의학박사 학위논문

백서에서 풍선이관성형술 후 조직병리 변화에 대한 연구

Serial Histopathologic Changes after Eustachian Tube Balloon Dilation in Rats

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이 논문을 의학박사 학위 논문으로 제출함

2021 년　1 월

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Summary

Purpose: Though balloon dilation has shown promising results for dilatory Eustachian tube (ET) dysfunction, it is unclear how the ET changes histologically after ET balloon dilation (ETBD). We aimed to evaluate the serial histopathologic changes of the ET after ETBD in a rat model.

Materials and Methods: Twenty male Wistar rats (10 weeks old) were housed according to the principles and procedures described in the National Institute of Health (NIH)'s Guide for the Care and Use of Laboratory Animals. The left ET was dilated with a balloon catheter of 1mm in diameter and 5mm in length and the right ET was used as a control. The rats were randomly assigned to 4 groups of 5 rats each. Five rats were sacrificed immediately after the balloon dilation, 5 after 1 week, 5 after 4 weeks and 5 after 12 weeks for histological examination. Histopathologic evaluation included changes of the epithelial cells, presence of squamous metaplasia, and the proportion of the goblet cells present in the epithelium; changes of the vascular structures and dimensions of the submucosa (degree of fibrotic changes); and presence of cartilage fracture and thickness of the encircling cartilage.

Results: Immediately after ETBD, we observed desquamation of nearly all epithelial cells. There were no significant changes in the submucosa. Four out of 5 tubal cartilages were fractured. At 1-week post-ETBD, there was partial recovery of ciliated epithelia cells along with squamous metaplasia and epithelial hyperplasia. Goblet cells were not recovered at this time point. The depth of the submucosa had increased; however, this was not statistically significant when the area was used for comparison. Vascular structures increased in the submucosa. There were no changes in the thickness or the area of cartilage. By 4-weeks post-ETBD, goblet cells were re-encountered, squamous metaplasia and epithelial hyperplasia decreased. There was persistent thickened submucosa, as measured both by depth and area. There were no changes in the thickness or the area of cartilage. At 12-weeks post-ETBD, the proportion of goblet cells, squamous metaplasia and epithelial hyperplasia were similar to the contralateral normal ET. There was persistent thickened submucosa. The surrounding cartilage was healed and no changes in the thickness or the area of cartilage were identified.

Conclusion: Immediate histopathologic changes of the epithelium after ETBD were de-

epithelialization which was recovered by squamous metaplasia and hyperplasia which was observed at 1-week post-ETBD. Goblet cells were recovered after 4 weeks post-ETBD. The submucosa was persistently thickened and vascular structures increased after ETBD. Cartilage fractures healed with no change in its dimensions. This study is the first report describing the serial histological changes after ETBD and would be helpful for understanding the histological changes after ETBD and planning future animal studies for histological examination after placing various types of stents which could be used for intractable ET dysfunction.

Keywords: Eustachian tube, Balloon dilation, Eustachian tube dysfunction, rat model

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INTRODUCTION

Eustachian Tube

The human middle ear is a functional system which consists of the nasopharynx and Eustachian tube (ET) situated anteromedially and the mastoid air cells posterolaterally.[1] In adults, the ET lies at an angle of 45 degrees to the horizontal plane, and at 0 degrees in infants.[2] Structurally, the ET comprises two portions: the bony ET and the cartilaginous ET. The bony portion is about one-third of the total ET and located in the petrous part of the temporal bone. The cartilaginous portion is about two-thirds and is surrounded by tubal cartilage and the tensor veli palatini muscle.[3,4]

In the healthy ET, the osseous portion is always open, whereas the cartilaginous portion is closed at rest and opens in response to movements such as swallowing, yawning, or doing the Valsalva maneuver. This opening of the ET equalizes the middle ear with the atmosphere. The cartilaginous portion plays the main role in the dynamic function of the ET with the tensor veli palatini muscle as its only active dilator.[1] The levator veli palatini can also aid ET opening by elevating the soft palate and medially rotating the cartilaginous lamina.[5] Passive closure of the tubal lumen is due to extrinsic forces of the surrounding tissues and the recoil of elastic cartilage.

