COMPARISON OF MICROFLAP HEALING OUTCOMES WITH TRADITIONAL AND MICROSUTURING TECHNIQUES: INITIAL RESULTS IN A CANINE MODEL

DANIEL J. FLEMING, MD

ANDREWS AIR FORCE BASE, MARYLAND

STAN MCGUFF, DDS

SAN ANTONIO, TEXAS

C. BLAKE SIMPSON, MD

SAN ANTONIO, TEXAS

A prospective, blinded study was designed to determine whether placement of a microsuture in epithelial defects created on canine vocal folds results in histologically demonstrable improved healing. Epithelial defects similar to those created during microflap removal were made by means of traditional microsurgical techniques on both vocal folds of 4 adult dogs. One vocal fold defect on each dog was then closed with a single microsuture placed through the laryngoscope. The larynges were harvested approximately 6 weeks later, and blinded histologic quantification of scar formation was performed. Microsutures resulted in less scarring in all but 1 of the larynges studied. Unsutured vocal folds exhibited a 75% larger average scar cross-sectional area. Although the sample size was insufficient to establish statistical significance, the observed difference in scar formation between microsutured and unsutured vocal folds suggests that primary closure with a microsuture in the canine model results in less scarring than when healing occurs by secondary intention.

KEY WORDS — healing, histology, larynx, microsuture, phonosurgery.

INTRODUCTION

Benign laryngeal lesions are important causes of voice disorders and make up a large proportion of the typical laryngologist's practice. These lesions respond in varying degree to behavior modifications, including smoking cessation, adequate hydration, and the elimination of vocal abuse. A role for proton pump inhibitors and antireflux precautions has also been elucidated. Unfortunately, many of these lesions eventually require surgical intervention.

Excellent voice quality is dependent on the generation of a complex mucosal wave during phonation.[1] Vocal fold scarring disrupts this wave by causing a deficit in the superficial layer of the lamina propria with scarring of the vocal ligament (middle and deep layers of the lamina propria) to the overlying mucosa, resulting in suboptimal voice quality.[2] The patient often attempts to compensate for the resulting vocal deficit by changing the shape and position of the larynx with the extrinsic muscles, increasing the infraglottic air pressure, or changing the shape of the supraglottic resonators, resulting in worsened vocal dysfunction.[3] The importance of minimizing vocal fold scarring in order to allow the production of excellent phonation is therefore paramount.

Microsurgical treatment of laryngeal lesions is a relatively new technique. Few studies have evaluated vocal fold healing after microsurgical techniques. The use of microsuturing for the repair of microflap defects has only recently been reported, and its use does not appear to be widespread. This study is designed to show the efficacy of endoscopic microsuturing for the reapproximation of mucosal defects, and to present the technique to a wider population of practicing otolaryngologists.

MATERIALS AND METHODS

Four unrelated, heartworm-free, adult male mongrel dogs weighing between 18 and 27 kg (40 and 60 lb) were held in quarantine for at least 1 week. Animal care was supervised by The University of Texas Health Science Center at San Antonio (UTHSCSA) Laboratory Animal Resources Department follow-

From the Departments of Otolaryngology–Head and Neck Surgery (Fleming, Simpson) and Pathology (McGuff), The University of Texas Health Science Center, San Antonio, Texas. Dr Fleming is currently in the Department of Otolaryngology, Malcolm Grow Medical Center, Andrews Air Force Base, Maryland. Microlaryngeal equipment donated by Pilling Corporation was used to complete the procedures described herein. This study was performed in accordance with the PHS 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 (7 U.S.C. et seq.); the animal use protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Texas Health Science Center at San Antonio.

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CORRESPONDENCE — C. Blake Simpson, MD, Dept of Otolaryngology–Head and Neck Surgery, The University of Texas Health Science Center, 7703 Floyd Curl Dr, San Antonio, TX 78229-3900.

