Development of an Animal Model for Type II Sulcus (Sulcus Vergeture)

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Summary: Objectives. To develop a reproducible experimental animal model for sulcus vergeture in rabbits.

Study Design. Experimental animal study.

Material Method. We evaluated three methods of inducing sulcus in twelve New Zealand white rabbits to produce a sulcus model. Experimental groups comprised: group 1 (n = 4) underwent submucosal injury following endolaryngeal epithelial incision; group 2 (n = 4) received submucosal injury via thyrotomy; group 3 (n = 4) received submucosal injury via thyrotomy, followed with horizontal mucosal lateralization suture. Right vocal folds of the rabbits underwent surgery to produce sulcus vocalis and left vocal folds were used for the comparison. In the sixth week after the procedure, the rabbits were sacrificed and the larynxes were harvested and analyzed histopathologically.

Results. No animals in group 1 or 2 developed sulcus vocalis. Sulcus formation was observed in all rabbits in group 3, under endoscopic examination and microscopic sulcus formation was demonstrated for the first, second, and third rabbits. An epithelial depression area was seen at the glandular ductal opening zone for the fourth rabbit, but it could also be accepted as a sulcus formation.

Conclusion. We successfully developed a reproducible survival model for sulcus using a submucosal injury via thyrotomy, followed with a horizontal mucosal lateralization suture. This model provides the groundwork for future research into the applicability of new approaches for sulcus management.

Key words: Experimental sulcus modeling—Larynx—Sulcus vocalis—Vocal fold.

INTRODUCTION

The term sulcus is used to describe the condition caused by a groove of the free edges of the vocal folds. This groove induces a defective area resulting in mucosal stiffness and bowing of the vocal folds during phonation. This condition leads to glottic insufficiency and decreased mucosal wave and amplitude.[1]

The etiology of sulcus vocalis has not been clearly defined. Whether sulcus vocalis is congenital or acquired remains controversial.[2] To clarify the pathophysiology, Ford et al[3] divided sulcus vocalis into three types: physiologic (type I), sulcus vergeture (type II), and sulcus vocalis (type III). Type I also known as physiologic sulcus is seen in patients with atrophy and is limited to the superficial layer of the lamina propria (LP). Sulcus vergeture (type II) is characterized by an epithelial grooving that affects entire membranous vocal folds. It often penetrates the superficial layer with extensions to the intermediate and deep layer.

The majority of cases have a patient history of long-term hoarseness beginning in childhood, and records from family cases indicate that it is a developmental and possibly inherited condition. Type III sulcus, configures a more localized pouch that extends through the vocal ligament or muscle. This is most likely an acquired phonotraumatic process, as it is frequently accompanied by inflammation. To date, this has been the most widely used classification system.

Both non-surgical and surgical treatment modalities have been investigated for sulcus. Surgical modalities generally aim to treat either glottic insufficiency or vocal fold pliability or both. There are several options like injection laryngoplasty with different materials including autologous fat,[4] hyaluronic acid,[5] medialization thyroplasty[6] resection, and resections with replacement of the LP with collagen,[7] fat,[8] autologous fascia,[9] and small intestine submucosa.[10] Although many surgical treatment approaches for sulcus exist in the literature, there is still room for new treatment modalities. For developing better treatment modalities animal models would be beneficial.

Different animal models, such as bovines, canines, ovines, pocines, deers, rats, and rabbits have been investigated to understand physiology of human voice production and simulate laryngeal pathologies.[11-14] Among them, the canine larynx has been studied extensively owing to its similarity in size, gross structure, and vibrational characteristics to human larynx.[11] However, considering histology of layered lamina propria the rabbit vocal fold is more suitable to simulate human vocal fold pathologies.[15,16] In spite of various animal studies focused on vocal fold scars, there are no models described for any type of sulcus. Therefore, in our study, we aimed to create a morphological sulcus experimental animal model in rabbits.

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MATERIAL METHOD

Twelve New Zealand White rabbits (3.5±0.5 kg) were included in the study. The study was approved by the Animal Care and Use Committee and the Bioethics Committee of Bezmialem Vakif University and was performed in the Bezmialem Vakif University Research Center, Animal Experiments laboratory. We investigated three different methods of causing laryngeal injury to establish a sulcus vocalis formation throughout the research. The following were the characteristics of the experimental groups: group 1 (n = 4) underwent submucosal injury following endolaryngeal epithelial incision; group 2 (n = 4) received submucosal injury via thyrotomy; group 3 (n = 4) received submucosal injury via thyrotomy, followed with horizontal mucosal lateralization suture.

The right vocal folds of the rabbits underwent surgery to produce sulcus vocalis, the left vocal folds underwent no surgical procedures and these contralateral unoperated vocal folds were used for the comparison.

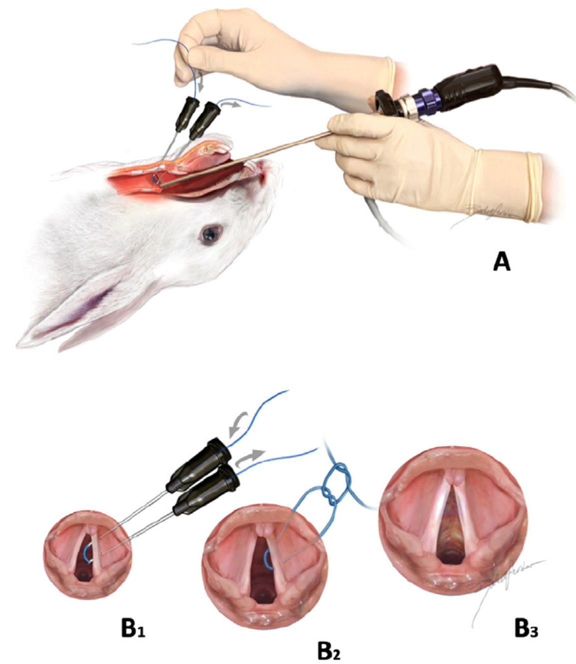

FIGURE 1. Illustration of the horizontal mucosal lateralization suture (A). Extracting the needle tips to the air column near the vocal process and placement of the suture material in the needle (B1). Knotting of suture material delivered from both holes (B2). Sulcus vocalis formation on the right vocal fold (B3). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Anesthesia