As the sole connector of middle ear to the nasopharynx, a functioning ET is important for maintaining a healthy well-aerated middle ear.[6] The functions of the ET include not only the middle ear ventilation, but also the transport of secretion and protection against pathogens and nasopharyngeal reflux.[7,8] Therefore, dysfunction of the ET can lead to the development of acute and chronic otitis media, one of the most common disease entities encountered in otolaryngology practice.[9]

Eustachian tube Dysfunction

Conditions that interrupt proper opening, such as inflammatory response of the lumen of the tube by irritants and infectious reactions, can result in ET dysfunction (ETD). Irritant reactions include the response to allergens, gastro-esophageal reflux disease, chemicals such as smoke and infectious reactions involve viral or bacterial infection.[5,10,11] The inability of the ET to open results in the failure

of gas equilibrium between the middle ear and nasopharynx. The atmospheric gases diffuse across venous capillary cell membranes in the middle ear, so dilatory dysfunction of the ET results in net negative pressure within the middle ear, leading to symptoms such as aural fullness or 'popping sounds', reduced hearing, tinnitus, autophony, otalgia, and imbalance.[11]

Existing literature on the prevalence of ETD in adults is limited. Overall prevalence of ETD among adults in the United States was estimated to be 4.6%.[12] Results from other studies range from 0.9%[13] to 48.5% in patients with chronic rhinosinusitis.[14] Until recent years, the otolaryngologist's understanding of the Eustachian tube dysfunction (ETD) was limited and few treatment options were available.[15-20] However, , surgical management is now possible with the introduction of the Eustachian tube balloon dilation (ETBD).[21]

Eustachian Tube Balloon Dilation

Since Ockermann et al. reported their first experience of ETBD in 2010,[22] many studies have shown the procedure to be feasible and safe for the treatment of ET dysfunction.[23-30] The reported success rates were between 36% and 80%.[25,31,32] The reported effect of ETBD differs according to which outcome parameters were used. In the studies with a minimum of 12-month follow-up, the Valsalva maneuver improved in 80-98%, subjective symptoms in 73-98%, otoscopic findings in 90%, tympanometry in 24-54%, and tubomanometry in 28-43% of the patients.[32]

Some of ETD patients treated with ETBD are unresponsive, and in some cases, the effect of balloon dilation gradually even decreases over time. Although these studies report that ETBD is superior to conventional medical management, there is still a population of ETD patients who do not respond to this dilation treatment. No consensus has been made for further management of failure cases of ETBD. This is mainly due to limited understanding of mechanism behind ETBD due to the lack of histologic studies of ET after ETBD.

Proposed underlying mechanism of ETBD are microtears in the cartilaginous part of the ET[21] and a decrease in mucosal inflammation and reducing the load of biofilm infections.[33] One histopathologic

study by Kivekas et al. found thinning of the mucosa, shearing of epithelium and crush injury to the submucosa, especially to lymphocytic infiltrates as immediate response to balloon dilation, and healthy pseudocolumnar epithelium and replacement of lymphocytic infiltrate with a thinner layer of fibrous tissue as postoperative findings.[33] One drawback of this study was that the ET mucosa specimens were taken from the nasopharyngeal opening of the ET which does not include the true cartilaginous portion.

Aim of Study

Histopathologic exam of the whole human cartilaginous ET after ETBD is impossible. Therefore, an animal study is warranted to verify the histologic changes of the ET after ETBD. In this study, we aimed to evaluate the serial histopathologic changes of the ET after balloon dilation in a rat model.

MATERIALS AND METHODS

Animal Study

This study was approved by the Institutional Animal Care and Use Committee at our institution and conformed to US National Institutes of Health guidelines for humane handling of laboratory animals. All rats were supplied with food and water ad libitum and were maintained at 22 ± 2 °C with a 12-hour day night cycle.

Twenty male Wistar rats (10 weeks old) were housed according to the principles and procedures described in the National Institute of Health (NIH)'s Guide for the Care and Use of Laboratory Animals. The left ET was dilated with a balloon catheter of 1mm in diameter and 5mm in length. The rats were randomly assigned to 4 groups of 5 rats each for serial histological examination. Five rats were sacrificed immediately after the balloon dilation, 5 after 1week, 5 after 4weeks and 5 after 12weeks (Fig. 1).

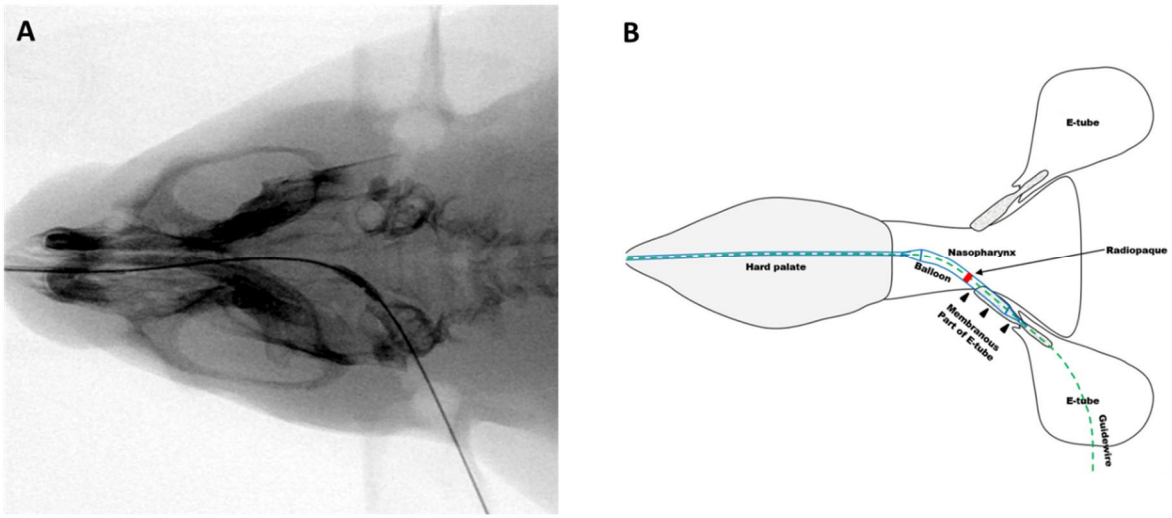
The contralateral ET was not ballooned, acting as control.

