured fibroblasts returns to normal or near normal absent or severely diminished mucosal waves in experimentally scarred vocal folds in canines. The role of this new and promising treatment modality remains to be further studied.

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