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Functional and Histological Evaluation following Canine Vocal Fold Reconstruction Using Composite Thyroid Ala Perichondrium Flaps

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Abstract

Objectives/Hypotheses.—We evaluated the effects of vocal fold reconstruction using a composite thyroid ala perichondrium flap (CTAP) after unilateral vocal fold stripping in beagles. We hypothesized that CTAP would improve glottic closure, decrease phonation threshold pressure, and decrease perturbation. In addition, vocal folds with CTAP would exhibit neovascularization and fat with increased von Willebrand factor (vWF) and smooth muscle actin (SMA), reflecting neoangiogenesis and flap viability.

Study Design.—Randomized controlled trial using beagles.

Setting.—University laboratory.

Methods.—Ten beagles underwent unilateral vocal fold stripping. Dogs in the scar-only group (n = 5) were sacrificed at 1 month. Dogs in the CTAP group (n = 5) underwent ipsilateral reconstruction with CTAP at 1 month and were sacrificed at 2 months. Excised larynx experiments evaluated vocal fold vibration using aerodynamic, acoustic, and mucosal wave measurements.

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Author Contributions

Matthew R. Hoffman, study design, data acquisition, data analysis, drafting manuscript, final approval of manuscript, accountable for all aspects of work; **Rachel Glab**, assisted on surgical procedures, data acquisition, data analysis, drafting of manuscript, final approval of manuscript, accountable for all aspects of work; **McLean Gunderson**, assisted on surgical procedures, data acquisition, data analysis, drafting of manuscript, final approval of manuscript, accountable for all aspects of work; **Allison L. Maytag**, data acquisition, data analysis, critical revision of manuscript, final approval of manuscript, accountable for all aspects of work; **David T. Yang**, study design, data acquisition, data analysis, critical revision of manuscript, final approval of manuscript, accountable for all aspects of work; **Jack J. Jiang**, study design, critical revision of manuscript, final approval of manuscript, accountable for all aspects of work; **Seth H. Dailey**, study design, performed surgical procedures, data interpretation, critical revision of manuscript, final approval of manuscript, accountable for all aspects of work.

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Disclosures

Competing interests: Matthew R. Hoffman, vice president of Wisconsin Voice and Swallow Innovations Group, Inc—this is a small startup company that develops devices for vocal fold medialization but was not involved in this study, did not contribute funding to this study, and has no financial interest in the autologous flap described in this study.

Qualitative evaluation of vocal fold morphology and quantitative analysis of elastin, collagen, glycosaminoglycans, vWF, SMA, and hyaluronic acid were performed.

Results.—Phonation threshold pressure ($P = .005$), percent jitter ($P = .010$), percent shimmer ($P = .007$), and open quotient ($P = .007$) were lower in the CTAP group. Neovascularization ($P = .0079$) and fat ($P = .1667$) occurred more with CTAP, although the difference in fat was not significant. von Willebrand factor was higher with CTAP vs contralateral normal fold ($P = .110$), although not statistically significant. Smooth muscle actin was higher with CTAP vs contralateral normal fold ($P = .038$) and scarred vocal folds ($P = .022$).

Conclusions.—Composite thyroid ala perichondrium flap restored glottic closure and vibratory periodicity following vocal fold scarring. Additional investigation on biologic response is warranted. Composite thyroid ala perichondrium flap offers an autologous, vascularized implant that can improve both vocal fold structure and function.

Keywords

vocal fold scar; glottic insufficiency; composite thyroid ala perichondrium flap; vocal fold augmentation; aerodynamic; acoustic

Vocal fold scar disrupts normal vocal fold structure with consequent adverse effects on voice production, including decreased vibratory amplitude, vocal fatigue, impaired vocal control, and breathiness.^{1–3} Scar has multiple etiologies, including vocal abuse, inflammation, radiation, and iatrogenic injury.⁴ Scar remains a clinical challenge,^{2,3,5} with inconsistent and suboptimal outcomes.^{2,5,6}

Fat augmentation of the lamina propria has shown promise in animals and humans. Fat has several advantages, including availability, low cost, autologous nature, and viscoelastic properties similar to native lamina propria.⁷ Jiang et al⁸ and Woo et al⁹ reported improved laryngeal function after fat implantation in canines with scar. Benefits in humans include improvements in perceptual evaluation and glottic closure.¹⁰ Although these results are promising, fat implantation has limitations. Most notably, resorption can occur¹¹ and may limit durability. In addition, traditional fat grafts require a secondary harvest site.