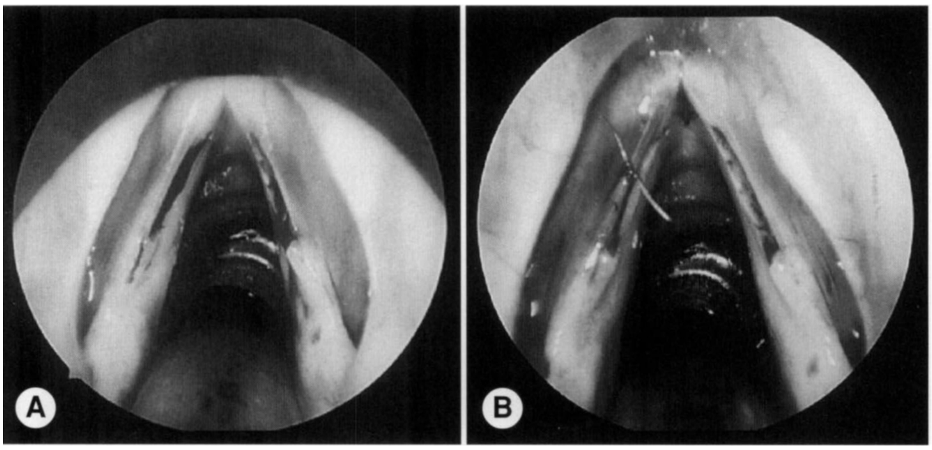

Fig 1. (Dog 2) Microflap epithelial defects. **A)** Symmetric defects consistent with those created by microflap techniques were made on each subject's true vocal folds. **B)** Alternating sides were closed with microsuturing technique.

ing a protocol approved by the UTHSCSA Institutional Animal Care Program.

No food or water was given for 12 hours before surgery. The dogs were premedicated with acepromazine maleate (5 mg subcutaneously) and anesthetized with intravenous sodium pentobarbital to a level of corneal sensation loss (approximately 10 to 20 mg/kg).[4,5] They were maintained at this level with halothane, ventilator-administered through a small-bore, cuffed endotracheal tube placed with its tip resting 5 cm below the vocal folds.[6] Direct laryngoscopy was performed to confirm normal laryngeal anatomy, and the laryngoscope was stabilized with a Lewy suspension apparatus. Photodocumentation was achieved with a Canon camera with a 90-mm lens modified for attachment to a 0° Hopkins telescope, model 8712AA (Karl Storz–Endoscopy America, Culver City, California).[7] The microflap defects were made in a manner consistent with currently used techniques[8] along the leading edge of the vocal fold at a depth to include the epithelium and sparing as much of the underlying lamina propria as possible. It should be noted that canine vocal folds do not have a well-defined vocal ligament; therefore, the flap elevation was kept immediately subepithelial, 1 to 2 mm lateral and medial-inferior to the defect margins. This flap involved surface dimensions of 4 to 7 mm in length and 2 to 3 mm in width (Fig 1). The vocalis muscle was preserved.[2] The anterior 3 to 5 mm of each vocal fold was not violated, as this has consistently been shown to result in anterior glottic scarring.[9]

On alternating sides from dog to dog, the microflap defect was closed with 1 microsuture. A microscopic flap elevator was used to undermine the epithelium 1 to 2 mm medial and lateral to the defect in a manner analogous to undermining skin edges for plastic closure of skin defects. Elevation was performed in the loose connective tissue layer immediately subepithelial with a blunt 30° elevator (Pilling Company, Fort Washington, Pennsylvania). Hemostasis was achieved with 1:10,000 epinephrine-soaked cottonoids and microsuction. A 6-0 double-armed fast-absorbing gut suture on a TG 140-8 needle (Ethicon Inc, Somerville, New Jersey) was loaded in micro–alligator forceps after removal of 1 needle. This suture is commonly used by ophthalmologists and is readily available. The suture knot was tied by a modification of a technique originally described by Woo et al[10] (Fig 2). The two ends of the suture were then cut with a microscissor. The vocal folds were sprayed with a premixed ampule of 4% lidocaine hydrochloride, and the laryngoscope was removed. The dogs were then awakened, extubated, and housed in the Laboratory Animal Resources Department, with arrangements made for adequate food and exercise.

Between postoperative days 39 and 49, each dog was euthanized with sodium pentobarbital after photodocumentation of the vocal folds was performed. A vertical midline incision was made in the neck, and the strap muscles were retracted laterally. The larynx was harvested from superior to the thyroid cartilage to the second tracheal ring. After fixation for several days in formalin, each larynx was split in the anteroposterior dimension. A sagittal section of each hemilarynx, including the section of the vocal fold that had undergone the microflap procedure and the adjacent supraglottic and subglottic structures, was created with a No. 15 blade. This sample was then cut in a coronal plane at the midpoint of the microflap defect. Mucosal biopsy specimens were fixed in hematoxylin and eosin. Masson's trichrome–stained slides were also made to allow a better description of the histologic architecture (Fig 3).[3,11]

A pathologist blinded to the specimen side that underwent microsuturing evaluated the resulting slides. Tissue from an unoperated canine vocal fold was also processed and compared to known normal canine vocal fold samples.[2,7,12-14] The defect sites were compared to prior investigators' findings regarding canine laryngeal wound healing patterns.[7,14-16]