General anesthesia was ensured with the administration of 7 mg/kg intramuscular xylazine HCl (Rompun 2%, Bayer) and 35 mg/kg intramuscular ketamine HCl (Alfamine 10%, Ege Vet). Endolaryngeal examination and all surgical interventions were performed using a Storz 0° rigid telescope (Karl Storz, Tuttlingen, Germany) and recorded using portable video endoscope system (Euroclinic, Bologna, Italy).

Sulcus modeling

The endolaryngeal approach was used in group 1. A longitudinal mucosal incision was carried out with a sickle knife. Micro flap was elevated and micro pick was used to perform submucosal injury. In the other 2 groups, a transthyroid approach was used. Neck region shaving and disinfection were followed by the hyperextension of the neck and a vertical midline incision was made in the eight rabbits. Strap muscles were lateralized and the larynx was carefully dissected from the soft tissue and released. After the thyroid cartilage was skeletonized, two holes with 2 mm intervals horizontally were drilled at the level of the vocal fold by using a 1 mm cutter tip (Medtronic, Minnesota, USA). The vocal fold level was determined with the line at the midpoint of the vertical length between the thyroid notch and the lower border of the thyroid cartilage. The holes were drilled just under this line. A micro pick was entered through the drilled holes and carried out to the vocal fold under 0° endoscopic vision (Karl Storz, Tuttlingen, Germany). After the tip of the pick is noticed under the vocal fold mucosa, anterior and posterior horizontal movements were performed along the vocal fold to create a submucosal scar in the lamina propria. In group 3, after submucosal injury was performed by micro pick via thyrotomy like in group 2, a 22-gauge black needle was inserted from the frontal hole into the air column through the vocal fold level under endoscopic vision. The suture material 3/0 polydioxanone (Ethicon, Inc., Somerville, NJ) was inserted into the needle cannula from the back of the needle tip and was also delivered endo-larynx (Figure 1A). In the same way, another needle was inserted from the posterior hole in the thyroid cartilage to reach the air column from a point close to the vocal process of the arytenoid cartilage at the vocal fold level. After the cannula at the tip of the posterior needle became fully visible under endoscopic vision, the larynx was reached through the mouth with alligator forceps, and the suture material that was delivered to the endo-larynx from the frontal hole was held with forceps and inserted into the posterior needle cannula (Figure 1A, 1B_1). The suture material was taken from the back of the posterior needle tip and the needles were removed from the thyroid cartilage. In this way, the endo-laryngeal suture material was delivered outside of the thyroid cartilage. A sulcus-like 1 mm groove was formed in the right vocal fold by ligating the suture material on the right lamina of the thyroid cartilage (Figure 1B_2, 1B_3). The strap muscles and skin were sutured and antibacterial thiaphenicol skin spray (Piyedif, Ceva-Dif) was administered.

Preoperatively, 20 mg methylprednisolone (Prednol-l 20 mg, MN Pharmaceuticals), and postoperatively, trimethoprim sulfadoxine (Animar 25 mL, Ceva-Dif) was administered every second day for 1 week to all rabbits. All rabbits were given feed and water ad libitum at 45-60% humidity and with 12-hour-dark 12-hour-light periods at 21-24°C. Daily feed and water were added and the rabbits were monitored for the prevention of inflammation and stridor.

After fourth week the rabbits were examined endoscopically under general anesthesia and sulcus vocalis formations were assessed in group 3, the old incision line in the neck area was passed and the suture material was cut out. The rabbits were followed up for 2 weeks more and then reexamined. At the end of the 6 weeks, it was shown that the sulcus vocalis formation did not disappear. All rabbits were sacrificed with 100 mg/kg intramuscular ketamine HCl, after the skin incision, the larynx was reached and harvested from under the cricoid cartilage and stored in 10% formaldehyde.

The laryngectomy materials that were stored in 10% formaldehyde were examined macroscopically in the pathology laboratory, and the area in which the sulcus was found from the view of the supraglottic region was marked with black ink. The laryngectomy materials were cut in 2 portions antero-posterior direction at the middle point of the sulcus formation that was marked in black macroscopically. Two paraffin blocks were prepared for each laryngectomy material. Coronal sections taken from areas colored black ink were stained with hematoxylin-eosin (H&E) (Merck, Darmstadt, Germany), evaluated by light microscopy, and photographs were taken for non-consecutive sections along the coronal plane of the sulcus vocalis formation.

RESULTS

No animals in group 1 (endolaryngeal submucosal injury) and group 2 (transthyroid submucosal injury) developed any sulcus formation. Conversely, endoscopic and microscopic sulcus formations were revealed in group 3 (transthyroid subepithelial injury and lateralization suture). Comparable to the contralateral unoperated vocal fold, sulcus vocalis formation was shown by endoscopic images (Figures 2A, 3A, 4A, and 5A) and histologic sections (Figures 2B, 3B, 4B and 5B) for group 3 .

