

## FAT IMPLANTATION INTO REINKE'S SPACE: A HISTOLOGIC AND STROBOSCOPIC STUDY IN THE CANINE

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The scarred vocal fold is a cause of persistent dysphonia after laryngeal trauma, microsurgery, and tumor resection. The loss of Reinke's space with scar and stiffness is the primary cause. This study explores the technical aspects of endoscopic implantation of fat as an autologous implant for restoring the bulk and pliability of the vocal folds. Fat from the buccal area was harvested and prepared in 6 dogs. A mucosal flap submucosal pocket was prepared by means of microlaryngoscopy instrumentation. The fat implant was placed into the submucosal pocket. The mucosal flap was sutured endoscopically. Six weeks later, the larynx was harvested and mounted, and vocal fold oscillation was studied while driven by a humidified flow source. The vibratory patterns were studied by stroboscopy. Histologic sections of the vocal folds were made in the coronal plane. All 6 specimens had histologic evidence of viable implanted fat and/or fibrous tissue at the implant site. The site of implantation was in the superior aspect of the vocal fold, but contributed to mass in vocal fold bulk. This increase in bulk histologically corresponded to stroboscopic evidence of increased mass. On stroboscopy, the implant side continued to demonstrate good vibratory function. The study shows that fat implantation can be carried out as an endoscopic procedure. Fat implantation may be useful as a surgical procedure for restoration of Reinke's space. It may be applicable in patients with scars, sulcus vocalis, or vocal fold atrophy.

KEY WORDS — dysphonia, fat implantation, Reinke's space, scarred vocal fold, stroboscopy.

### INTRODUCTION

The vibratory function of pliable vocal folds is important in the production of a clear sound. Structural abnormalities remaining after microlaryngeal surgery, trauma, and tumor resection often contribute to residual dysphonia. Scarring and fibrosis of the vocal folds are the principal causes of postoperative dysphonia.<sup>1</sup> The body-cover theory of vocal cord vibration has been demonstrated in histologic detail by Hirano and Kakita.<sup>2</sup> Histologic examination of the human vocal folds has demonstrated the unique layered structure of the vocal fold cover. When scar and vocal fold stiffness is the cause of persistent dysphonia, the resultant vocal fold oscillation demonstrates loss of mucosal amplitude and wave secondary to disruption or loss of Reinke's space. The operative options in patients with scarred vocal folds are limited. The use of collagen injection for glottal incompetencies has been advocated by Ford.<sup>3</sup> Recently, there has been interest in the use of fat as an injectable material in the augmentation of the vocal folds. This has been used in patients with glottal incompetencies due to vocal fold paralysis, as well as other causes.<sup>4,5</sup> Fat injection into the vocalis muscle, as augmentation

material, does not answer the quest for a more pliable material to substitute for the superficial layer of the lamina propria in the vocal fold. Injection of fat into a scar is often difficult to perform. Some data suggest that the resorption rate of fat injected into the vocal fold is high.<sup>6</sup> Although the success of fat as a permanent injectable augmentation alternative to Teflon has not been fully demonstrated, fat has many advantages. The viscoelastic properties of fat are close to those of the superficial layer of the lamina propria. Fat is softer than Teflon, collagen, or Gelfoam. It is readily available as an autologous tissue. Experience with fat over time has been well researched.<sup>7</sup> The use of fat as an implant rather than in the injected form has theoretical and practical advantages. Fat implantation is the placement of fat into a surgically created pocket. By dissection of a pocket to receive the fat implant, the fat may be placed into the defect in a precisely measured amount.

Fat implantation into the vocal folds has been studied in animal models by previous authors. In 1989, Wexler et al<sup>8</sup> studied the fate of fat in a canine scarring model. They showed conclusively that the scarred canine larynx may be rehabilitated by thy-

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Presented at the meeting of the American Broncho-Esophagological Association, Orlando, Florida, May 6-7, 1996.

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rotomy, submucosal fat implantation into Reinke's layer, and suture closure of the wound. The fat implant survived and restored vocal fold vibration. Histologic examination showed viable fat in Reinke's space. In another study, the same group showed the vibratory capabilities of the fat-implanted vocal fold to be superior to those of the scarred vocal fold model.<sup>9</sup> Their surgical approach, however, requires open thyrotomy and tracheostomy. The possibility of improved vocal function with fat implantation propelled the search for a better surgical alternative for performing fat implantation than open thyrotomy and tracheostomy. One possibility is endoscopic fat implantation. With improved instrumentation and endoscopic suturing techniques, endoscopic fat implantation may be possible. It would retain the advantages of fat implantation without the need for tracheostomy or laryngofissure. The purpose of this study was to explore the limits of an endoscopic approach to fat implantation in the vocal fold in a canine model. In addition, the fate of the fat implant and the effect of the fat implant on the vibratory function were tested after surgery by stroboscopy and histologic examination.