Figure 1. Time points at which histologic examinations were carried out and the number of animals sacrificed at each time point.

Fluoroscopic Eustachian tube balloon dilation

All procedures were performed under fluoroscopic guidance by an interventional radiologist and otologist. Anesthesia was induced by means of intramuscular injection of 50mg/kg zolazepam and tiletamine (Zoletil 50; Virbac, Carros, France) and 10mg/kg xylazine (Rompun; Bayer Healthcare, Leverkusen, Germany). After disinfection of the left external auditory canal with 0.05% chlorhexidine hydrochloride, left tympanic membrane was punctured by micro puncture needle (Cook, Bloomington, US). The needle was directed 45 degrees anteriorly, and in horizontal plane under fluoroscopic guidance. Tip of the needle was located inside tympanic cavity and about 0.2 ml of contrast media was injected to the tympanic cavity to visualize the tympanic orifice of the ET. Then, tip of the needle was relocated close to the tympanic orifice. A 0.014-inch microguidewire (Transend, Boston Scientific, Fremont, US) was gently advanced through the ET and came out of the nasal cavity. After removal of micro puncture needle, micro balloon catheter for coronary balloon (Genoss, Suwon, Korea) was introduced over the guidewire from the nasal side and located at middle portion of the ET to cover whole ET. Balloon catheter was inflated at pressure of 10 atmosphere for 1 minute (Fig. 2). During inflation of the balloon catheter, waist formation and subsequent disappearing of the waist was observed. Micro balloon catheter and micro guidewire were removed after deflation of the balloon.

Figure 2. Fluoroscopic image (A) and schematic image (B) of the Eustachian tube balloon dilation (ETBD). The micro balloon catheter of 1 mm in diameter and 5 mm in length was located at middle portion of the Eustachian tube and inflated at pressure of 10 atmosphere for 1 minute.



Histopathologic examination

Rats were sacrificed by administering inhalable pure carbon dioxide and decapitated surgically. The mandible and anatomical structures anterior to the soft palate were resected. The nasopharyngeal opening of the ET was identified. Soft tissue structures around the ET were dissected off taking care not to disrupt the anatomy of the ET. Head samples were fixed in 10% neutral buffered formalin for 48 hours, followed by decalcification for 2 weeks (Osteosoft, Merck, Germany). The samples were coronally sliced from nasopharynx to posterior wall of the tympanic cavity every 200 μm. Paraffin blocks were prepared from the sliced head samples and 5 μm-thick slices obtained with an MR2258 microtome (Histoline, Pantigliate, Italy). The slides were stained with hematoxylin-eosin.

Whole slides of the ET were reviewed. The cartilage surrounding the ET was first identified. The section in which the sectioned ET cartilage was in the form of a 'comma' shape, showing both the cartilaginous and nasopharyngeal epithelia, was selected for analysis (Fig. 3). Evaluation included changes of the epithelial cells, changes of dimensions of the submucosa (degree of fibrotic changes), and changes of the encircling cartilage.

In the epithelium, presence of goblet cells, squamous metaplasia and epithelial hyperplasia was searched. In order to quantify the changes of these findings in the epithelium, the length of the epithelium exhibiting goblet cells, squamous metaplasia or epithelial hyperplasia was measured and divided by the whole perimeter of the ET lumen of the corresponding slide. The proportion of the epithelium with goblet cells, squamous metaplasia and epithelial hyperplasia was compared among 5 groups: normal group, immediate, 1 week, 4 weeks, and 12 weeks after ETBD.