In rabbit 1 (group 3), the microscopic examination revealed that the sulcus vocalis formation was filling the lamina propria and was in contact with the muscle layer. This image was evaluated as type II sulcus vergeture. Fibroblast growth and neo-angiogenesis were followed in the submucosal area at the depth of the sulcus formation (Figures 2B and 2C). Thyroid cartilage destruction, the trace made by the needle extending

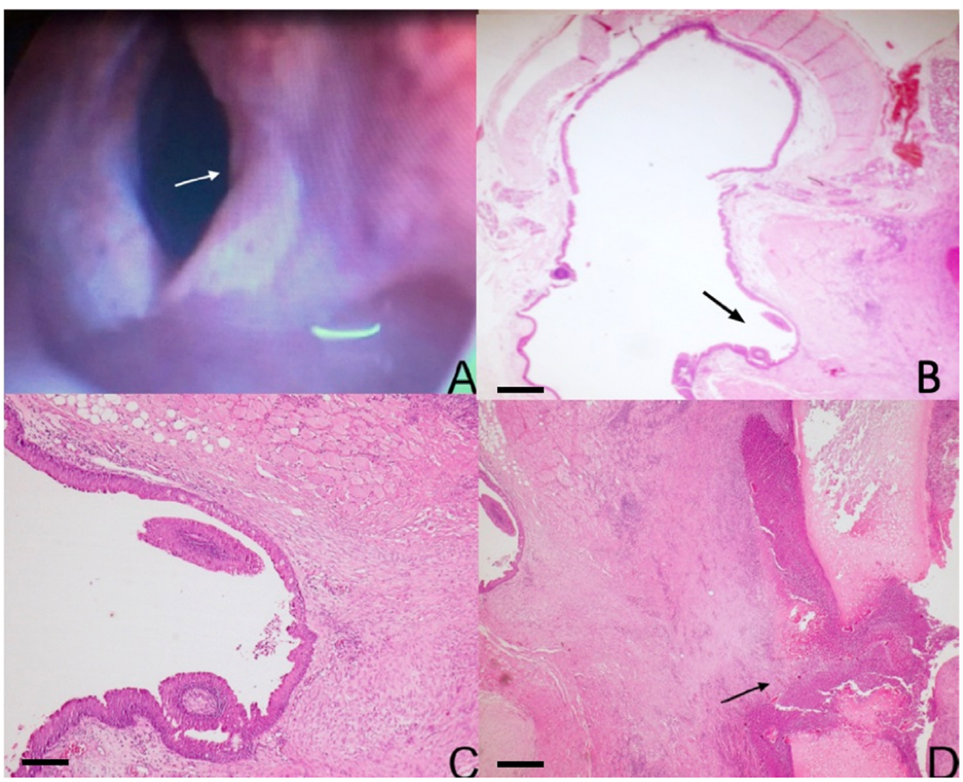

FIGURE 2. Endoscopic view of the sulcus vocalis formation (white arrow) of rabbit 1 (group 3) (A). Coronal histopathologic section of larynx that was obtained from middle point of sulcus groove) sulcus vocalis formation in the right vocal fold (black arrow) and normal left vocal fold (Scale bar, 1000 μm) (B). Epitelial invagination, sulcus formation, and fibroblast growth in the submucosal area at the depth of the sulcus formation (Scale bar, 220 μm) (C). The trace made by the needle extending from thyroid cartilage hole to the vocal fold and sulcus formation at the end of the trace (black arrow) (Scale bar, 500 μm) (D). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

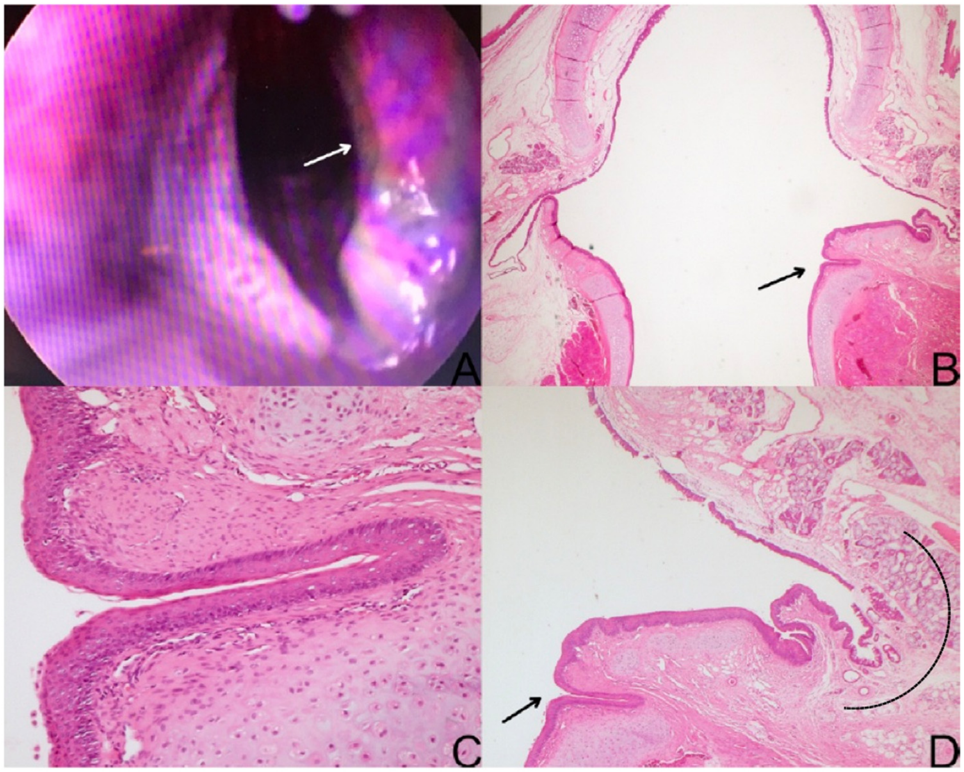

FIGURE 3. Endoscopic view of the sulcus vocalis formation of rabbit 2 (group 3) (white arrow) (A). Coronal histopathologic section of the larynx that was obtained from posterior point of sulcus groove, right sulcus vocalis formation (black arrow), and left normal vocal fold (H&Ex30) (B). Fibroblast growth and neoangiogenesis in the submucosal area of the sulcus vocalis formation (H&Ex50) (C). The trace made by the needle and sulcus formation at the end of the trace (black arrow) and connective tissue in the ventricle and vocal membrane just above the trace (right bracket) (H&Ex40) (D). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