#### MATERIAL AND METHODS

**Animal Surgery.** Six adult conditioned laboratory dogs were used for this study. The body weights ranged from 14 to 20 kg. All animal surgery and care procedures were carried out in accordance with institutional policies on care of animals. In each experiment, the left vocal fold served as the control side, and the right side served as the experimental side. The animals were anesthetized with Nembutal and maintained under a state of anesthesia with spontaneous ventilation by periodic intravenous maintenance of Nembutal. A buccal fat pad measuring 3 × 10 × 5 mm was harvested through a buccal incision. The fat donor site was closed with a 3-0 chromic suture. The fat pad was preserved in saline gauze for later implantation. The adult Jako laryngoscope (Pilling, Ft Washington, Pa) was placed into position, and suspension microlaryngoscopy was carried out. With standard microlaryngeal instrumentation and operative magnification, the endoscopic procedure to create a pocket for fat implantation commenced. A submucosal pocket was created by making a 1-cm incision on the superior surface of the right true vocal fold. The incision was expanded by blunt and sharp dissection to create a submucosal pocket along the medial edge of the vocal fold. With a cup forceps, the mucosal flap was retracted medially. The microdissector and microscissors were used to create a pocket medially and inferiorly along the contour of the vocal fold. Care was taken to stay in the submucosal plane and avoid

vocal ligament injury. Once the submucosal pocket had been created, adrenaline-soaked (1:1,000) cotton pads were placed into the pocket to achieve hemostasis. The autogenous fat was shaped into a 6 × 4 × 2-mm implant and fashioned to fit into the submucosal pocket. The fat was placed into the submucosal pocket with cup forceps. The mucosal flap was placed into position. To prevent fat dehiscence from the incision and to obtain complete edge-to-edge approximation of the mucosal edge, one 4-0 chromic suture was placed endoscopically to suture the edges of the vocal folds.<sup>10</sup>

**Endoscopic Evaluation and Excised Larynx Preparation.** The animals were examined at 6 weeks after endoscopic surgery under general anesthesia. The larynx was harvested after euthanasia. Excess soft tissue was trimmed from the larynx, and the larynx was mounted in a Plexiglas larynx holder. The vocal folds were approximated by a suture across the vocal process. The excised larynx phonation model is fashioned after Baer.<sup>11</sup> The laryngeal holder has pressure taps for subglottic pressure, flow metering, and adjustment of flow. A heated, humidified airflow source (Bird heater humidifier controller) was used to drive the vocal folds. The air temperature was set at 36°C at 100% humidification. The subglottic pressure necessary to initiate the onset of steady vocal fold oscillation was recorded. During sustained vocal fold oscillation, a stroboscopic light source and video camera were used to record vocal fold vibratory behavior (Power Instrumentation, Skokie, Ill). Stroboscopic endoscopy examination was used to compare the vibratory behavior of the control side with that of the experimental side as to vibratory capability, amplitude, mass, and mucosal wave.

**Histology.** After the studies of vibratory behavior were completed, the larynx was fixed in 10% formalin, dehydrated, and processed for histology. The larynx was mounted and cut in the coronal plane. Sections were made in 10- $\mu$ m increments. Histologic sections were obtained at the same level for both the control (left) and experimental (right) vocal folds through the membranous vocal folds. The slides were stained by hematoxylin and eosin. The laryngeal sections were examined. Comparison was made between the experimental side and the control side for 1) evidence of the fat implant, 2) the existence of inflammation, and 3) the thickness of the submucosal layer. Qualitative histologic changes in Reinke's space were recorded.