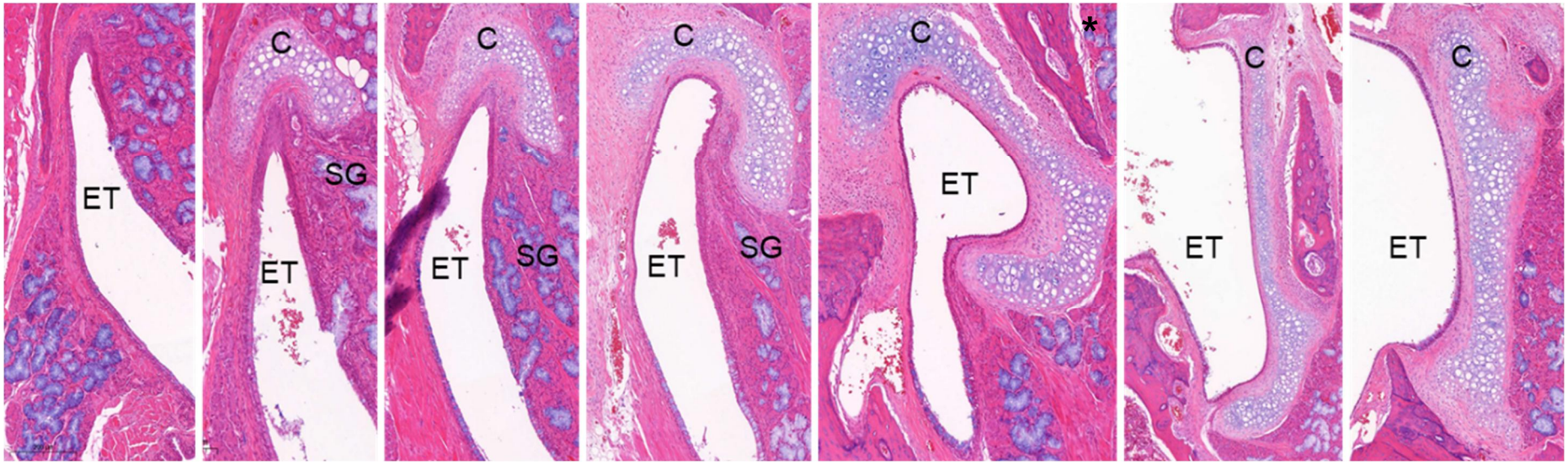
In the submucosa, vessels were identified. Vessels under the tubal cartilage were counted and analyzed as numbers per slide.

In order to evaluate the extent of fibrosis caused by ETBD, the depth of the submucosa was measured. The depth of the submucosa of the left ET (balloon) was measured at 9 points in total. The depth of submucosa at medial, middle and lateral points of the surrounding cartilage was measured. The same was done for sections 200 μm proximal and distal to the reference slide. Then the 9 measurements

were averaged to obtain the representative depth of submucosa. The depth of the submucosa of the right ET (normal) corresponding to the points measured on the left side was also obtained for normal value.

Due to the variation of the angles at which the ETs were sectioned among slides, using only linear measurements may not accurately represent the thickness of the submucosa. Therefore, another measurement, the area of the submucosa, was also calculated. The histomorphometric analysis was obtained through CaseViewer (version 2.4, 3DHISTECH, Budapest, Hungary). The area of the cartilage, epithelium and lumen were recorded. The relative proportions of observed absolute values were also compared.

Figure 3. Normal histology of the right-side Eustachian tube sectioned from the nasopharynx (left) to the tympanic bulla (right). Sectioned coronally at 200 μm interval, in the caudal direction from the medial to lateral sections. C; cartilage, ET; Eustachian tube, SG; submucous glands. * The section in which the ET cartilage was in the form of a 'comma' shape, showing both the cartilaginous and nasopharyngeal epithelia, was selected for analysis.



Stastical analysis

The changes were assessed by the Mann Whitney U test among groups. SPSS version 22.0 software (IBM Corporation, Armonk, NY) was used for all statistical analyses. P values of <0.05 were considered statistically significant.