from thyroid cartilage hole to the vocal fold, and sulcus formation at the end of the trace (Figure 2D) may be an indicator of the assumption of forming a sulcus vocalis.

In rabbit 2 (group 3); microscopic sulcus vocalis formation which extended into the lamina propria, inflammation along the trace of needle oriented to sulcus, fibroblast growth, and neo angiogenesis were seen in Figure 3B, 3C, 3D. The fibrosis following the thyroid cartilage destruction was lied down between the muscle layers and did not contain glandular structure, unlike the connective tissue in the ventricle and vocal membrane (Figure 3D). In rabbit 3 (group 3); histologic sections of the larynx revealed that the sulcus formation was limited to the superficial layer of the lamina propria (Figure 4B). Inflammation along the trace of needle oriented to sulcus, fibroblast growth, neo angiogenesis, and thyroid cartilage destruction was seen (Figure 4C, 4D).

In rabbit 4 (group 3); the depression in the epithelia was at the mucosal end of the needle trace that come from the thyroid cartilage hole (Figure 5B, 5D). Thyroid cartilage destruction, fibroblast growth, and neo angiogenesis were also seen in Figure 5C, 5D. An epithelial depression area was seen at the glandular ductal opening zone but it was the mucosal end of the needle tract so it could also be accepted as a sulcus formation.

Microscopic examination of four larynxes in group 3 revealed sulcus formation at the end of the trace made by the needle extending from thyroid cartilage hole to the vocal fold (Figure 6, A1-D3). The fibrosis following the thyroid cartilage destruction was lied down between the muscle layers and did not contain glandular structure, unlike the ventricle and vocal membrane. Microscopic examination of four larynxes in group 2 revealed thyroid cartilage destruction, the trace made by the needle extending from thyroid cartilage hole to the vocal fold but no sulcus vocalis formation with comparing to the contralateral unoperated vocal fold. (Figure 6, E1-H2). The fibrosis following the thyroid cartilage destruction was containing fibroblast growth, and neo-angiogenesis and lied down between the muscle layers, and did not contain glandular structure, unlike the ventricle and vocal membrane. Histologic sections of four larynxes in group 3 revealed fibroblast growth and neo-angiogenesis at the submucosal area but not thyroid cartilage destruction and sulcus vocalis formation (Figure 6, 1A-D) Endoscopic examinations of group 1 and 2 revealed normal endoscopic vocal fold view for both sides.

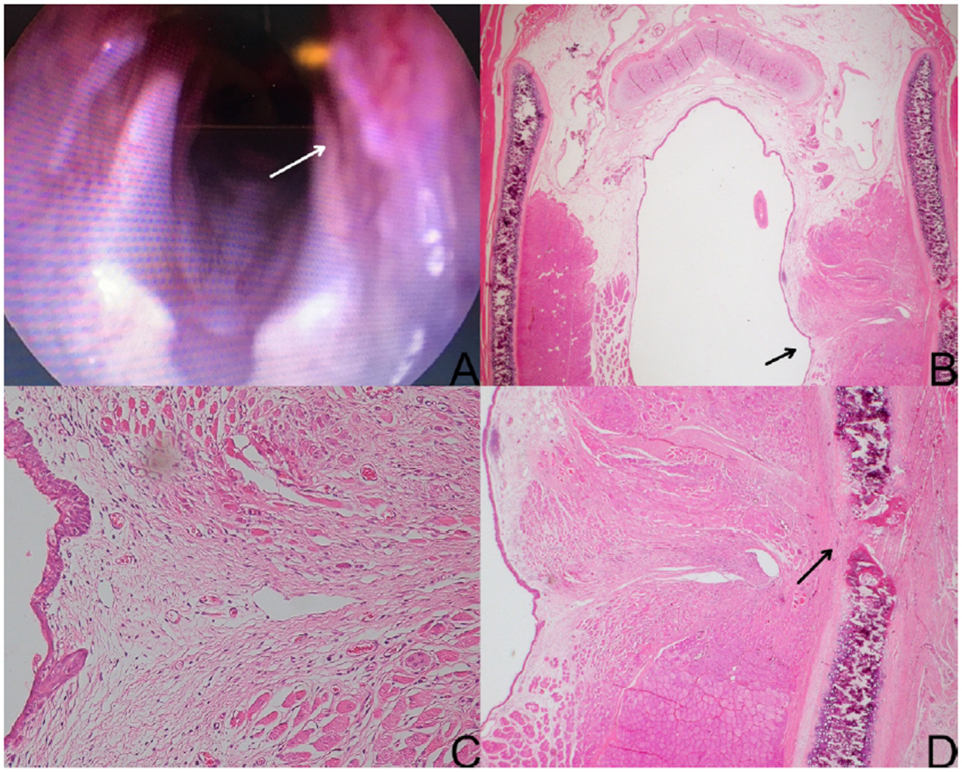

FIGURE 4. Endoscopic view of the sulcus vocalis formation of rabbit 3 (group 3) (white arrow) (A). Coronal histopathologic section of the larynx that was obtained from anterior point of sulcus groove, right sulcus vocalis formation (black arrow) and left normal vocal fold (H&Ex30) (B). Fibroblast growth and neoangiogenesis in the submucosal area of the sulcus vocalis formation (H&Ex50) (C). Destruction of thyroid cartilage, fibroblast growth throughout the needle trace and sulcus formation at the end of the trace (arrow) (H&Ex40) (D). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

