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ORIGINAL RESEARCH

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Establishment of a survival rabbit model for laryngotracheal stenosis: A prospective randomized study

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Abstract

Objective: To develop a reproducible survival rabbit model for laryngotracheal stenosis (LTS).

Methods: Seventy New Zealand white (NZW) rabbits were randomly divided into experimental groups ($n = 30$) and a control group ($n = 40$). In experimental groups, a nylon brush was inserted retrograde from the tracheotomy through the subglottis and rotated until a full layer circumferential mucosal injury to cartilage exposure, assisted by fiberoptic laryngoscopy (FOL) visualization. Experimental group 1 ($n = 10$), rotated 10 times; group 2 ($n = 20$), rotated 20 times. The control group underwent tracheotomy only without nylon brush scraping. The rabbits underwent FOL at 1st, 4th, 8th, and 12th week postinjury respectively to observe the formation of LTS. They were euthanized and the larynxes and tracheas were subjected to gross and histopathological examination at 12 weeks postinjury.

Results: The control group all survived, while five cases in experimental groups died from LTS and/or mucous plug. Histological observation showed that the control group had intact laryngotracheal mucosal epithelium without any stenosis; the experimental groups showed proliferation of fibroblasts and thickening of collagen fibers. The mean stenosis in control group was 9.31 ± 0.98%, while that in experimental

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group 1 was 32.78 ± 7.07% and 58.25 ± 8.96% in experimental group 2. The difference between the three groups was statistically significant ($\chi^2 = 47.98$, $p < .05$).

Conclusions: We successfully developed a reproducible survival rabbit model for LTS using a nylon brush through FOL visualization combined with tracheostomy. This model can provide a mature and stable animal model for the exploration of wound-healing pathophysiology and the effect of interventions.

Level of evidence: NA.

KEYWORDS

fiberoptic laryngoscopy, laryngotracheal stenosis, New Zealand white rabbit, nylon brush scraping, tracheotomy

1 | INTRODUCTION

Since the advent of prolonged intubation (ETI) as a method of long-term ventilatory support in neonates, the incidence of acquired laryngotracheal stenosis (LTS) in childhood has increased significantly.[1] This clinical condition is caused by ETI, long-term tracheostomies, trauma, airway burns, and some systemic diseases.[2] However, the most commonly etiology of LTS is prolonged or repeated ETI,[3] resulting in mucosal inflammation, ulceration, and necrosis.[4] These pathologic changes finally induce the formation of excessive granulation, which narrows the laryngotracheal cavity.

LTS is a life-threatening disease in the pediatric population and may lead to chronic disability. Although various methods have been used for treating LTS, the management continues to be challenging.[5] Treatment for these patients may require multiple procedures with associated morbidity,[6–8] ranging from serial endoscopic balloon dilations, tracheostomy, to laryngotracheal reconstruction.[9] The treatment methods have changed little over the last 50 years, and there is inconsistent evidence of efficacy from medical therapies.[6,10] An effective and less invasive technique for the treatment of LTS in children is therefore needed. The main barrier in developing better therapies for LTS is that there still exists a very limited understanding of the mechanisms behind the formation of fibrosis.[6] Given that these experiments cannot be simulated in vitro or easily in humans,[3,11] it is urgent to establish experimental animal models in which pathologic changes should be similar to those seen in clinical cases. However, prospective, experimental, and histopathological studies of the pathogenesis and therapeutic management of LTS are lacking.[11]

The New Zealand White (NZW) rabbit has been the most often used animal model to investigate pediatric airway stenosis given its anatomical similarities to an infant's airway.[12] Some protocols of creating an animal model of LTS have been attempted with many open or endoscopic techniques,[2,11,13–17] but with various mortality rates and reliability of stenosis.[11,13–16,18,19] Endoscopic approach alone requires operator with rich experience, as it is impossible to visualize the full thickness of the mucosa and determine the depth of the injury.[20] Moreover, previous authors have not, however, consistently evaluated the postinjury endoscopic appearance, postinjury respiratory symptoms, and histologic findings.[15] Therefore, regardless of the damage mechanism, the primary challenge is producing a significant degree of LTS securely during a controlled time period.[16] In view of the above-mentioned facts, we aim to create a rabbit model for LTS that was reproducible and survivable over a long-term follow up period using an open approach combination of FOL visualization and to document this model symptomatically, endoscopically, and histologically.

2 | MATERIALS AND METHODS

All animal procedures were conducted in accordance with the guidelines published by Shanghai Jiaotong University School of Medicine Institutional Animal Care and Use Committee (Protocol: JUMC2023-193). This study was approved by our institutional Research Ethics Board (Approval Letter of Ethics Review Committee, Children's Hospital of Shanghai/Shanghai Children's Hospital, Shanghai Jiao Tong University; Approval No: SHCH-IACUC-2022-XMSB-85, Validity of the approval: 2023-01-01 to 2025-12-31).

2.1 | Experimental design

Our study included 70 New Zealand white rabbits (Shanghai Jiagan Biotechnology Co., Ltd., license key: SCXK Shanghai 2020-0006), which were fed feed (Suzhou Shuangshi Experimental Animal Feed Technology Co., Ltd) and given water ad libitum. All animals were given 1 week to acclimate to their new environment in box cages before airway intervention.

This was a randomized block, single-blind, prospective animal experimental study. About 30 NZW rabbits were in the experimental groups and underwent laryngotracheal injury and the remaining 40 animals served as uninjured controls. Experimental groups comprised: group 1 ($n = 10$), which rotated 10 times; group 2 ($n = 20$), which rotated 20 times. The experimental groups consisted of four main steps: tracheostomy, FOL visualization, nylon brush scraping, and tracheal closure. The control group underwent tracheotomy only without nylon brush scraping, and then closed the trachea. The two groups underwent FOL under general anesthesia to dynamically

observe the formation of LTS. At an interval of 12 weeks postinjury, the three groups were euthanized and larynxes and tracheas were subjected to gross and histopathological examination. The pathologist reporting the morphological and histopathological examination was blinded to the group to which the specimen belonged. The controls at the time of sacrifice were used primarily to establish a normative data base of the cross-sectional areas of the trachea and subglottis in the rabbit.