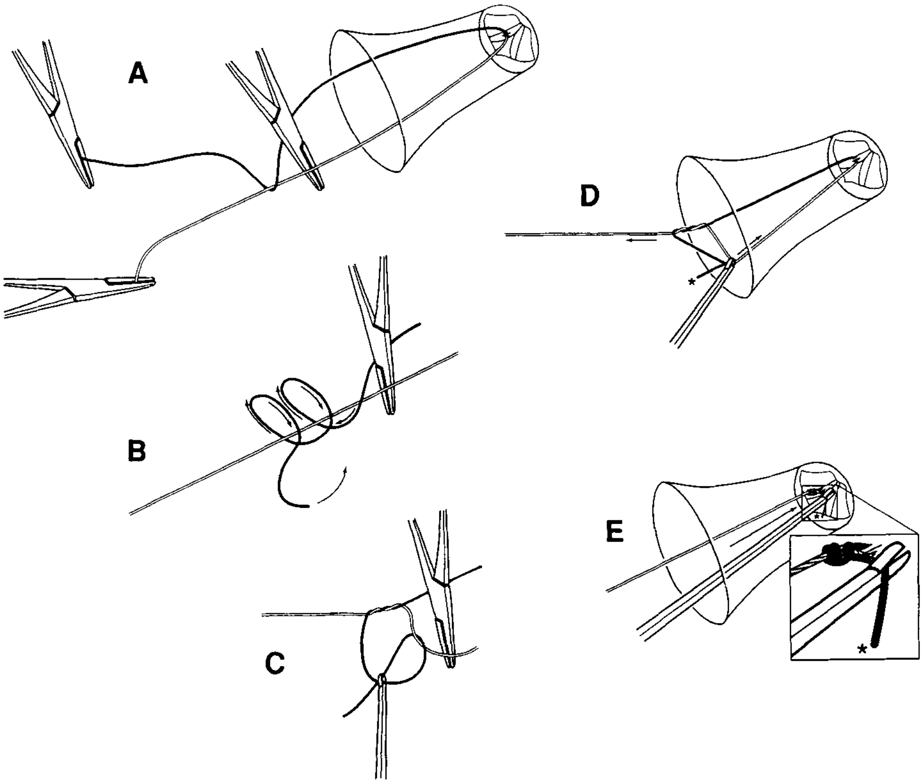

Fig 2. Endoscopic knot technique. **A)** Both suture ends are grasped with mosquito hemostats, and additional hemostat is placed between sutures distally, close to opening of laryngoscope. Black suture represents left side, and white suture is right. Left suture (black) is looped around right suture (white) in clockwise fashion. **B)** Left (black) suture is looped around right suture total of 3 times clockwise. Free end of left (black) suture is advanced toward initial (distal) crossing of two strands of suture. **C)** Free end of left (black) suture is used to create slipknot around open loop, adjacent to distal stationary hemostat, as shown. Micro–alligator forceps are used to grasp left (black) suture at its final "crossing"; this step prevents knot from forming in laryngoscope barrel and subsequently breaking. **D)** "Loose" knot assembly is advanced down laryngoscope by pushing distally with micro–alligator forceps and pulling back on other (white) suture. When level of true vocal folds is reached, one releases knot assembly with micro–alligator forceps, exercising care not to allow free end of left (black) suture (asterisk) to untie. Microscope can be brought back into field at this point. **E)** Free end of left (black) suture is grasped with micro–alligator forceps and advanced distally, while providing countertension on opposite (white) suture. Secure knot will form at level of vocal fold.

Quantitative evaluation of the extent of scarring was achieved for each slide by measuring the area of tissue involved with scar. Scar was identified as immature, plump, spindled to stellate fibroblasts with a loose myxoid collagenous stroma containing few elastic fibers. Each section was evaluated with a Zeiss microscope at 40 times magnification. A micrometer was used to determine the cross-sectional area of the scar in square millimeters. A percent difference in vocal fold healing between the two sides in each dog was calculated, and a paired *t*-test was performed.

RESULTS

The data for each dog are presented in the Table. Unsutured vocal folds exhibited a 75% larger average scar cross-sectional area than did microsutured vocal folds. The sample size was insufficient to establish statistical significance with a paired *t*-test, resulting in a p value of .092 with a 95% confidence interval of –0.027 to 0.207.