Recently proposed vascularized composite thyroid ala perichondrium flaps (CTAPs) address both these limitations.¹² Thyroid cartilage outer perichondrium with preepiglottic fat has its own blood supply and is harvested at the same site as the primary operation. Furthermore, the graft can be implanted through a modified minithyrotomy that allows access to both the lamina propria and paraglottic space.¹³ Initial canine experiments demonstrated that CTAP remained viable and did not negatively alter normal vocal fold structure or function¹²; however, this study was performed on normal larynges, and effects on scar have not been evaluated. Although no significant benefits of CTAP over thyroid ala perichondrium (TAP) alone were observed in that initial study, CTAP includes fat and thus has the theoretical advantage of matching the viscosity of native lamina propria.⁷ Added bulk from the preepiglottic fat may also be beneficial if a large-volume defect must be corrected.

In this study, we assessed aerodynamic, acoustic, vibratory, and histologic outcomes in canines undergoing vocal fold stripping and CTAP-based reconstruction compared with

vocal fold stripping alone. We hypothesized that CTAP would restore glottic closure, decrease aerodynamic power necessary for phonation, and improve voice stability. In addition, fat and neovascularization would be present after reconstruction. Last, vocal folds with CTAP would have higher von Willebrand factor (vWF) and smooth muscle actin (SMA), reflecting neoangiogenesis and flap viability.

Materials and Methods

Study Design

This study was approved by the University of Wisconsin Institutional Animal Care and Use Committee. Ten male beagles weighing 9.2 to 11.7 kg and aged 8 to 20 months were included. Sample size was calculated based on previously published acoustic data from a similar study,¹⁴ using the minimum number of animals required to attain 80% power with a 2-sided independent samples *t* test and $\alpha = 0.05$. Dogs received a normal diet, exercise, and social interactions except for 1 to 2 days after surgery, when they received softer, easier to swallow food. All dogs underwent unilateral vocal fold stripping and healed for 1 month, forming a unilateral scar. Five dogs were euthanized (“scar-only group”) and the other 5 underwent vocal fold augmentation via unilateral CTAP implantation (“CTAP group”). These dogs recovered from surgery for 1 month prior to being euthanized (Figure 1). Euthanasia was followed by endoscopy, larynx harvest, excised larynx experimentation, and histologic analysis.

For histology, the vocal fold undergoing CTAP-based reconstruction is the “CTAP group.” The contralateral normal vocal fold in those dogs is the “control group.” Last, the vocal fold undergoing vocal fold stripping alone is the “scar group.”

Vocal Fold Stripping

Dogs were premedicated with buprenorphine (0.02–0.03 mg/kg) and dexmedetomidine (0.005–0.015 mg/kg), induced with intravenous propofol (6.6 mg/kg), intubated, anesthetized with isoflurane, and placed in dorsal recumbency.

A rigid endoscope (SDC Pro 2; Stryker Medical, Portage, Michigan) with digital camera (988; Stryker Medical) and light source (Q5000; Stryker Medical) documented laryngeal anatomy. Intramuscular enrofloxacin (7.5–10 mg/kg) and intravenous dexamethasone (0.07–0.15 mg/kg) were administered preoperatively. Micro-forceps were used to remove the epithelium and lamina propria down to the thyroarytenoid muscle along the length of 1 vocal fold. Inhalational anesthesia stopped once removal of the epithelium and lamina propria was confirmed endoscopically.

Vocal Fold Repair

Animals were premedicated, anesthetized, and positioned as with the scarring procedure; prepped for sterile surgery; and given lactated Ringer’s solution (10 mL/kg/h).