Inclusion criteria:

a. 3–4 months old;
b. NZW rabbit;
c. 2.5–3.5 kg.

Exclusion criteria:

a. Not in good overall general health;
b. Died midway due to LTS or/and mucous plug.

Outcome:

a. The primary outcome for this study was change in cross-sectional area (CSA) post-injury, expressed as mean percent change in CSA;
b. The secondary outcome was histopathological change at 12 weeks post-injury;
c. Laryngotracheal appearance by FOL;
d. Postoperative symptoms, including respiratory stridor/distress, subcutaneous emphysema, poor oral intake.

2.2 | Surgical procedure

2.2.1 | Tracheostomy

After the induction of general anesthesia with intramuscular injection of ketamine (10–50 mg/kg) and xylazine (2–10 mg/kg), the rabbit was laid supine on the surgical table (Figure 1). The anterior neck of each rabbit was shaved and disinfected. After making a midline skin incision in the anterior of the neck, expose the larynx and the trachea, being careful not to injure the common carotid artery, sternohyoid and sternothyroid muscles. We made a transverse incision along the tracheal cartilage, with a length of 1/2–2/3 of the circumference. The incision point was located 1.5 cm below the bottom edge of the cricoid cartilage.[2]

2.2.2 | Nylon brush scraping

Experimental groups received manually induced laryngotracheal injury with a nylon brush (Quzhou Shuanghesheng Grinding Tools Trading Co., Ltd). Under the FOL direct vision assistance, a nylon brush was inserted into the laryngotrachea by way of the incised edge toward the mouth, and then the laryngotracheal mucosa was circumferentially scraped by pushing and pulling the brush 10 times and rotating 10 times in group 1 ($n = 10$) or 20 times in group 2 ($n = 20$). The depth of the injury was full-thickness mucosal injury with cartilage exposure. To limit collateral mucosal injury, the modified nylon brush applied in experimental groups had a diameter of 6.0 mm and a length of 10 mm. Cotton swabs soaked in ephedrine were used for topical hemostasis if needed. The control group underwent tracheotomy only without nylon brush scraping.

2.2.3 | Tracheal closure

After confirmation of hemostasis, the incised trachea was closed with four to five interrupted sutures using 6–0 monofilament polypropylene thread. Muscle, subcutis, and skin were closed with continuous suture with each layer using 3–0 polyamide thread.[2]

2.3 | Perioperative management

Postoperatively, all cases were treated with as-needed buprenorphine for pain, cefuroxime at 100 mg/kg and dexamethasone at 2 mg/kg for respiratory distress[18] by our attending veterinarian. Rabbit weight was followed through the study to measure good general health.

2.4 | Fiberoptic laryngoscopy

We transferred three groups of animals to their respective cages and conducted a 12-week follow-up there (Figure 2). The experimental groups of rabbits underwent fiberoptic laryngoscopy (FOL) at weeks 1, 4, 8, and 12, respectively to dynamically observe the formation of LTS using the same anesthesia induction method described above. We performed FOL before tracheostomy, 4th and 12th week postinjury in the control group. A skilled assistant held the rabbit in a supine position, extended its head, and opened its mouth with silk thread wrapped around its front teeth. We softly pulled out the tongue to the left side, imported anesthesia laryngoscope, and the FOL was performed to visualize the larynx and trachea. Images from airway endoscopies were recorded.

2.5 | Specimen acquisition

The rabbits were then euthanized with sodium pentobarbital (100 mg/kg) while the animals remained under general anesthesia, and the larynxes and tracheas were subjected to gross and histopathological examination at an interval of 12 weeks postinjury (Figure 3). The larynxes and tracheas were harvested from the level of the hyoid bone to 2 cm below the cricoid. We immersed the specimens in 4% paraformaldehyde (PFA)[11] for fixation and transferred to the laboratory for morphological and histopathological examination within 24 h.