DISCUSSION

The etiology of the sulcus is unclear and highly debated. There are opposing hypotheses that it is either a congenital malformation or an acquired disorder by phonotrauma. Bouchayer et al[17] hypothesized that sulcus was a congenital disorder as a result of faulty development of the fourth and sixth branchial arches and a consequence of ruptured epidermoid cysts, which consist of keratin fragments. As evidence for their hypothesis, 55% of their patients had early-onset childhood dysphonia, and 15% had type II sulcus with the frequent association of cysts or mucosal bridges. Familial cases also reported for sulcus vergeture which also increase the possibility of the genetic predisposition for the disease. This predisposition is also supported by the wide interval of incidence of sulcus in cadaveric studies in the literature.[2,18,19] On the other hand, increased prevalence of type III sulcus was shown in professional voice users and vocal abusers support the possibility of having phonotrauma one of the factors in sulcus vocalis.[20]

The heterogeneity and lack of clarity in pathophysiology make the development of the experimental sulcus model difficult. It is especially true for a model that mimics the pathophysiology of the disease. Creating a congenital sulcus formation as an experimental model or modeling a cyst formation and destruction of the cyst and then waiting for a sulcus formation is not realistic. Although creating a morphological model is still possible, no studies were exploring this.

The clinical appearance of a sulcus is strikingly similar to that of a scar on the vocal folds. Vocal fold scar etiology is centered on vocal fold abuse and trauma, which are also key etiologic causes of sulcus. This is especially true in the case of the sulcus vocalis (type III). Vocal fold scar has been studied extensively using a variety of animal models, and the characterization of the vocal scar animal model has been well described.[21,22] These models have been successfully utilized and experienced multiple times. Despite the cumulative experience on vocal fold scarring, there have been no experimental studies on the sulcus, specifically for sulcus vergeture, a form of type II sulcus that is morphologically distinct from scar and type III sulcus.

The goal was to develop an animal model capable of recreating the morphometry of the sulcus on the vocal folds. To reshape morphometry, surgical manipulation of tissue is required, which is extremely difficult in the vocal folds. On the other hand, in order to obtain a reliable animal model, in our study lamina propria histological architecture is prominent issue. Considering various animal models in terms of vocal fold lamina propria, the rabbit vocal fold is histologically similar to human vocal folds with regard to

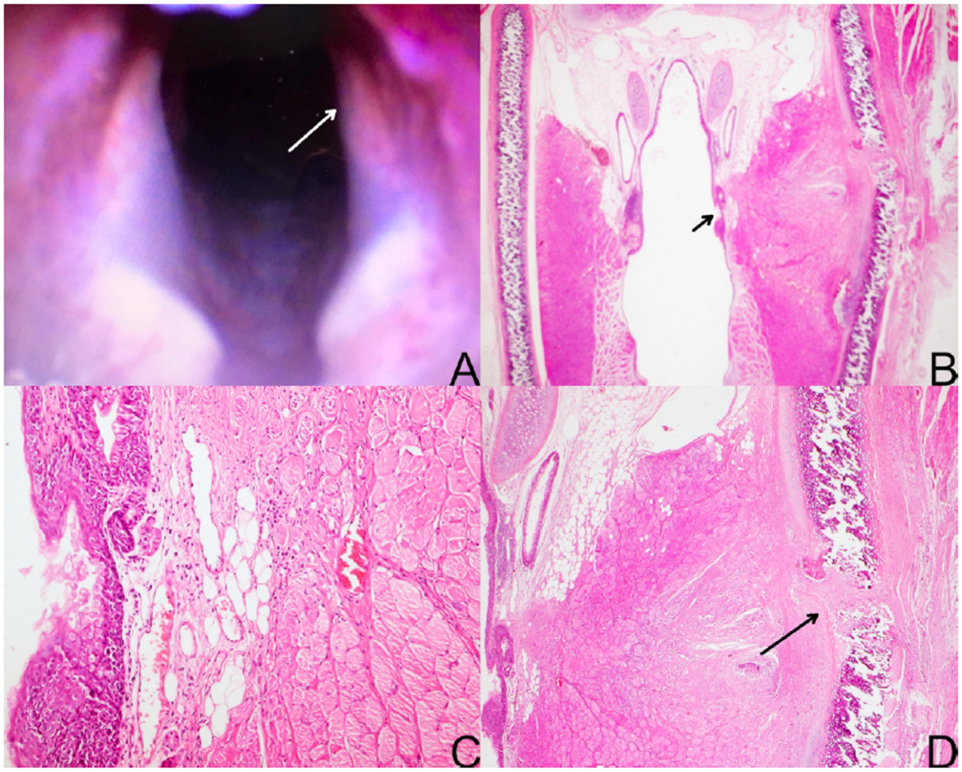

FIGURE 5. Endoscopic view of the sulcus formation (white arrow) of rabbit 4 (group 3) (A). Coronal histopathologic section of the larynx that was obtained from anterior point of sulcus groove, right vocal fold epithelial depression area and ductal opening zone (black arrow) and left normal vocal fold (H&Ex10) (B). Fibroblast growth and neoangiogenesis (H&Ex50) (C). The trace made by the needle extending from thyroid cartilage hole to the vocal fold and epithelial depression area and ductal opening zone at the end of the trace ((black arrow) (H&Ex40) (D). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