Unilateral scar was confirmed endoscopically. Each animal received enrofloxacin intramuscularly (7.5–10 mg/kg) and dexamethasone intravenously (0.07–0.15 mg/kg). A 6-cm fullthickness transverse incision was made using a No. 15 blade. Blunt dissection

exposed the sternohyoid muscles, which were bluntly separated and retracted laterally. Dissection continued to expose the thyroid ala perichondrium.

An inferiorly based CTAP was harvested using a Freer elevator and fine bipolar cautery, as described previously.¹³ The incision extended beyond the superior border of the thyroid cartilage into the anteromedial preepiglottic space (Figure 2). The flap was retracted and a 27-gauge needle inserted into the lamina propria. Once needle placement in the vocal fold was confirmed endoscopically, the needle was removed and a 5 × 6-mm minithyrotomy was created using a No. 15 blade.¹³ Dilators introduced over a blunt stylet progressively enlarged a pocket superficial to the thyroarytenoid muscle.

A custom titanium helix was attached to the CTAP with 5–0 polydioxanone suture to anchor it.¹³ The screw, flap, and stylet used to deploy the helix were all inserted into the pocket. The helix was deployed into the anterior surface of the arytenoid, and the stylet and driver were removed. Proper flap placement was confirmed and effect on glottal edge was evaluated endoscopically before closure.

A 0.5-inch Penrose drain was inserted through a separate stab incision and secured with 2–0 nylon suture to prevent subcutaneous emphysema. Sternohyoid muscles were apposed with 4–0 Vicryl. The wound was closed using subcutaneous running 3–0 Vicryl and cutaneous simple interrupted 2–0 Dermalon layers. Animals were given buprenorphine (0.005–0.02 mg/kg) before stopping inhalational anesthesia. Enrofloxacin (5–20 mg/kg) was administered orally for 7 days. Carprofen (4 mg/kg) was given orally for 2 to 3 days. The drain was left in place up to 96 hours. Dogs were fed canned dog food and allowed only solitary exercise for 2 to 3 days after surgery.

Euthanasia

Dogs were premedicated with buprenorphine (0.02–0.03 mg/kg) and dexmedetomidine (0.005–0.015 mg/kg) intramuscularly or intravenously and then given intravenous Euthasol (pentobarbital sodium and phenytoin sodium; 1 mL/10 lbs). Postmortem endoscopy was followed by larynx removal.

Excised Larynx Experiment

Larynges were placed in saline until excised larynx experimentation later that day. The epiglottis, corniculate and cuneiform cartilages, and false vocal folds were removed to expose the true vocal folds. Larynges were mounted on the apparatus described previously.¹⁵ A hose clamp stabilized the trachea to a tube connected to a constant-pressure source. Arytenoids were placed in a phonatory position using micrometers. Vocal fold elongation was controlled using a suture inferior to the thyroid notch and maintained at 115% of resting length.

Air was passed through 2 humidifiers (Concha Therm III; Fisher & Paykel Healthcare, Laguna Hills, California) and measured using an airflow meter (FMA-1601A; Omega Engineering, Stamford, Connecticut). Pressure was measured immediately below the larynx (Series 1110; Hans Rudolph, Kansas City, Missouri).

Acoustic data were collected with a microphone 10 cm from the glottis (RTA-M; dbx Professional Products, Sandy, Utah) and amplified by a preamplifier (302; Symetrix, Mountlake Terrace, Washington). A data acquisition board (AT-MIO-16; National Instruments, Austin, Texas) and customized software (LabVIEW; National Instruments) recorded aerodynamic data at 100 Hz and acoustic data at 40,000 Hz. High-speed video of vocal fold vibration was recorded at 4000 frames/s (Fastcam-ultima APX; Photron, San Diego, California). Experiments were performed in a triple-walled sound-attenuated room.

Trials were 5 seconds of phonation followed by 5 seconds of rest. Five trials were performed per larynx. During each trial, airflow was increased gradually until phonation onset. Saline was applied to prevent dehydration.

Data Analysis

Airflow and pressure at phonation onset were recorded as phonation threshold flow (PTF) and phonation threshold pressure (PTP), respectively. Phonation threshold power (PTW) was calculated as the product of PTF and PTP.