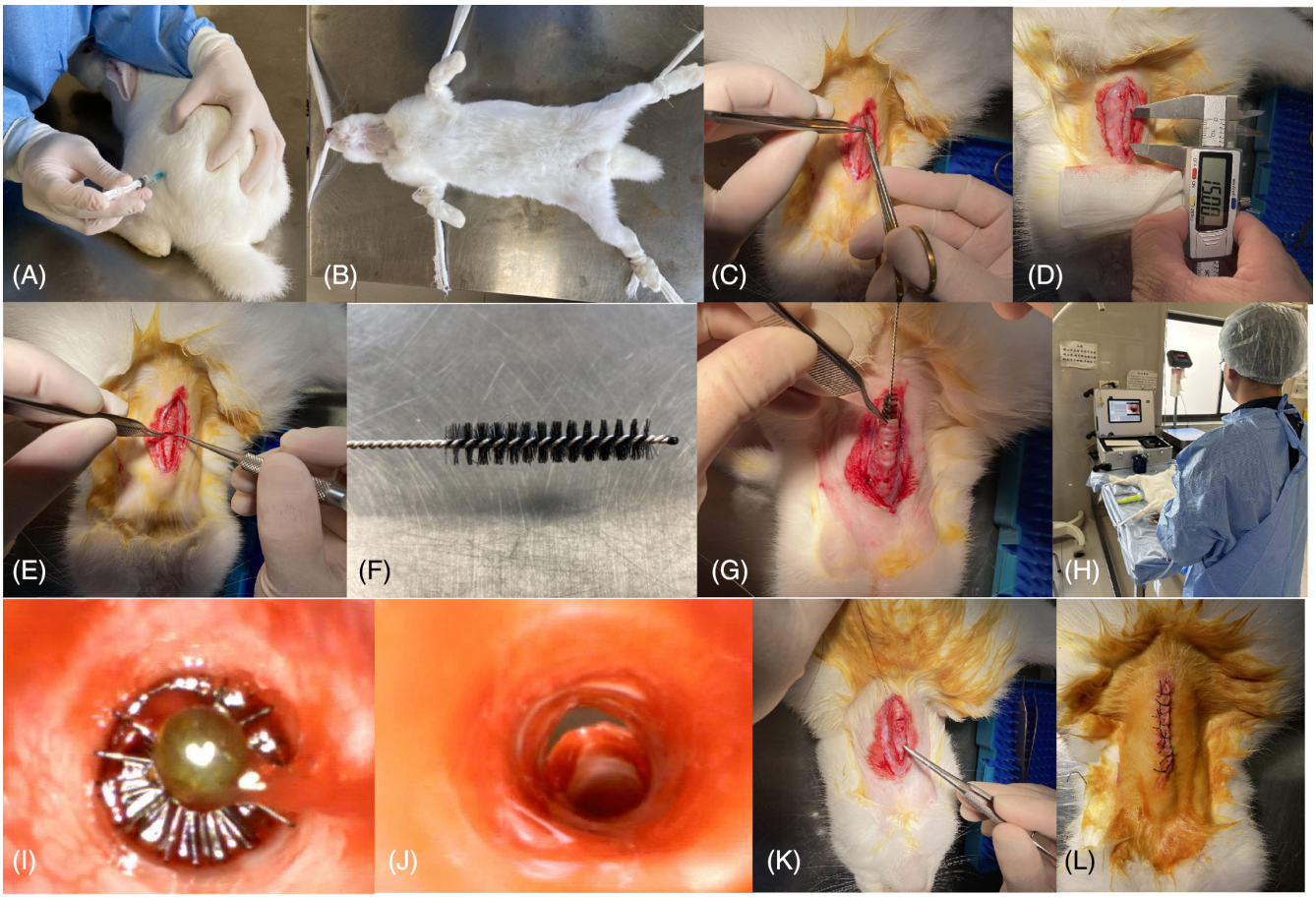

FIGURE 1 Surgical procedure. After the induction of general anesthesia by intramuscular injection of ketamine and xylazine (A), the rabbit was placed supine on the operating table (B). The anterior neck of each rabbit was shaved and disinfected. After a midline skin incision in the anterior neck, the larynx and the trachea were exposed carefully (C). The trachea incision point was located 1.5 cm caudal to the bottom edge of the cricoid cartilage (D). The trachea was incised transversely along the tracheal cartilage with an incised length of 1/2–2/3 of the circumference (E). A modified nylon brush with a diameter of 6.0 mm and length of 10 mm (F) was inserted into the laryngotrachea by way of the incised edge toward the mouth (G). By assistance of FOL visualization (H), we realized relatively precise manually scraping and localization (I). To control mucosal injury, the laryngotracheal mucosa was circumferentially scraped by pushing and pulling the brush 10 times and rotated 10 times in group 1, 20 times in group 2. The depth of the injury was full-thickness circumferential mucosal injury with cartilage exposure (J). After confirmation of hemostasis, the incised trachea was closed with four to five interrupted sutures using 6-0 monofilament polypropylene thread (K). Muscle, subcutis, and skin were closed with continuous suture with each layer using 3-0 polyamide thread (L).

2.6 | Pathological analysis and stenosis degree calculation

Each specimen was sliced and fixed in 10% buffered formalin, embedded in paraffin, and cut into 3 μm sections (Figures 2 and 3). Pathology slides were analyzed under an optical microscope (OLYMPUS, BX53, Japan) using Hematoxylin eosin (HE) staining, and measured by Image Viewer_X64_V2.0.4.0630 (Shenzhen Shengqiang Technology Co., Ltd., China) software. Histopathological changes were recorded by our pathologist who was blinded to the group. These areas comprised the narrowest part of the cricoid and the narrowest part of the trapped tracheal rings. The cross-sectional area (CSA) of the trachea and subglottis were measured. The following parameters were measured: the internal transverse and anteroposterior diameter of the lumen and the circular arc formed by the laryngotracheal cartilage.

Using photographs of the transversely sectioned laryngotrachea by ImageViewer_X64_V2.0.4.0630 software, we evaluated the degree of LTS defined as follows[2]: $(1 - s/S) \times 100$, where s is the luminal area of the intratracheal cavity and S is the area of the circular arc formed by the laryngotracheal cartilage. That is $(1 - [r_l + r_s]/[R_l + R_s]) \times 100\%$, where r_l and r_s are the longest caliber and the shortest caliber of the laryngotracheal lumen, respectively, and R_l and R_s are the longest diameter and the shortest diameter of the circular arc formed by the laryngotracheal cartilage, respectively.

2.7 | Data analysis

Statistical analysis was conducted using the SAS 9.13 software package (SAS institute Inc., USA). The quantitative data of normally