RESULTS

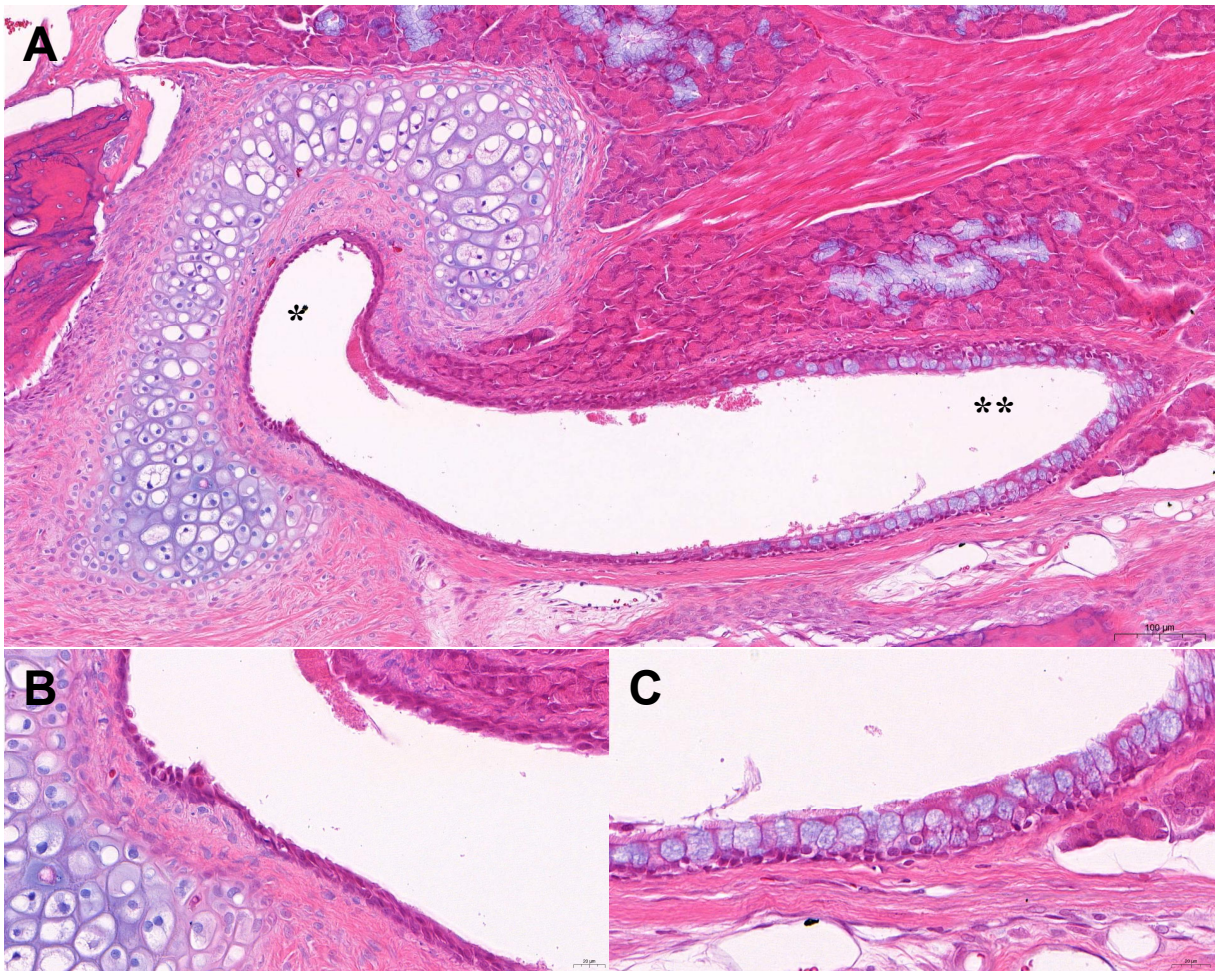
Normal Histological Findings of the Rat Eustachian Tube

The epithelium of the rat ET is ciliated respiratory epithelium. The ET epithelium can be divided into the cartilaginous end and the nasopharyngeal end (Fig. 4). The epithelial cells at the nasopharyngeal end are columnar and has goblet cells (secretory mucous cells). The epithelial cells at the cartilaginous end are more cuboidal in shape and contains little secretory mucous cells.

The ET is surrounded by cartilage in the posterosuperior direction. There is thin submucosa between the epithelium and the cartilage. The submucosa consists of fibroblasts, collagen fibers and a few vessels. There was no lymphocytic involvement in the ET.

As the ET extends toward the nasopharynx, the cartilage disappears and submucous glands appear, especially in the medial portion.

Figure 4. (A) Normal histology of the rat Eustachian tube. The ET consists of the cartilaginous end (*) and the nasopharyngeal end (**). (B) High-power field view of the epithelium at the cartilaginous end. The cells are cuboidal and contains no goblet cells. (C) High-power field view of the epithelium at the nasopharyngeal end. The cells are columnar and include goblet cells.



Changes of the Epithelium after ETBD

At the nasopharyngeal end, the epithelial cells were desquamated immediately after ETBD. At 1 week post-balloon, the morphology of the epithelial cells was recovered but goblet cells were not seen. At 4 weeks post-balloon, the goblet cells could be identified but in a less orderly manner. After 12 weeks, the columnar epithelium and goblet cells were fully recovered (Fig. 5)

Figure 6 shows the changes in the portion of the epithelium with the goblet cells present in proportion to the whole perimeter of the ET lumen. The proportion of the goblet cells in the epithelium decreased to 0 immediately after ETBD and remained 0 until 1week. At 4 weeks, the proportion of the goblet cells increased significantly (28.7 ± 16.5 %) so that no statistical differences could be recognized compared to the normal group (32.0 ± 3.4 %). At 12 weeks, the proportion of the goblet cells remained at a similar level compared to the normal group (31.9 ± 6.8 %).

At the cartilaginous end, the epithelial cells were desquamated immediately after ETBD. At 1-week post-balloon, the epithelial cells changed their morphology into squamous metaplasia. At 4 weeks, the epithelial hyperplasia decreased, and the original morphology was finally recovered at 12 weeks post-ETBD (Fig. 7).