Acoustic signals were trimmed to three 1-second segments per trial (GoldWave, St John's, Canada), and these segments were analyzed (TF32, Madison, Wisconsin) to obtain signal-to-noise ratio (SNR), percent jitter, and percent shimmer.

Videos were analyzed to determine open quotient and qualitatively evaluate vibratory amplitude. The PTP for the scar-only group was too high to allow for direct quantitative comparisons of amplitude, since the pressure required to initiate oscillation in the scar-only group would cause chaotic vibration in the CTAP group, and subglottal pressure affects vibratory amplitude.¹⁶

Statistical Analysis

Two-tailed independent samples *t* tests with $\alpha = 0.05$ determined if parameters were different between CTAP and scaronly groups. If data did not meet assumptions for parametric testing, a Wilcoxon-Mann-Whitney rank sum test was used.

Histologic Analysis

After the excised larynx experiment, larynges were placed in 10% neutral buffered formalin. Approximately 24 hours later, larynges were dissected to isolate the true vocal folds, which were sectioned coronally into thirds (anterior, middle, and posterior) and placed into cassettes for histologic processing and paraffin embedding. Serial 5-micron sections were stained with hematoxylin and eosin (H&E) as well as Alcian blue (American Master*Tech Scientific, Lodi, California) with and without hyaluronidase digestion, Masson's trichrome, and elastic Verhoeff Van Gieson (EVG) (Newcomer Supply, Middleton, Wisconsin).

Hyaluronidase digestion.—Two sets of slides were deparaffinized and hydrated. The first set was incubated at 37°C for 1 hour in a buffer solution with pH 6.0 (94 mL of 0.1 M potassium phosphate and 6 mL of 0.1 M sodium phosphate). The second was incubated in hyaluronidase digestion (0.025 g testicular hyaluronidase; Sigma-Aldrich, St Louis,

Missouri) and 50 mL buffer solution with pH 6.0. Slides were washed and stained with Alcian blue.

Immunolabeling.—Serial 5-micron tissue sections were created, deparaffinized, and rehydrated. Antigen retrieval was performed using heat-induced epitope retrieval with 10 mM sodium citrate, pH 6.0, in a Decloaking Chamber (Biocare Medical, Walnut Creek, California). Nonspecific staining was blocked using 10% bovine serum albumin followed by 5% nonfat milk. Sections were incubated with primary antibodies pancytokeratin clone AE1/AE3 (1:40 dilution, mouse monoclonal; Dako (Carpinteria, California)) and SMA (1:50,000 dilution, mouse monoclonal; Sigma-Aldrich) incubated 1 hour at room temperature or vWF (1:700 dilution, rabbit polyclonal; Dako) incubated 50 minutes at room temperature. Sections were washed with Tris-Buffered Saline/Tween and endogenous peroxidase with 3% H₂O₂. Antibodies were detected using ImmPRESS anti-rabbit or anti-mouse IgG biotin-free horseradish peroxidase (HRP) polymer detection systems (Vector Labs, Burlingame, California) for 30 minutes and visualized with ImmPACT DAB substrate (Vector Labs). Tissue sections were counterstained with hematoxylin, dehydrated, cleared, and cover-slipped.

Qualitative analysis.—Qualitative analysis was performed on the anterior sections since this was most likely to exhibit change after CTAP implantation. We evaluated whether the following were present or absent (binary rating system): fat, cartilage, architectural disruption, neovascularization, and intact epithelium. We hypothesized that the CTAP group would be more likely to exhibit fat, architectural disruption, and neovascularization compared with control and scar groups. Furthermore, only perichondrium and not cartilage would be found in the CTAP group. Last, all vocal folds would have intact epithelium; this is important for the CTAP group to exclude the possibility of implant extrusion. Lamina propria cellularity was evaluated and graded on a 3-point scale with 1+ = nuclei occupy less than one-third, 2+ = nuclei occupy one-third to two-thirds, and 3+ = nuclei occupy more than two-thirds of the region. All analyses were performed by a pathologist (D.T.Y.).