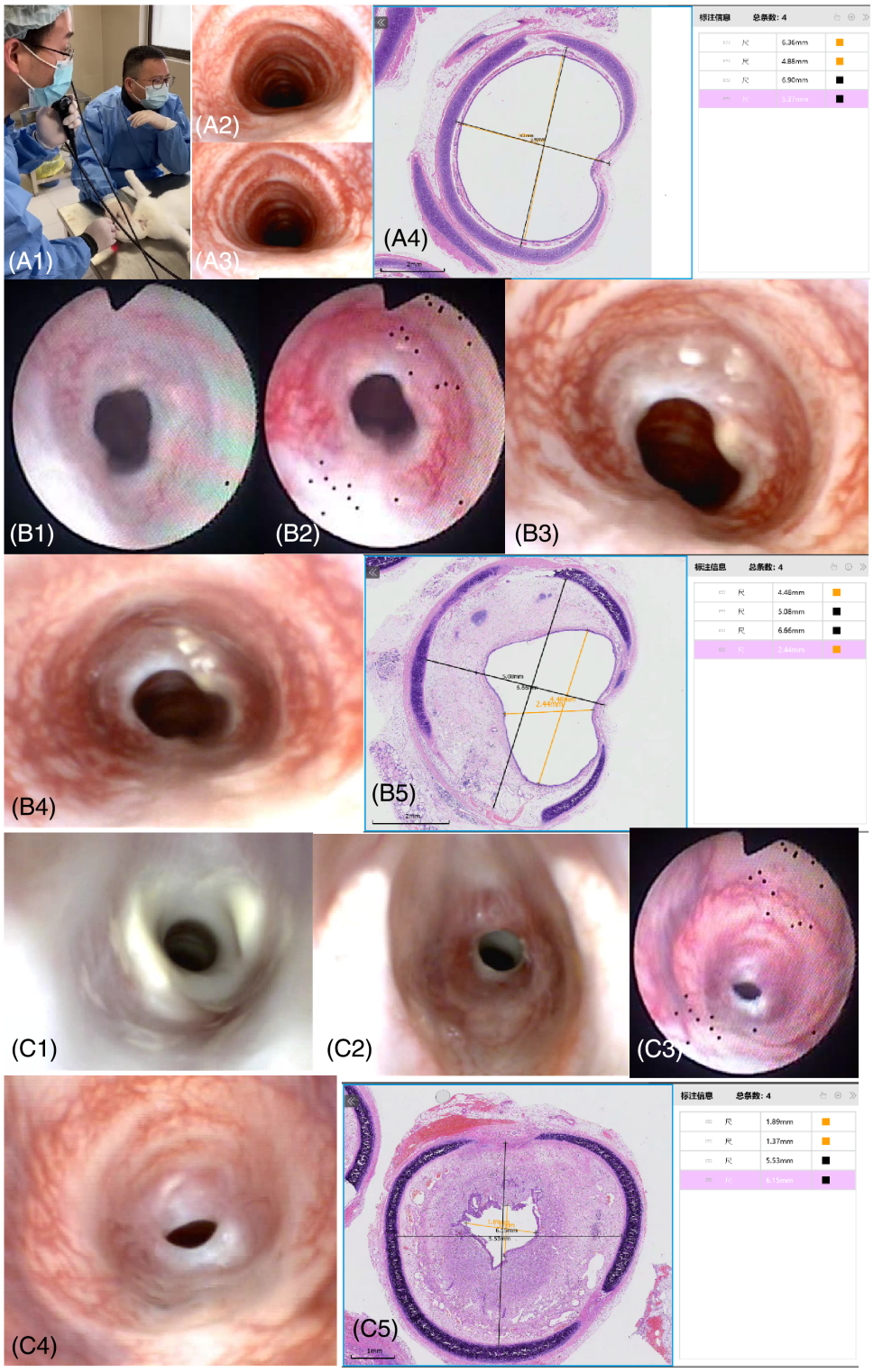

FIGURE 2 Dynamically observe the formation of LTS by FOL. We performed FOL under general anesthesia (A1), 4th and 12th week post-injury in the control group (A2, preoperative native laryngotracheal, A3, 12th weeks post-tracheotomy; A4, the degree of stenosis, 8.39%), and 1st, 4th, 8th, 12th week post-injury in experimental groups. (B1–B5/C1–C5) LTS 1st week post-injury, 4th week post-injury, 8th week post-injury, 12th week post-injury, the degree of stenosis 41.06%/72.09%.

distributed were expressed as (mean ± standard deviation). Compare the degree of stenosis in three completely randomized groups (experimental group 1, 2, and the control group) using K-W test. Wilcoxon rank sum test was used to compare intraluminal area (mm^2) in the trapped ring and cricoid between experimental group 1 and the control group, experimental group 1 and experimental group 2, $p < .05$ was considered statistically significant.

3 | RESULTS

3.1 | General situation

Control group: all subjects in the control group survived to the 12-week follow-up period and no animals developed any stenosis. Experimental groups: 5 rabbits showed poor oral intake in the first

few postoperative days but recovered completely in 5 days. Three rabbits developed subcutaneous emphysema after surgery which disappeared within the following week. Experimental group 1 ($n = 10$): 9 rabbits survived to complete the study while one case died of mucous plug blocking the airway, and the overall mortality rate was 10% (1/10). Experimental group 2 ($n = 20$): 16 rabbits survived to complete the study while 4 cases died midway due to LTS and phlegm blockage, and the overall mortality rate was 20% (4/20). Within 7 days after surgery, 12 rabbits began to develop respiratory stridor, and four rabbits died 9, 10, 12, and 17 days postinjury from delayed respiratory failure. The larynx and trachea of each of those five died rabbits were removed on the day of death, and histologic examination was performed. Immediate postmortem necroscopy revealed near complete airway stenosis and mucous plug blocking the airway (Figure 4). Excluding the 5 deaths mentioned above, the remaining 25 rabbits were enrolled and euthanized 12 weeks after the scraping operation. The average weight at the beginning of the study period was 2.8 kg (range 2.5–3.5 kg), average weight at the endpoints was 3.3 kg (range 2.8–4.1 kg), showing an increase in weight gain.

3.2 | FOL findings

Images from airway FOL were recorded. In the control group, the laryngotrachea was unobstructed, no scar tissue proliferation was found, and the mucosa was not congested or edematous. The experimental groups showed obvious congestion and edema at the scraping and brushing site, with scar tissue proliferation 1 week after the scraping operation. The narrow part was limited to the scraped area, excluding the suture area. In the experimental group 2, more severe and homogeneous narrow was observed (Figure 2).

3.3 | Pathological findings and calculation of the degree of stenosis

Histological observation showed that the control group had intact laryngotracheal mucosal epithelium without inflammation or edema. However, various degrees of inflammatory tissue and edema were confined within the scraped region in the laryngotrachea. The experimental groups showed intraluminal narrowing, submucosal thickening, proliferation of fibroblasts, thickening of collagen fibers, cartilage deformity/new cartilage formation, perichondritis, and aggregates of inflammatory cells (Figure 3).

The NZW rabbits of controls were used to establish a normative data base of the cross-sectional areas of the trachea and subglottis concurrently. Microscopic measures of the cricoid and of the trapped rings are shown in Table 1. We measured the internal transverse and anteroposterior diameter of the native airways, with a diameter of 5.69 ± 0.49 and 4.25 ± 0.43 mm, respectively in the trachea, while that of 4.77 ± 0.54 and 5.77 ± 0.66 mm, respectively in the subglottis (Table 1).

The mean percentage of airway stenosis in control group was 9.31 ± 0.98% (Table 2), while that in experimental group 1 was 32.78 ± 7.07%, range, 18.29%–40.81% (Table 3); in experimental group 2 was 58.25 ± 8.96%, range, 41.06%–72.09% (Table 4). There was a significant difference between the three groups ($\chi^2 = 47.98$, $p < .0001$). The experimental group 2 developed more severe and homogeneous narrow ($S = 45$, $p < .0001$) (Table 5).