Figure 8 shows the changes in the portion of the epithelium with squamous metaplasia in proportion to the whole perimeter of the ET lumen. The proportion of squamous metaplasia significantly increased at 1-week post-dilation (33.7 ± 6.4 %), then decreased significantly to 10.1 ± 7.7 %. At 12 weeks after ETBD, the proportion of squamous metaplasia decreased to 4.2 ± 3.7 %, which showed no statistical difference compared to its normal control.

Figure 9 shows the changes of the maximum cell layer count of the epithelium. Immediately after ETBD, all epithelium fell off therefore the maximum cell layer count was 0. There was significant increase of the maximum cell layer count at 1-week post-dilation (5.0 ± 0.9), which decreased to 2.8 ± 0.4 and 2.4 ± 0.5 at 4 weeks and 12 weeks, respectively.

Figure 5. Serial changes of histopathology of the nasopharyngeal end of the rat ET. Left column shows low-power field and the right column, high-power field view of the epithelium. The epithelial cells were desquamated immediately after ETBD. At 1week post-balloon, the morphology of the epithelial cells was recovered but goblet cells were not seen. At 4- and 12-weeks post-balloon, the columnar epithelium and goblet cells were fully recovered.

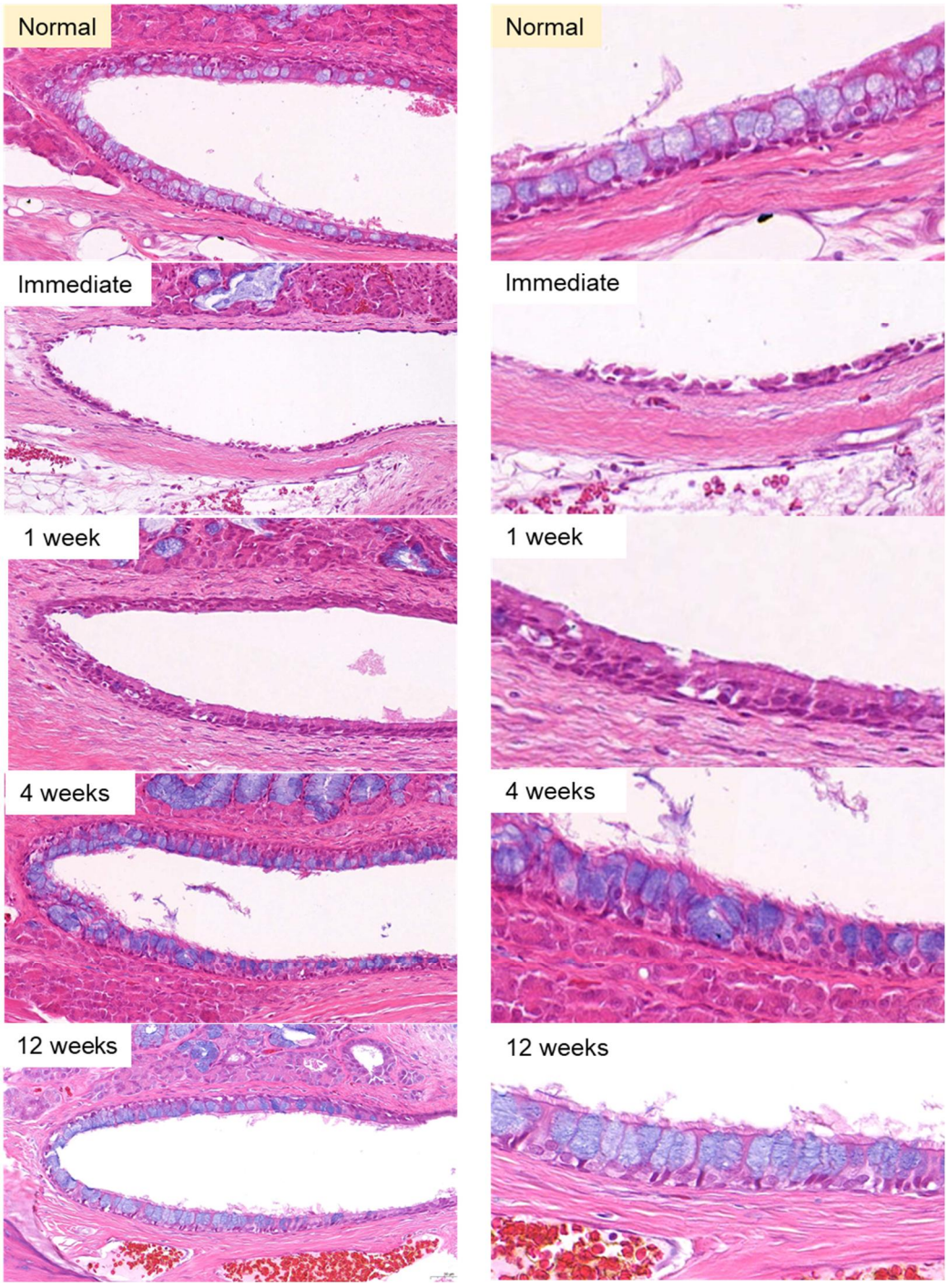


Figure 6. Serial changes of the proportion of the goblet cells present in the epithelium. * $p<0.05$.