DISCUSSION

The myoelastic-aerodynamic theory of phonation was advanced by van den Berg[17] in 1958. This theory postulated that a complex interaction between airflow from the lungs and the mass and tension of the vocal folds produced vibration, resulting in voice production.[5] In 1975, Hirano[18] published his landmark description of the anatomy of the human vocal fold. He demonstrated that the vocal fold consisted of epithelium, the lamina propria, and the thyroarytenoid (or vocalis) muscle.[18] The lamina propria was further subdivided into superficial, middle, and deep layers. Most benign laryngeal lesions have been shown to involve only the superficial layer of the lamina propria (Reinke's space),[19] whereas fibroblasts necessary for the production of scar are primarily confined to the intermediate and deep layers of the lamina propria.[20]

Polyps, nodules, cysts, granulomas, and polypoid corditis (Reinke's edema) make up the majority of

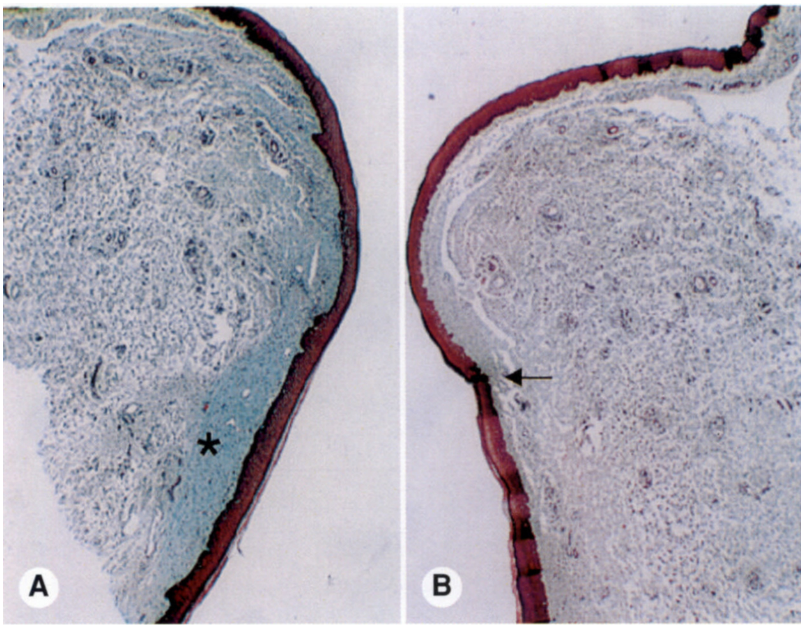

Fig 3. (Dog 3) Comparative regions of mucosal scarring (Masson's trichrome stain, original ×20). Representative cross sections of region of scar for **A)** unsutured vocal fold (asterisk) and **B)** sutured vocal fold (arrow). Note relatively smaller area of scar on sutured vocal fold. Scar tissue was differentiated from native connective tissue and conus elasticus by means of high-magnification evaluation of histomorphology with tri-elastic histochemical stain. Scar tissue was composed of immature, plump, spindled to stellate fibroblasts with loose myxoid collagenous stroma containing few elastic fibers. In contrast, native connective tissue and conus elasticus were composed of mature spindled fibroblasts with more densely collagenous stroma containing numerous elastic fibers. These differentiating features are not apparent in these low-magnification photomicrographs.

benign laryngeal lesions. Through the mid-1970s, vocal fold "stripping" was the most accepted method for treating benign laryngeal lesions.[20] This involved the removal of the vocal fold mucosa sometimes down as deep as the vocal ligament or even the muscular layer, resulting in a fibroblastic response, significant vocal fold scarring, and disruption of the mucosal wave that could result in a poor vocal outcome.[2]

Current surgical techniques emphasize the preservation of the middle and deep layers of the lamina propria (the vocal ligament) by approaching lesions through the superficial layer of the lamina propria via a lateral or medial microflap, as described by Bouchayer and Cornut[21] in France and Courey et al[19] in the United States. The goal of this technique is restoration of normal vocal fold anatomy and histology with minimal disruption of the normal surrounding structures.[19] When lesions are appropriately selected, excellent results in terms of postoperative voice and vocal fold vibratory characteristics can be obtained. Blinded perceptual analysis of paired voice samples obtained before and after medial microflap surgery by Courey et al[8] revealed improvement in 16 of 17 patients. All of the patients discussed in that study showed maintenance or return of the mucosal wave, and 15 of the 17 had improved glottic closure patterns. An earlier study by Courey et al[19] found that 28 of 30 patients who underwent microflap procedures rated themselves as clinically improved.