4 | DISCUSSION

Pediatric LTS develops by various causes such as ETI, long-term tracheostomies, airway burns, trauma, tumors, and some systemic diseases.[2,21] Due to widespread adoption of prolonged ETI for respiratory support, LTS in children has become increasingly more prevalent over the past quarter century.[1] Post-intubation injury is the most common reason for LTS,[21,22] the endotracheal tube (ETT) size and duration of intubation are the major risk factors for the formation of LTS.[7,23,24]

LTS is a complex narrowing of the airway, which may manifest as a potentially life-threatening emergency.[25] Although various techniques have been used for treatment of LTS in children, it is a challenging clinical entity with limited number of effective treatment modalities.[22] The accepted contemporary treatment of LTS via tracheotomy in most cases is laryngotracheoplasty (LTP),[26] yet a significant number of children are unable to be decannulated, in part, because of suboptimal mucosal wound healing that results in re-stenosis. Future research efforts to further improve decannulation rates in children with LTS should be directed, at least in part, toward a better understanding of mucosal wound healing. In contrast to skin, mucosal wound healing has not been extensively studied.[20] High quality studies of LTS are lacking and almost impossible to conduct in humans,[11] we desperately need animal models of LTS. A critical preclinical approach in understanding the pathogenesis of LTS and translating investigative technologies from the bench to the bedside is the use of these animal models.[25]

The NZW rabbit has been the primary animal model to investigate pediatric LTS given its easily handled, readily available and relatively inexpensive.[12,22,27–30] In addition, the rabbit larynx and trachea are almost similar to those of infants and children under 2 years old.[11,22,29,31,32] The average subglottic diameter of the rabbits, which changes minimally by weight, ranges from 5.4 to 5.8 mm.[29,31] In the present study, the internal transverse and anteroposterior diameter of native airways in NZW rabbit was (5.69 ± 0.49) mm and (4.25 ± 0.43) mm, respectively (Table 1), which was consistent with the above literature reports.

Multiple techniques for inducing airway stenosis in animal models has been described, including ETI,[11,19] nylon brush scarping,[2] silver nitrate[33] or hydrochloric acid,[20] electrocautery,[20,34,35] and carbon-dioxide laser.[30,36,37] These methods are applied by an open approach[2,34] or endoscopically,[19] however, they remain limited by high mortality and morbidity, large variability in the degree of stenosis, and subjectivity of the endoscopic stenosis grading methods.[11,15,19,20,33,38]

Methods involving a brush-scraping technique have been employed in open and endoscopic approaches. McIlwain et al.[16]

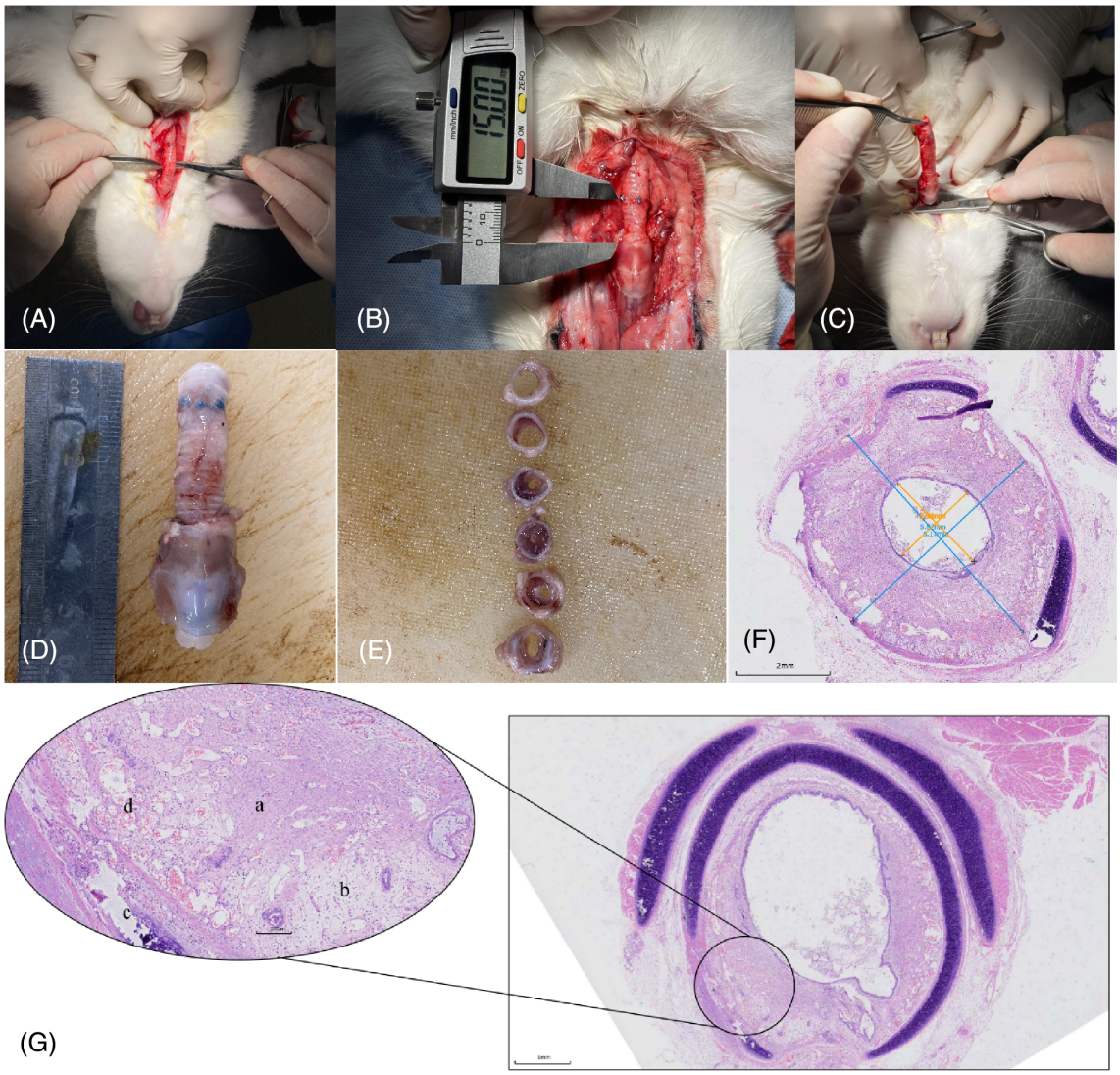

FIGURE 3 Specimen collection and gross/pathological examination. At an interval of 12 weeks postinjury, the rabbits were then euthanized with sodium pentobarbital. The larynxes and tracheas were harvested from the level of the hyoid Bone to 2 cm below the cricoid (A–C), and subjected to gross and histopathological examination (D–F). Histologic examination showed submucosal hypertrophy caused by proliferation of fibroblasts and thickened collagen fibers (a), intensive infiltration of inflammatory cells (b), cartilage deformity/new cartilage formation (c), and increased number of capillaries (d).