Quantitative analysis.—The following stains were performed: EVG for elastin, trichrome for collagen, Alcian blue for glycosaminoglycans, vWF to identify blood vessels¹⁷ and serve as a marker of angiogenesis, SMA to identify vascular smooth muscle cells and myofibroblasts^{18,19} and serve as a marker for blood supply and wound healing, and Alcian blue with hyaluronidase for hyaluronic acid. Slides were scanned at 5× magnification (PathScan Enabler IV; Meyer Instruments, Houston, Texas) and uploaded into Photoshop CS5 (Adobe Systems, San Jose, California) to isolate the lamina propria. ImageJ 1.42q (National Institutes of Health, Bethesda, Maryland) segmented images into 4 layers using K-means clustering to calculate staining density, expressed as a percentage of total pixels. Stain density was calculated at the anterior, middle, and posterior thirds of the vocal fold.

Statistical analysis.—Qualitative variables were compared between the CTAP and control groups and the CTAP and scar groups using 2-tailed Fisher exact tests with $\alpha = 0.05$.

Differences in stain density were evaluated between the CTAP and control groups and the CTAP and scar groups. Comparisons between CTAP and control were performed using paired *t* tests. If assumptions for parametric testing were not met, Wilcoxon-Mann-Whitney signed rank tests were performed. Comparisons between CTAP and scar were performed using 2-tailed independent samples *t* tests with $\alpha = 0.05$. If assumptions for parametric testing were not met, Wilcoxon-Mann-Whitney rank sum tests were performed.

Results

Safety

There were no significant complications or deaths. In 1 dog in the CTAP group, the drain was kept for 48 hours for subcutaneous emphysema.

Excised Larynx

Summary data are presented in Table 1. The PTP (Figure 3) and PTW were lower for larynges with CTAP. Percent jitter and percent shimmer were lower with CTAP (Figure 4), while SNR was higher. Open quotient was higher in the scar-only group. Kymograms revealed decreased amplitude of the scarred vocal fold and increased vibratory amplitude in the larynges undergoing CTAP-based reconstruction (Figure 5).

Histology

Summary data are presented in Tables 2 and 3. Architectural disruption and neovascularization were seen more in the CTAP group (Figure 6). Fat and cartilage were more common in the CTAP group, but these differences were not statistically significant.

Smooth muscle actin was higher in the CTAP group vs the control group at the anterior vocal fold (Figure 7). von Willebrand factor was higher in the CTAP group compared with control, but this was not statistically significant (Figure 7). Smooth muscle actin was higher in the CTAP group vs the scar group at the anterior third. von Willebrand factor was lower in the CTAP vs the scar group.

Discussion

This is a follow-up to the initial canine study evaluating CTAP-based vocal fold reconstruction.¹² Importantly, this study evaluated the effect on scar. Safety was also further evaluated. Subcutaneous emphysema prompting prolonged drain placement occurred in 1 dog, but no other adverse events occurred, and there were no mortalities or dysphagia. This provides additional evidence for safety.

Excised larynx experimentation demonstrated the benefits of CTAP on scar. Decreases in PTP and PTW reflect increased efficiency of voice production.²⁰ Average PTF decreased, but variability was relatively large, and this difference was not statistically significant. Notably, changes in vocal fold stiffness may have been greater than changes in glottic gap. While PTF is more sensitive to glottic gap,²¹ healing following scarring combined with preserved innervation modeled by symmetric arytenoid adduction led to relatively minimal glottic incompetence. Scarring, though, likely produced significant changes in stiffness and

thus PTP.^{22,23} Decreases in perturbation parameters indicate increased phonatory stability with CTAP. Increased SNR is likely due to both improved vibratory function and decreased air leak.

Changes in vocal fold stiffness and improved glottic closure were further demonstrated by improved vibration (Figure 5) and decreased open quotient. This suggests that changes in aerodynamic and acoustic parameters may be due not only to improved glottic closure (a mechanical medialization effect) but also increased vocal fold pliability (restoration of vibratory function).

Several histologic findings merit consideration. Fat was observed in 3 larynges in the CTAP group. Although one may attribute absence of fat as resorption or lack of flap viability, there were no obvious differences in the acoustic or aerodynamic parameters in these 2 larynges compared with the other 3. Thus, multiple or alternative mechanisms may be involved. Adipose tissue-derived stem cells have been isolated from both humans²⁴ and canines²⁵ and could be causing local changes in the lamina propria, thus creating a lamina propria favorable for vocal fold vibration despite the absence of fat. Of note, there was increased lamina propria cellularity compared with the contralateral vocal fold.