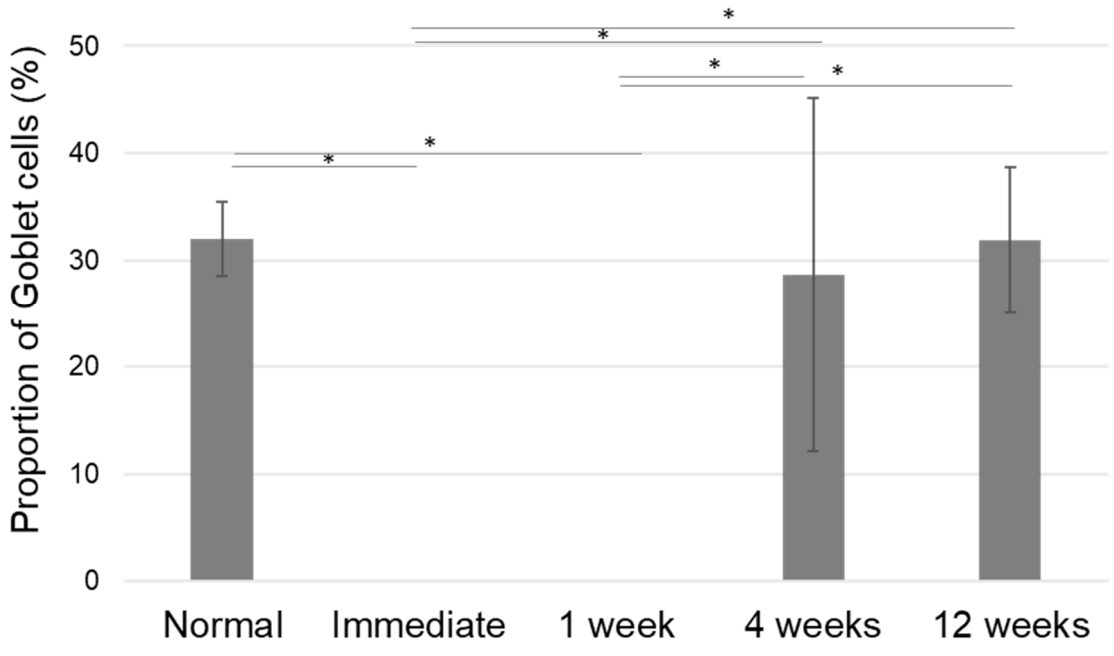


Figure 7. Serial changes of histopathology of the cartilaginous end of the rat ET. Left column shows low-power field and the right column, high-power field view of the epithelium. The epithelial cells were desquamated immediately after ETBD. At 1-week post-balloon, the epithelial cells changed their morphology into squamous metaplasia. At 4 weeks, the epithelial hyperplasia decreased, and the original morphology was finally recovered at 12 weeks post-ETBD.