described a technique that a nylon/polypropylene brush (diameter, 6 mm) was inserted into the laryngotrachea and rotated 40 times endoscopically until a circumferential mucosal injury obtained in 16 NZW rabbits. They found that using a polypropylene brush can realize more consistent and severe stenosis than the nylon brush. However, this still caused a 25% early death rate and 100% of the surviving rabbits needed an emergent airway intervention to escape additional mortality. Nakagishi et al.[2] used an open approach to brush the trachea in eight rabbits and reported wide variability in the grades of stenosis produced, ranging from 20% to 70% obstruction. Steehler et al.[38] used a nylon brush to develop tracheal stenosis. The rabbits injured through an open approach did not show stenosis, while the rabbits receiving endoscopic access showed a wide range of tracheal stenosis (10–80% obstruction). The techniques using a brush in a full circumferential injury consistently showed early death or sacrifice rates of 16.7%–33%,[2,15,16,38] and variability in the grade of stenosis induced.[38,39] Schweiger et al.[15] found that they were unable to establish an ideal rabbit model for LTS, which may be due to differences in brushing techniques or the types of brushes used. Mady et al.[25] attempted to design and investigate a fully endoscopic, reliable, and accurate method for inducing SGS in the NZW rabbit model using a polypropylene brush (6.35 mm diameter). The use of this mechanical device potentially eliminated the variability introduced by manual airway scraping.[25] Yet, full revolutions set at 120 rpm for 120 cycles was attempted, which led to severe trauma associated with mucosal avulsion and even a torquing rotation of the entire airway.[25] At present, it is still very difficult to establish a significant LTS animal model without compromising the survival of the animal for a minimum period of

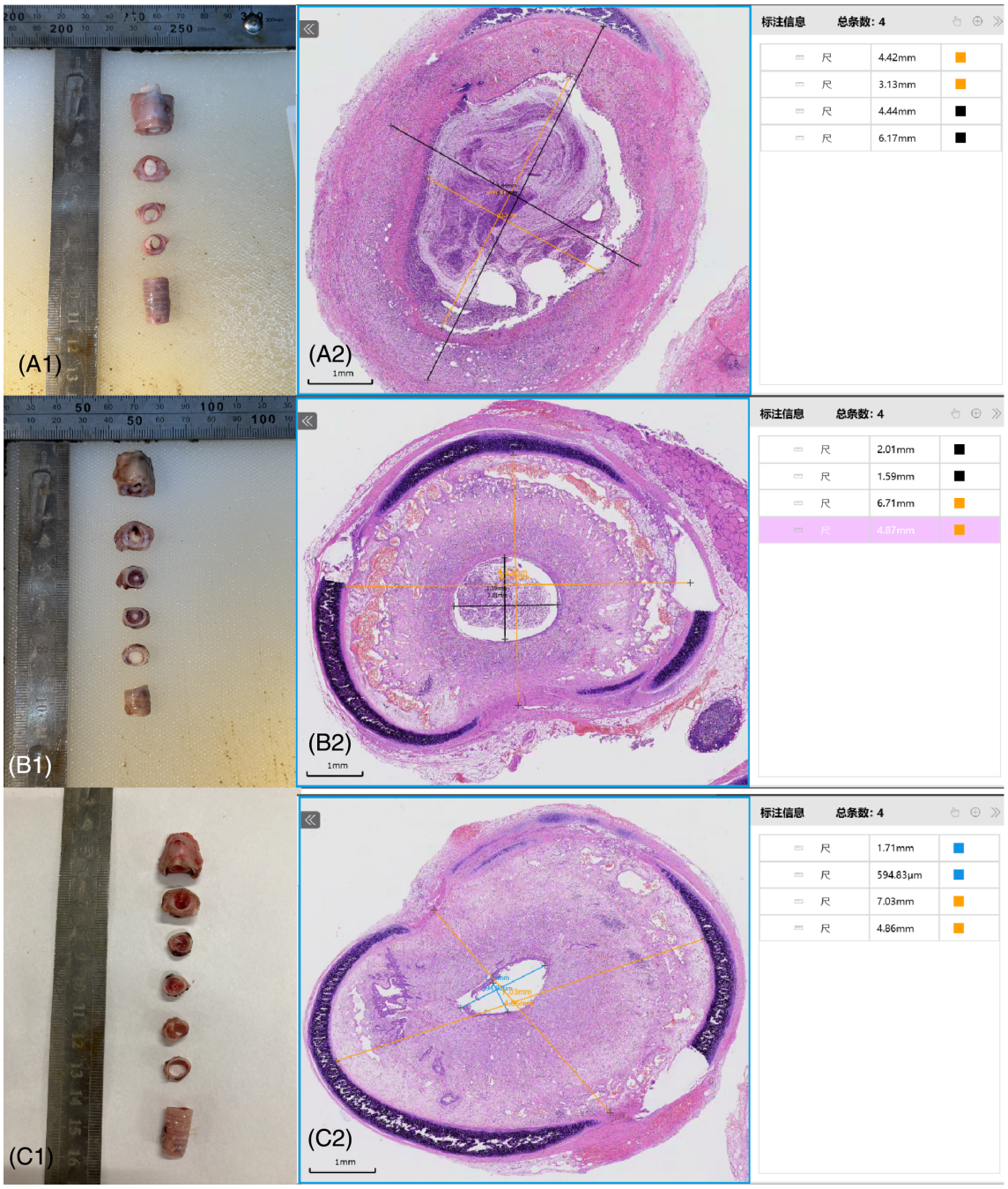

FIGURE 4 The excluded cases died midway due to LTS or/and mucous plug. The larynx and trachea of each of those five rabbits died in the experimental groups were removed on the day of death, which were subjected to gross and histopathological examination. Immediate postmortem necroscopy revealed near complete airway stenosis (C) and/or mucous plug blocking the airway (A, B) (percentage of stenosis: A, 28.84%; B, 68.91%; C, 80.55%).