Three vocal folds in the CTAP group showed cartilage. This could be displaced thyroid cartilage or the result of perichondrium-induced chondrogenesis.^{26,27} This was not observed in the initial study.¹² Fat may protect against chondrogenesis, but the presence of cartilage did not correlate with absence of fat, nor was it associated with adverse phonatory outcome. Only vocal folds with the CTAP showed architectural disruption or neovascularization, occurring as a result of local response to the flap and process of repair. Quantitative analysis revealed significantly increased SMA and discernibly increased vWF in the CTAP group, consistent with flap viability and neoangiogenesis. Otherwise similar composition of the CTAP group vs the control group demonstrates that flap insertion does not adversely affect the native extracellular matrix.

This study has several limitations. A modest sample size was included, and there was no long-term follow-up. While a control was included where only vocal fold stripping was performed, there was no sham group including a scar and manipulation of Reinke's space without CTAP implantation. This would allow for more detailed histologic assessment of the effects of CTAP-based reconstruction. Stripping the vocal fold down to the thyroarytenoid muscle may not be an ideal model of vocal fold scar; however, it standardized injury depth. Flap placement, though, was not as precise as in human patients, where the flap can be placed deep to the epithelium within the superficial lamina propria. Dogs in the scar-only and CTAP groups were also sacrificed at different time points after vocal fold stripping. As remodeling occurs up to or beyond 6 months after scarring,²⁸ it is possible that improved outcomes observed in the CTAP group were at least partially related to ongoing remodeling and not solely CTAP-based repair. Last, we did not assess local biological effects. Following implantation of the vascularized flap, local paracrine effects may occur that cause scar remodeling.

Despite the limitations, this study shows that CTAP can treat scar effectively and safely, producing superior aerodynamic, acoustic, and vibratory outcomes compared with the scar-only group without causing any significant adverse effects. While potentially valuable treatments for scar are being developed,^{29–31} methods for CTAP are already available, and preliminary human outcomes are promising.³² Furthermore, flap materials can be harvested intraoperatively at no additional cost, and safety issues related to stem cells such as potential malignant transformation are avoided. Additional investigations on biologic effects of flap placement and long-term effects in humans are warranted.

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Figure 1.

Study design, including time points of procedures and euthanasia for the scar-only and composite thyroid ala perichondrium flap (CTAP) groups.

Figure 2. (Left) Image demonstrating procedure to harvest the inferiorly based composite thyroid ala perichondrium flap (CTAP) and (right) schematic demonstrating placement in the vocal fold through a minithyrotomy. The black arrow points to the CTAP.

Figure 3.

Phonation threshold pressure was significantly lower in the composite thyroid ala perichondrium flap (CTAP) group compared with the scar-only group.

Figure 4. Percent jitter and shimmer were significantly lower in the composite thyroid ala perichondrium flap (CTAP) group compared with the scar-only group.

Figure 5.
Sample kymograms from the composite thyroid ala perichondrium flap group (top) and the scar-only group (bottom).

Figure 6.
Hematoxylin and eosin stain of coronal section of anterior third of vocal fold showing position of composite thyroid ala perichondrium flap within the vocal fold (arrow).

Figure 7.

Coronal sections of anterior vocal fold showing staining for smooth muscle actin (SMA) and von Willebrand factor (vWF). The control group represents the contralateral normal vocal fold for larynges in the composite thyroid ala perichondrium flap (CTAP) group. The CTAP and control images for both SMA and vWF are from 1 dog in the CTAP group. Scar images for both SMA and vWF are from 1 dog in the scar-only group.

Table 1.

Summary Results from Excised Larynx Experiment.