#### RESULTS

**Operative and Postoperative Findings.** All the animals tolerated the procedure without difficulty. The operation lasted from 30 to 60 minutes. The

SUMMARY OF SUBGLOTTIC PRESSURE AND FLOW RATE AT THRESHOLD OF PERIODIC VOCAL FOLD OSCILLATION

Specimen	Subglottic Pressure (cm H <sub>2</sub> O)	Flow Rate (mL/s)	Vibration of Vocal Fold
1	8	240	Yes
2	12	300	Yes
3	10	310	Yes
4	10	220	Yes
5	9	250	Yes
6	14	350	Partial decrease

wide-bore operating laryngoscope gave good binocular vision for creating the mucosal flap. Although the superior aspect of the submucosal pocket was easy to create, operating on the undersurface of the vocal fold was more arduous and required blunt probes and dissectors. A submucosal pocket was, however, made in each experiment without difficulty. The fat implant could be placed easily into the submucosal pocket to add bulk to the vocal fold. After the fat was implanted, there was a visible bulge of the vocal folds medially. A suture was necessary to keep the fat from herniating through the incision. The endoscopically placed sutures were positioned on the superior surface of the vocal folds. Bleeding was minimal. The dissection in the submucosal plane was able to be used to create submucosal pockets of various sizes. In all the specimens, Reinke's space was easily found. Endoscopic fat implantation could be performed with existing microinstruments.

The postoperative courses were uncomplicated. None of the animals had difficulty with the airway or deglutition. All the animals were noted to have voice after the procedure. Because of the technical difficulty of recording voice in the animal shelter, only qualitative impressions of the voice could be taken.

There was minimal voice change compared to the preoperative state after the first week.

**Videostroboscopy Findings.** Videostroboscopy recordings were generated from the excised canine preparation. Stable vibration was seen in all the specimens. The subglottic pressure threshold necessary for stable oscillation ranged from 8 to 14 cm H<sub>2</sub>O (see Table). Prior experience (unpublished data) using the same experimental setup in normal canine larynges showed thresholds of oscillation at 5 to 10 cm H<sub>2</sub>O. The induced vocal fold vibration showed a vibratory pattern that was remarkably similar to that of normal vocal folds. The implanted side showed good mucosal amplitude and an intact mucosal wave. The implanted side appeared to be slightly thicker than the control side, giving the impression of greater mass. All the specimens were capable of stable periodic oscillations. The operated sides had the appearance of greater mass, but were equal in amplitude, mucosal wave, and vibratory capability to the control side. These differences were occasionally noted between the two sides. The operated side, being greater in mass, appeared to have a delay in the phase of vocal fold vibration compared to the opposite side. In all the preparations, the vocal fold amplitude was rated as the same as or greater than that of the nonoperated side. However, the degree to which apparent mass was added to the vocal folds showed considerable variation, which could not be accounted for by the size of the surgical implant placed into the pocket. Figure 1 is a series of videostroboscopy images for specimen 2. The glottal cycle shows a good vibratory amplitude, a symmetric phase of vibration, and an intact mucosal wave on both the control and implanted sides. The implanted side (right) has a slightly greater mass than the control side. The increased mass of the vocal fold may account for the increased subglottic pressure thresh-

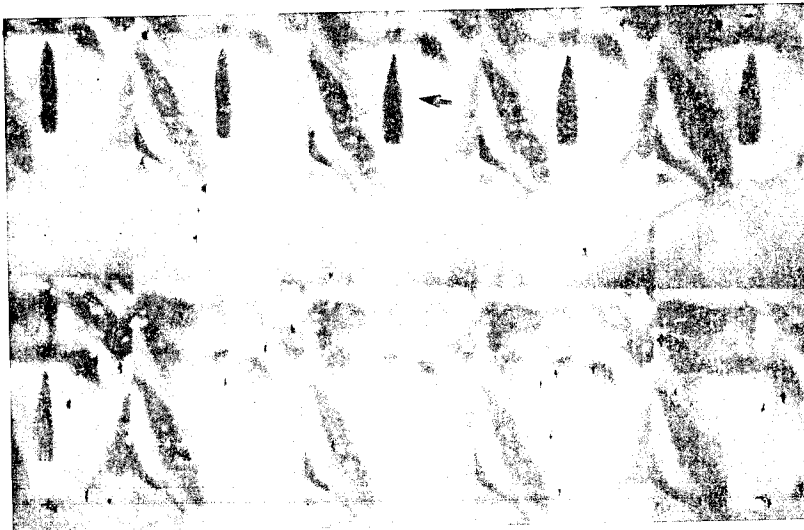


Fig 1. (Specimen 2) Stroboscopic sequences. Flow rate was 300 mL/s, subglottic pressure was 12 cm H<sub>2</sub>O, and frequency was 82 Hz. Implanted side (arrow) has vibratory amplitude and wave, with some increase in mass.

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