12 weeks.[11] Accomplishing this goal is more difficult than one may imagine because, on the one hand, one must impart an insult to the mucosa significant enough to result in a cicatricial stenosis grossly and histopathologically. Yet, on the other hand, the insult cannot induce such extensive injury that the acute inflammatory response and formation of granulation tissue critically obstructs the already narrow airway of small animals. If this occurs, they asphyxiate acutely, not surviving long enough for a mature LTS to develop (Figure 4). In this study, combination of open approach and endoscopic approach, we successfully established a survival rabbit model of LTS (Figure 1). After tracheal incision, with the help of FOL visualization, the laryngotracheal inner surface was scraped using a nylon brush, producing LTS of 18.29%–40.81% [average 32.78 ± 7.07%] in group 1 (Table 3) and 41.06%–72.09% [average 58.25 ± 8.96%] in group 2 (Table 4), 12 weeks after the scraping procedure. The overall mortality rate was 16.67% (group 1, 10%; group 2, 20%). The group 2 developed more

TABLE 1 Inner diameter of laryngotracheal in NZW rabbits.

	Trachea		Subglottis	
Case	Transverse diameter	Anteroposterior diameter	Transverse diameter	Anteroposterior diameter
1	5.88	4.20	4.77	4.97
2	5.29	3.34	5.18	5.30
3	6.01	3.78	4.82	5.35
4	5.96	3.76	4.87	5.58
5	6.15	4.51	4.21	6.23
6	6.57	4.12	5.55	6.23
7	6.41	5.01	4.09	5.91
8	6.61	5.25	4.81	6.95
9	5.43	5.00	4.64	5.67
10	5.28	4.45	4.77	5.29
11	5.64	4.60	4.05	5.90
12	5.60	3.97	4.80	5.21
13	5.93	3.74	4.20	4.87
14	5.09	4.60	4.58	5.77
15	5.72	4.20	4.73	6.93
16	5.55	4.05	5.06	5.88
17	6.62	3.94	5.44	6.56
18	4.79	4.39	4.15	4.98
19	4.44	3.56	4.35	4.82
20	4.81	4.00	3.33	4.23
21	5.08	4.43	4.26	5.90
22	5.35	4.22	4.54	6.34
23	5.52	4.67	4.70	5.23
24	5.51	3.34	4.63	5.39
25	5.73	4.47	4.02	5.54
26	5.88	4.26	5.40	6.09
27	5.46	4.10	4.41	5.05
28	5.89	4.66	5.31	6.13
29	5.86	4.43	4.87	5.24
30	5.11	4.26	4.72	5.57
31	6.21	4.56	5.85	6.72
32	5.71	4.45	5.63	7.10
33	5.59	3.98	4.62	5.55
34	5.77	4.52	5.70	6.19
35	5.99	3.92	5.21	5.86
36	5.71	3.98	4.42	5.30
37	5.42	3.92	4.64	5.70
38	6.01	4.47	5.05	6.34
39	5.55	3.86	4.83	5.99
40	6.36	4.88	5.57	6.87
$\bar{X} \pm S$	5.69 ± 0.49	4.25 ± 0.43	4.77 ± 0.54	5.77 ± 0.66

Note: Unit: millimeters.

severe and homogeneous narrow ($S = 45$, $p < .0001$) (Table 5, Figures 2 and 3). We suppose that we could had a lower mortality rate simultaneously without compromising model quality if we rotated 10–20 times. We will continue to explore the balance point between the ideal model and the lowest mortality rate in our subsequent experiments.

TABLE 2 Stenosis degree in the control group.

Case	The lumen of trachea		Tracheal cartilage ring		Stenosis degree (%)
	r_l	r_s	R_l	R_s	
1	5.88	4.20	6.25	4.80	8.78
2	5.29	3.34	5.65	3.90	9.63
3	6.01	3.78	6.47	4.34	9.44
4	5.96	3.76	6.80	3.96	9.67
5	6.15	4.51	7.24	4.70	10.72
6	6.57	4.12	7.39	4.45	9.71
7	6.41	5.01	7.02	5.46	8.49
8	6.61	5.25	7.17	5.74	8.13
9	5.43	5.00	6.26	5.35	10.16
10	5.28	4.45	5.73	4.86	8.12
11	5.64	4.60	6.28	5.18	10.65
12	5.60	3.97	6.17	4.36	9.12
13	5.93	3.74	6.58	3.99	8.51
14	5.09	4.60	5.73	5.02	9.86
15	5.72	4.20	6.47	4.61	10.47
16	5.55	4.05	6.20	4.56	10.78
17	6.62	3.94	6.92	4.58	8.17
18	4.79	4.39	5.27	4.73	8.20
19	4.44	3.56	4.86	4.00	9.71
20	4.81	4.00	5.60	4.27	10.74
21	5.08	4.43	5.48	4.93	8.65
22	5.35	4.22	6.12	4.48	9.72
23	5.52	4.67	6.39	4.97	10.30
24	5.51	3.34	5.85	3.80	8.29
25	5.73	4.47	6.17	4.96	8.36
26	5.88	4.26	6.45	4.78	9.71
27	5.46	4.10	6.01	4.58	9.73
28	5.89	4.66	6.64	5.04	9.67
29	5.86	4.43	6.55	5.00	10.91
30	5.11	4.26	5.71	4.74	10.33
31	6.21	4.56	6.70	4.91	7.24
32	5.71	4.45	6.18	4.99	9.04
33	5.59	3.98	6.05	4.55	9.72
34	5.77	4.52	6.26	5.07	9.18
35	5.99	3.92	6.36	4.39	7.81
36	5.71	3.98	6.13	4.45	8.41
37	5.42	3.92	5.85	4.33	8.25
38	6.01	4.47	6.55	4.93	8.71
39	5.55	3.86	6.15	4.39	10.72
40	6.36	4.88	6.90	5.37	8.39

Note: Calculation formula of stenosis degree is $(1 - [r_l + r_s]/[R_l + R_s]) \times 100\%$, where r_l and r_s are the longest caliber and the shortest caliber of the laryngotracheal lumen, respectively, and R_l and R_s are the longest diameter and the shortest diameter of the circular arc formed by the laryngotracheal cartilage, respectively.