Parameter	Scar Only (n = 5), Mean ± SD	CTAP (n = 5), Mean ± SD	P Value
PTP, cmH ₂ O	21.34 ± 9.21	5.01 ± 2.20	.005
PTF, L/min	50.4 ± 37.5	23.4 ± 9.1	.156
PTW, cmH ₂ O*/L/min	1221.5 ± 1365.0	133.3 ± 110.9	.032
F0, Hz	285 ± 74	200 ± 74	.105
SNR	5.57 ± 3.85	15.74 ± 4.93	.007
% Jitter	0.08 ± 0.04	0.02 ± 0.01	.010
% Shimmer	0.35 ± 0.19	0.09 ± 0.04	.008
Open quotient	0.92 ± 0.12	0.64 ± 0.14	.007

Abbreviations: CTAP, composite thyroid ala perichondrium flap; PTF, phonation threshold pressure; PTP, phonation threshold flow; PTW, phonation threshold power; SNR, signal-to-noise ratio.

Table 2.Summary Results from Qualitative Histologic Analysis.^a

Parameter	CTAP (n = 5)	Control (n = 5)	P Value	Scar (n = 5)	P Value
Fat	3/5	0/5	.1667	0/5	.1667
Cartilage	3/5	0/5	.1667	0/5	.1667
Architectural disruption	5/5	0/5	.0079	0/5	.0079
Neovascularization	5/5	0/5	.0079	0/5	.0079
Intact epithelium	5/5	5/5	1.000	5/5	1.000
Lamina propria cellularity	1+ = 2	1+ = 4	.5238	1+ = 2	1.000
	2+ = 3	2+ = 1		2+ = 3	
	3+ = 0	3+ = 0		3+ = 0	

^aValues for fat, cartilage, architectural disruption, neovascularization, and intact epithelium are presented as a fraction. *P* values represent result of comparison to the composite thyroid ala perichondrium flap (CTAP) group.

Table 3.Summary Results from Quantitative Histologic Analysis.^a

Parameter	CTAP (n = 5)	Control (n = 5)	P Value	Scar (n = 5)	P Value
Anterior					
Elastin	26.1 ± 6.6	24.4 ± 6.4	.268	25.9 ± 8.2	.967
Collagen	37.3 ± 12.0	39.7 ± 5.2	.663	45.9 ± 3.2	.159
Glycosaminoglycans	3.1 ± 10.4	32.5 ± 13.0	.878	31.0 ± 11.5	.955
von Willebrand factor	10.9 ± 1.6	6.9 ± 3.5	.110	9.0 ± 5.9	.497
Smooth muscle actin	8.5 ± 2.5	5.7 ± 1.2	.038	5.1 ± 1.0	.022
Hyaluronic acid	25.6 ± 11.5	20.2 ± 4.3	.466	22.2 ± 5.4	.590
Middle					
Elastin	28.0 ± 8.2	29.6 ± 5.9	.641	27.2 ± 8.4	.096
Collagen	42.2 ± 6.1	41.7 ± 2.9	.902	46.5 ± 3.6	.164
Glycosaminoglycans	18.0 ± 7.2	28.3 ± 12.0	.313	32.2 ± 9.3	.416
von Willebrand factor	12.3 ± 6.6	8.3 ± 2.0	.313	12.7 ± 6.2	.916
Smooth muscle actin	6.5 ± 5.0	4.9 ± 1.2	.554	7.3 ± 2.8	.052
Hyaluronic acid	18.0 ± 12.2	23.1 ± 9.2	.625	25.1 ± 5.5	.367
Posterior					
Elastin	29.7 ± 11.7	24.4 ± 6.4	.511	29.5 ± 9.2	.977
Collagen	41.2 ± 8.5	41.7 ± 6.3	.938	46.5 ± 4.7	.251
Glycosaminoglycans	22.0 ± 7.0	23.9 ± 7.8	.741	29.5 ± 8.3	.159
von Willebrand factor	14.0 ± 4.7	9.5 ± 5.3	.274	10.7 ± 4.9	.298
Smooth muscle actin	8.4 ± 4.9	9.8 ± 12.1	.767	5.4 ± 1.6	.222
Hyaluronic acid	27.8 ± 11.9	12.0 ± 11.6	.002	24.0 ± 7.8	.572

^aData are percentages and are presented as mean ± standard deviation. P values represent results of comparison to the composite thyroid ala perichondrium flap (CTAP) group.