differentiated layered lamina propria, in which squamous surface epithelium lies over dense collagen fibers and loose connective tissue with deeper thyroarytenoid muscle.[16,23] Our experimental study tests three novel methods for developing a sulcus animal model, specifically mimicking type II known as sulcus vergeture. To create the sulcus vergeture model we aim to create controlled scar tissue in the vocal folds and thus to create a depression. We investigate 3 distinct approaches to find the optimum model for sulcus vergeture. Endolaryngeal mucosal and submucosal damage was employed in group 1, which is a standard approach for scar formation. A thyrotomy window was used to perform a submucosal-only damage in group 2. In the third group, we used laterization suture to produce a longitudinal depression on the vocal fold after submucosal damage, which is a commonly used surgical approach for bilateral vocal fold paralysis.

The surgical method in group 1 and 2 were not successful to form sulcus, however transthyroid subepithelial injury and lateralization suture was effective. The histologic postmortem findings demonstrated that this animal model was successful in developing a morphological model for the sulcus. In group 3, for the first three rabbits, we found a sulcus formation both endoscopically and microscopically. For the fourth rabbit, an epithelial depression area was seen at the same area as the glandular duct opening zone and it could also be accepted as a sulcus formation. The question of whether the sulcus, shown microscopically in Figures 2B, 3B, 4B, and 5B, reflected the macroscopic views in Figures 2A, 3A, 4A, and 5A. The difference can be explained by the contraction of the tissue associated with formalin and other chemical processes. Kansu et al[24] stated that the decrease of vocal fold thickness after formalin fixation was 9-24%, and after histologic processing, it was 0-14%. Based on this information, the microscopic views of sulcus formation were proven to correspond to the endoscopic views.

Phenomenology of the sulcus is not only affected by morphologic changes but also histologic alterations. Sulcus vocalis is a structural deformity of vocal fold edge resulting from the increase in collagenous fibers.[25] A study by Sato and Hirano[26] established that the thickness of the basement membrane and decrease in the quality and quantity of elastic tissue were associated with sulcus. Moreover, they found degeneration of fibroblasts in the macula flavae and increases collagenase activity. This mechanism is similar to age-related degeneration of the vocal fold. Increased vascular ingrowth and fibrosis, and the correlation between the depth of sulcus and severity of changes in subepithelial tissue were shown in Sato et al's study.[26] The study for the clarification of histopathologic characteristics of the sulcus

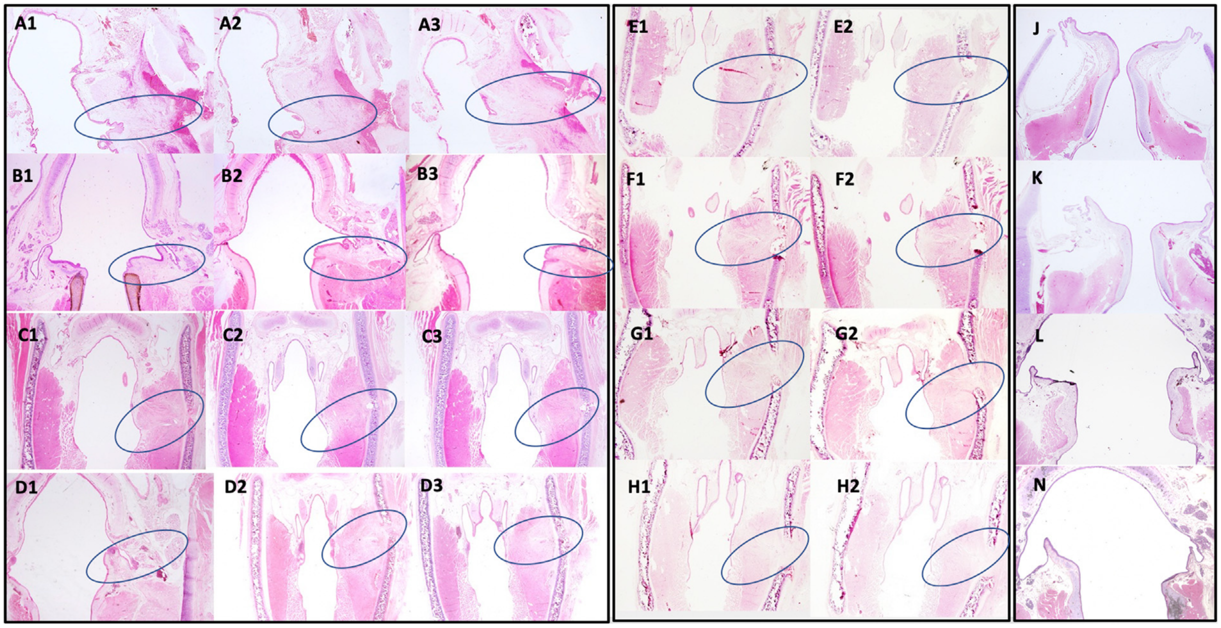

FIGURE 6. Coronal histopathologic sections of the larynxes for group 3 rabbits (A1-D3). Three coronal sections that were obtained from posterior to anterior direction of sulcus groove for group 3, rabbit 1 (A1-A3), for group 3, rabbit 2 (B1-B3), for group 3, rabbit 3 (C1-C3), for group 3, rabbit 4 (D1-D3) show normal vocal fold on left side and trace made by the needle from thyroid cartilage hole to sulcus formation on right side. Coronal histopathologic sections of the larynxes for group 2 rabbits (E1-H2). Two coronal sections that were obtained from posterior to anterior direction of sulcus groove for group 2, rabbit 1 (E1-E2), for group 2, rabbit 2 (F1-F2), for group 2, rabbit 3 (G1-G2), for group 2, rabbit 4 (H1-H2) show normal vocal fold on left side and trace made by the needle from thyroid cartilage hole but no sulcus vocalis formation on right side. Four coronal histopathologic sections of the larynxes for group 1 rabbits (J-N) show not thyroid cartilage destruction and sulcus vocalis formation (each figure shows different rabbit in the group) (H&Ex20). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