Dohar et al.[20] observed that depth and not circumferential extent was best correlated with the pathogenesis of LTS. Other animal studies also noted that injuries to the mucosa and submucosa typically resulted in normal healing. In contrast, injuries that reached the underlying lamina propria, perichondrium, or cartilage, resulting in fibrotic repair, not regeneration.[16,20,40,41] In this study, the experimental

TABLE 3 Stenosis degree in the experimental group 1.

Case	The lumen of trachea		Tracheal cartilage ring		Stenosis degree (%)
	r_l	r_s	R_l	R_s	
1	3.72	3.17	6.07	5.57	40.81
2	4.91	3.25	6.84	4.57	28.48
3	5.02	2.29	6.64	5.44	39.49
4	3.94	2.54	5.43	4.59	35.33
5	5.08	2.80	6.72	5.58	35.93
6	4.70	2.54	5.58	4.52	28.32
7	4.23	3.36	6.36	4.62	30.87
8[a]	4.42	3.13	6.17	4.44	28.84
9	5.09	2.15	6.77	4.81	37.48
10	5.08	3.50	5.85	4.65	18.29

Note: Calculation formula of stenosis degree is $(1 - [r_l + r_s]/[R_l + R_s]) \times 100\%$, where r_l and r_s are the longest caliber and the shortest caliber of the laryngotracheal lumen, respectively, and R_l and R_s are the longest diameter and the shortest diameter of the circular arc formed by the laryngotracheal cartilage, respectively.
[a]Died from sputum bolt.

TABLE 4 Stenosis degree in the experimental group 2.

Case	The lumen of trachea		Tracheal cartilage ring		Stenosis degree (%)
	r_l	r_s	R_l	R_s	
1	3.02	1.03	6.23	4.76	63.15
2	3.05	2.29	6.22	5.40	54.04
3	2.40	2.00	5.82	5.17	59.96
4	4.48	2.44	6.66	5.08	41.06
5	3.48	2.70	5.76	5.15	43.35
6	2.50	1.92	6.12	5.43	61.73
7	2.89	2.30	5.65	5.02	51.36
8	2.96	2.41	5.52	5.15	49.67
9[a]	2.16	1.68	6.33	5.21	66.72
10	2.05	1.69	6.10	5.19	66.87
11	2.13	1.52	5.58	5.47	66.97
12[a]	2.07	1.73	6.19	5.52	67.55
13	2.85	2.15	6.90	6.06	61.42
14	3.06	2.15	6.85	6.29	60.35
15	3.20	2.29	6.90	4.97	53.75
16[b]	1.71	0.59	7.03	4.86	80.66
17	1.89	1.37	6.15	5.53	72.09
18[a]	2.01	1.59	6.71	4.87	68.91
19	1.90	1.55	6.10	5.25	69.60
20	3.14	2.07	6.79	5.23	56.66

Note: Calculation formula of stenosis degree is $(1 - [r_l + r_s]/[R_l + R_s]) \times 100\%$, where r_l and r_s are the longest caliber and the shortest caliber of the laryngotracheal lumen, respectively, and R_l and R_s are the longest diameter and the shortest diameter of the circular arc formed by the laryngotracheal cartilage, respectively.
[a]Died from stenosis and sputum bolt.
[b]Died from stenosis.

groups had full thickness mucosal injuries with cartilage exposure confirmed by FOL (Figure 1). Therefore, we considered that cartilage involvement was a critical component and triggered the development of LTS. We found the intraluminal stenosis mainly included proliferation of fibroblasts, thickening of collagen fibers, cartilage deformity/new cartilage formation, increased number of capillaries, and

TABLE 5 Comparison of stenosis between control group and experimental groups.

Group	N	Stenosis degree (%)	χ^2/S	p
Control group	40	9.31 ± 0.98	47.98[a]	<.0001
Experimental group 1	9	32.78 ± 7.07		
Experimental group 2	16	58.25 ± 8.96		
Control group	40	9.31 ± 0.98	405[b]	<.0001
Experimental group 1	9	32.78 ± 7.07		
Experimental group 1	9	32.78 ± 7.07	45[b]	<.0001
Experimental group 2	16	58.25 ± 8.96		

[a]Variance unequal, Kruskal–Wallis test (χ^2).
[b]Wilcoxon rank sum test (*S*).

inflammatory cells (Figure 3), which was consistent with literature reports.[2,15,22] In previous literature reports,[11,31,33] sizing of the airway with ETT or subjective endoscopic evaluation was performed and documented. We believe that this method is not accurate enough. We brought ImageViewer_X64_V2.0.4.0630 software to measure the internal transverse and anteroposterior diameter of the lumen and the native airways. By which we can accurately calculated the degrees of LTS. Meanwhile, we establish a normative data base of the cross-sectional areas of the trachea and subglottis in the natural rabbits (Tables 1 and 2), which paves the way for further research assessing 3D printed drug-loading coating stent for the treatment of LTS.

The present study has several limitations. Firstly, the number of rabbits used is small. Secondly, the laryngotracheal injury disrupts the cartilaginous exoskeleton by open approach. However, most airway injuries occur intraluminal and not extraluminal illustrating a limitation of the model. Our team will explore the establishment of animal models for LTS through endoscopic technology alone in the future. Finally, deciding when scar tissue becomes mature and chronic is not just a matter of time, but also what happens at the histological level at different timepoints of an injured tissue. This study did not describe the differences in wound healing histopathological changes at different timepoints, nor did it determine when mature scars formed.

5 | CONCLUSIONS

We successfully developed a novel method to establish a reproducible survival rabbit model of LTS over a long-term follow-up period. This method consists of the processes of tracheotomy, direct visualization with FOL, nylon brush scraping of laryngotracheal mucosa, and closure of the trachea. We achieved relatively precise scraping depth and localization through FOL visualization combined with tracheostomy. We believe that our model can be used to help elucidate mechanisms and pathophysiology of LTS, and test novel minimally invasive therapeutic modalities.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

All data generated or analyzed during this study are included in this published article.

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