SCAR CROSS-SECTIONAL AREA

	Dog 1	*Dog 2*	*Dog 3*	*Dog 4*	*Average*
Sutured fold (mm^2)	0.17	0.05	0.12	0.16	0.12
Unsutured fold (mm^2)	0.24	0.17	0.30	0.16	0.22
Absolute difference (mm^2)	0.07	0.12	0.18	0.00	0.09
Percentage difference	41	240	150	0	75

When recurrent or scarred lesions and large benign vocal fold lesions are removed with a microflap technique, significant mucosal defects may result. Current practice is to allow these defects to granulate and heal by epithelial migration.[10] This can result in a nonvibratory segment of the vocal fold that prevents propagation of the mucosal wave, causing aperiodicity of the glottal sound.[10] In 1995, Woo et al[10] suggested that the placement of a microsuture would result in improved wound healing by allowing primary healing and reducing tension on the wound site during the healing process. They also proposed that a microsuture could allow the surgeon to position the scar away from the vocal fold edge, resulting in less trauma to the wound. In a series of 18 patients who underwent this procedure, Woo et al reported excellent results, with all patients reporting a subjective improvement in their voices. Videostroboscopic evaluation of these patients showed consistent improvement in mucosal wave, amplitude and vibratory function. Unfortunately, the study of Woo et al was not controlled, and no comparison can be made to standard microflap techniques.

Because of the relative inaccessibility and indispensable nature of the human larynx, the development of an easy-to-use, functionally representative, and generally accepted model of this organ is important. Leonardo da Vinci was the first to record attempts to examine the source of the human voice using excised cadaver larynges.[5] Others have used this model also, but basic postmortem changes in vocal fold vibratory properties[22] and muscular tension[23] have been extensively documented, resulting in its limited use. Mathematic modeling has allowed description of basic vocal fold vibratory properties, but remains limited in its ability to characterize voice production.[5]

Ferrien was the first to use a canine model, in 1741, when he used canine larynges to show that vocal intensity was influenced by glottal width and air velocity.[5] Since then, this model has become the most accepted experimental model of the human larynx. The canine larynx is similar in size and histology to the human larynx, but exhibits a thicker lamina propria. It vibrates on a 2-mass system, as does the human larynx. A critical difference between human and canine vocal folds is the fact that in dogs, there is no well-defined vocal ligament.[5] Because of this key difference, one must interpret canine data with caution. These differences in the lamina propria can have important implications that limit the clinical utility of canine vocal fold studies. Canine vocal fold vibratory characteristics in vivo are nonetheless very similar to those found in humans, so they have been studied extensively.[5,12] Canine larynges also have been found to exhibit many pathological conditions similar to those found in humans, including squamous cell carcinoma and polyps.[24,25] Finally, canine vocal fold healing has been shown to be similar to human vocal fold healing.[7]

Traditional evaluation of epithelial tissue is accomplished by hematoxylin and eosin staining, but this technique is often deficient in evaluating vocal fold healing because of extensive artifact. Van Gieson and Masson's trichrome stains of connective tissue allow a better description of the histologic architecture.[3,11] Standardized scoring systems have been proposed for characterizing gross and histologic findings for injured vocal folds,[7] and vocal fold scar quantification techniques have been developed by Coleman et al.[26]

The purpose of developing microlaryngeal techniques is to minimize surgically induced trauma to the delicate microstructure of the vocal folds. The microsuturing technique presented here provides a straightforward technique based on sound wound healing principles to reduce vocal fold scarring and improve phonation. The main disadvantage to this technique is the time involved in placing the microsuture. A learning curve is encountered, but it can be partially alleviated by practicing the technique outside the operating room with larger suture before attempting it intraoperatively. With practice, a suture can be passed, tied, and tightened in less than 5 minutes consistently.

In our series, 3 of the 4 canine larynges studied demonstrated less scarring when epithelial defects were closed primarily with a microsuture instead of being left to heal by secondary intention. In the fourth larynx, there was no difference between the sutured and unsutured sides. While these data did not achieve statistical significance ($p = .092$), they do suggest a relationship between microsuturing and improved healing. Future efforts may achieve statistical significance through the use of an increased sample size.

CONCLUSION

These data suggest that vocal fold epithelial defects in a canine model demonstrate less scarring when primary closure with a microsuture is performed than when healing occurs by secondary intention. Although the sample size was insufficient to establish statistical significance, there was an observed difference in quantified scar formation between microsutured and unsutured vocal folds of 0.12 mm^2 and 0.21 mm^2, respectively ($p = .092$, 95% confidence interval of −0.027 to 0.207). This preliminary evidence suggests that microsuture closure of epithelial defects of the vocal folds may decrease scarring, at least in a canine model. Further research is warranted to determine whether suturing will improve wound healing, phonatory quality, and mucosal wave characteristics in human subjects.

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