by Sunter et al showed increased vascularization and fibrosis and collagen fibers around the lamina propria of the sulcus region in sections of the fresh cadaveric larynx.[19] Also in our study, inflammation, fibroblast growth, and neo-angiogenesis were observed. Therefore, we obtained a similar histopathologic appearance to the 'real' cadaveric sulcus. At the same time, our endoscopic evaluation revealed an exact sulcus appearance.

The microscopic images in our model were variable, but sulcus formations in cadaveric larynx studies have also shown the same variability.[3] Based on our histologic images, the contralateral unoperated folds have irregularities, but there are some irregularities in the luminal surface of the larynx even in human cadaveric sections in the literature.[19] Some of these irregularities are associated with the invagination of the ventricle or aryepiglottic region in coronal sections. Especially laryngeal ventricle which consists of connective tissue can be mistaken for sulcus formation with the indentation to the laryngeal mucosa. Vocal membranes that variation in vocal fold morphology in nonhuman mammals are thin lightweight upward extensions of the membranous portion of the vocal folds and they consist of connective tissue without muscle fibers.[27] The indentation of this extension can be mistaken for sulcus formation as well. In the present study, whole larynx sections were used to evaluate sulcus formation and show localization. The discrimination was made by connective tissue of the sulcus formation that filled the lamina propria and lied down between the muscle layers, and did not contain glandular structure, unlike the ventricle and indentation of vocal membrane The connective tissue was containing fibrosis and neo-angiogenesis that started depth of the sulcus formation could be traced until thyroid cartilage hole. Thus, we could easily trace the sulcus and definitively distinguish the sulcus from the opposite unoperated vocal fold irregularities.

Moreover, the localization of the sulcus, which we were observed endoscopically, was confirmed microscopically as well. Endoscopic examination of group 3 larynxes revealed sulcus formations at the middle of the vocal fold for rabbit 1 (Figure2A), at the middle-posterior part of the vocal fold for rabbit 2 (Figure 3A), at the anterior part of the vocal fold for rabbit 3 and 4 (Figure 4A and 5A) and the microscopic evaluation of these larynxes were supporting the localization of sulcus formation. Sulcus formation was localized as middle and posteriorly, according to presence of posteriorly located vocal membrane, ventricle, and large epiglottic view on the histopathologic exam for rabbit 1 and 2 (Figure 2B, 3B); and sulcus formation was localized anteriorly according to presence of small epiglottic cartilage- petiole, and non-presence of ventricle and vocal membrane on the histopathologic exam for rabbit 3 and 4 (Figure 4B, 5B).

The study has its own limitation, especially with regard biomechanical features of the developed model. Rheoligic features of current model was not investigated in our study. Also, ex-laryngeal laryngeal vibration characteristic of the current sulcus model was not explored. This raises the question of whether the sulci will act similarly to the sulcus vergeture in an excised larynx setup. On the other hand, our technique/model appears to produce variability in scarring outcomes, not only in terms of scar severity but also in terms of scar position on the vocal fold surface. Further, discuss if method 3 results are similar enough to use for studying sulcus treatments. Are the differences between able to be identified microscopically? Obviously, they cannot be examined histologically until after the model is used for the investigational treatment.

Sulcus remains one of the difficult pathologies in the laryngology field to treat. And this reminds us the importance of animal models to study. We expect that our model will serve as a valuable tool for new and better treatments. Although the method we developed mimic the morphology with high success, the study has inherent limitations which need recognition. Our model is far from mimicking the disease at the molecular level and this limits our model's ability to serve as a testing ground for molecular or rejuvenating treatments. Future research should be devoted to clarifying the pathophysiology of the disease and the development of models that represent the disease at the cellular level.

CONCLUSION

Animal models represent an essential and valuable tool for studying the pathogenesis and treatment of disease. Although different methods have been attempted to achieve sulcus, an intervention method that provides definite results has not previously been identified. The development of an experimental sulcus animal model would provide the ability to establish new treatment techniques. This novel model can be used in future studies regarding the treatment of sulcus.

CONFLICTS OF INTEREST

The authors indicate no potential conflicts of interest.

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REFERENCES

1. Giovanni A, Chanteret C, Lagier A. Sulcus vocalis: a review. *Eur Arch Oto-Rhino-Laryngol*. 2007;264:337–344. https://doi.org/10.1007/s00405-006-0230-8.
2. Hsiung MW, Woo P, Wang HW, et al. A clinical classification and histopathological study of sulcus vocalis. *Eur Arch Oto-Rhino-Laryngol*. 2000;257:466–468. https://doi.org/10.1007/s004050000254.
3. Ford CN, Inagi K, Khidr A, et al. Sulcus vocalis: A rational analytical approach to diagnosis and management. *Ann Otol Rhinol Laryngol*. 1996;105:189–200. https://doi.org/10.1177/000348949610500304.
4. van den Broek E, Heijnen B, Hendriksma M. Bilateral vocal fold injection with autologous fat in patients with vocal fold atrophy with or without sulcus. *Eur Arch Oto-Rhino-Laryngol*. 2019;276:2007–2013. https://doi.org/10.1007/s00405-019-05479-5.
5. van den Broek E, Heijnen B, Hendriksma M, et al. Bilateral trial vocal fold injection with hyaluronic acid in patients with vocal fold atrophy with or without sulcus. *Eur Arch Oto-Rhino-Laryngol*. 2019;276:1413–1422. https://doi.org/10.1007/s00405-019-05347-2.
6. Su CY, Tsai SS, Chiu JF, et al. Medialization laryngoplasty with strap muscle transposition for vocal fold atrophy with or without sulcus vocalis. *Laryngoscope*. 2004;114:1106–1112. https://doi.org/10.1097/00005537-200406000-00028.
7. Ford C, Bless D, Loftus J. Role of injectable collagen in the treatment of glottic insufficiency: a study of 119 patients. *Ann Otol Rhinol Laryngol*. 1992;101:237–247. https://doi.org/10.1177/000348949210100307.
8. Pinto JA, Freitas S, Carpes AF, et al. Autologous grafts for treatment of vocal sulcus and atrophy. *Otolaryngol Neck Surg*. 2007;137:785–791. https://doi.org/10.1016/j.otohns.2007.05.059.
9. Tsunoda K, Baer T, Niimi S. Autologous transplantation of fascia into the vocal fold: Long-term results of a new phonosurgical technique for glottal incompetence. *Laryngoscope*. 2001;111:453–457. https://doi.org/10.1097/00005537-200103000-00014.
10. Pitman M, Cabin J, Iacob C. Small intestinal submucosa implantation for the possible treatment of vocal fold scar, sulcus, and superficial lamina propria atrophy. *Ann Otol Rhinol Laryngol*. 2016;125:137–144. https://doi.org/10.1177/0003489415601685.
11. Regner MF, Robitaille MJ, Jiang JJ. Interspecies comparison of mucosal wave properties using high-speed digital imaging. *Laryngoscope*. 2010;120:1188–1194. https://doi.org/10.1002/lary.20884.
12. Jiang JJ, Raviv JR, Hanson DG. Comparison of the phonation-related structures among pig, dog, white-tailed deer, and human larynges. *Ann Otol Rhinol Laryngol*. 2001;110:1120–1125.4. https://doi.org/10.1177/000348940111001207.
13. Welham NV, Montequin DW, Tateya I, et al. A rat excised larynx model of vocal fold scar. *J Speech Lang Hear Res*. 2009;52:1008–1020. https://doi.org/10.1044/1092-4388(2009/08-0049.
14. Hertegård S, Larsson H, Nagubothu SS, et al. Elasticity measurements in scarred rabbit vocal folds using air pulse stimulation. *Logoped Phoniatr Vocol*. 2009;34:190–195. https://doi.org/10.3109/14015430902913527.
15. Thibeault SL, Gray SD, Bless DM, et al. Histologic and rheologic characterization of vocal fold scarring. *J Voice*. 2002;16:96–104. https://doi.org/10.1016/s0892-1997(02)00078-4.
16. Hertegård S, Dahlqvist A, Laurent C, et al. Viscoelastic properties of rabbit vocal folds after augmentation. *Otolaryngol Head Neck Surg*. 2003;128:401–406. https://doi.org/10.1067/mhn.2003.96.
17. Bouchayer M, Cornut G, Witzig E, et al. Epidermoid cysts, sulci, and mucosal bridges of the true vocal cord:a report of 157 cases. *Laryngoscope*. 1985;95:1087–1094. PMID:4033333.
18. Ishii H, Baba T KI. Clinical observations in the sulcus vocalis. *J Otolaryngol Head Neck Surg*. 1967;70:911–919.
19. Sunter AV, Yigit O, Huq GE, et al. Histopathological characteristics of sulcus vocalis. *Otolaryngol - Head Neck Surg*. 2011;145:264–269. https://doi.org/10.1177/0194599811404639.
20. Selleck AM, Moore JE, Rutt AL, et al. Sulcus Vocalis (Type III): prevalence and strobovideolaryngoscopy characteristics. *J Voice*. 2015;29:507–511. https://doi.org/10.1016/j.jvoice.2014.09.015.
21. Rousseau B, Hirano S, Scheidt TD, et al. Characterization of vocal fold scarring in a canine model. *Laryngoscope*. 2003;113:620–627. https://doi.org/10.1097/00005537-200304000-00007.
22. Rousseau Bernard, Hirano Shigeru, Chan Roger W, et al. Characterization of chronic vocal fold scarring in a rabbit model.

J Voice. 2004;18:116–124. https://doi.org/10.1016/j.jvoice.2003.06.001.

23. Maytag AL, et al. 'Use of the rabbit larynx in an excised larynx setup. *J Voice*. 2013;27:24–28. https://doi.org/10.1016/j.jvoice.2012.08.004.
24. Kansu L, Aydın E, Akkaya H, et al. Shrinkage of nasal mucosa and cartilage during formalin fixation. *Balkan Med J*. 2017;34:458–463. https://doi.org/10.4274/balkanmedj.2015.1470.
25. Hirano M, et al. Sulcus vocalis: functional aspects. *Ann Otol, Rhinol and Laryngol*. 1990;99:679–683.
26. Sato K, Hirano M. Electron microscopic investigation of sulcus vocalis. *Ann Otol Rhinol Laryngol*. 1998;107:56–60. https://doi.org/10.1177/000348949810700111.
27. Neubauer, J. (2004) Nonlinear Dynamics of the Voice: Bifurcations and Mode Analysis of Complex Spatio-Temporal Signals. Humboldt-Universität zu Berlin. https://doi.org/10.18452/15343.