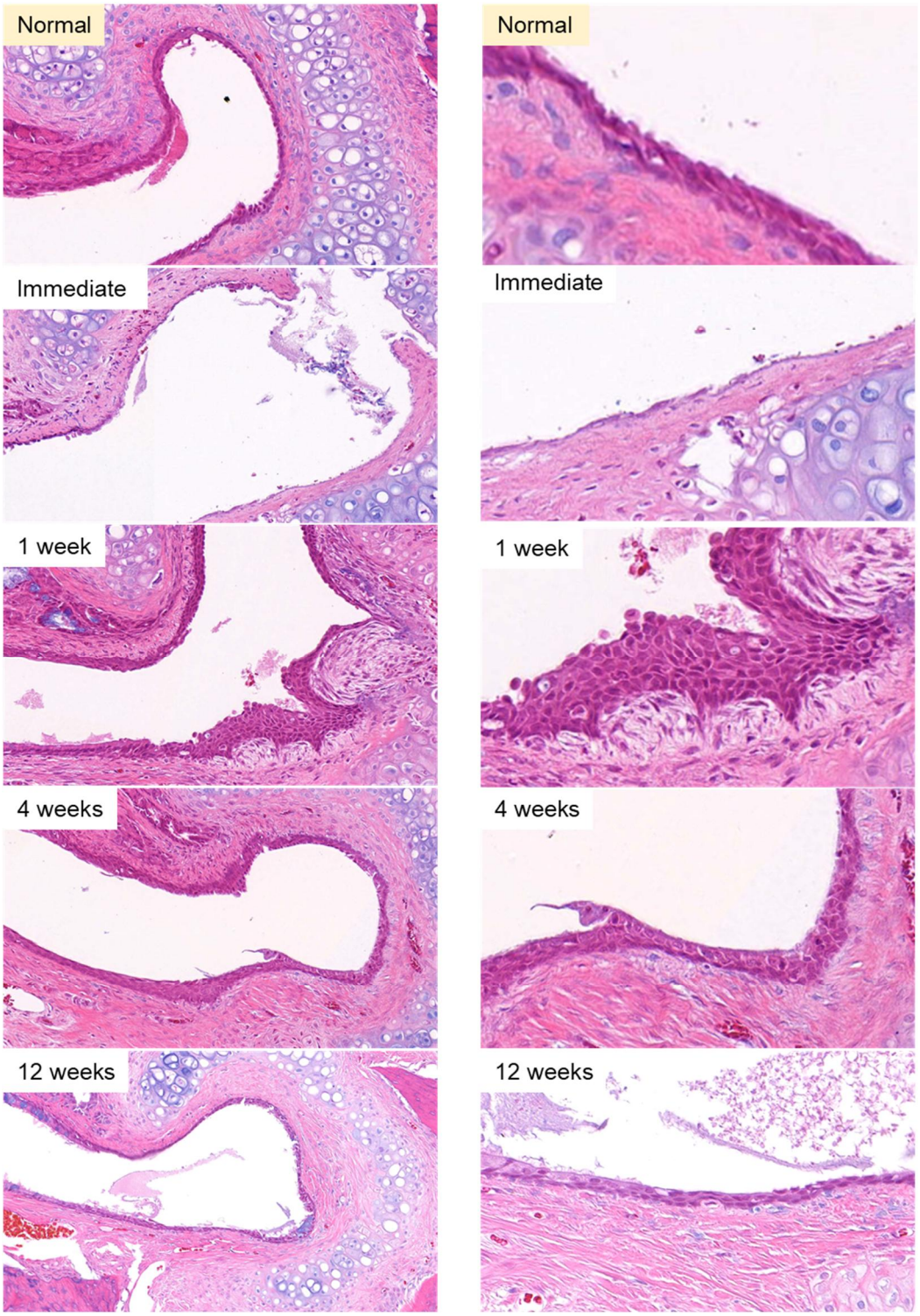


Figure 8. Serial changes of the proportion of squamous metaplasia present in the epithelium. * $p<0.05$.

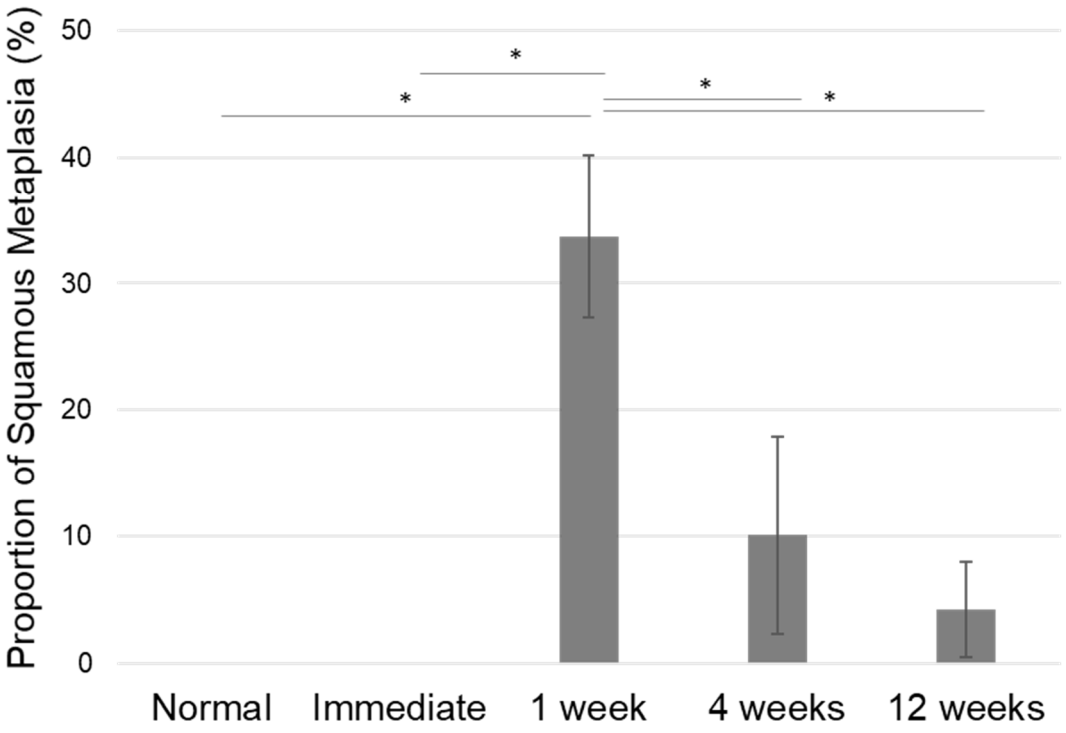
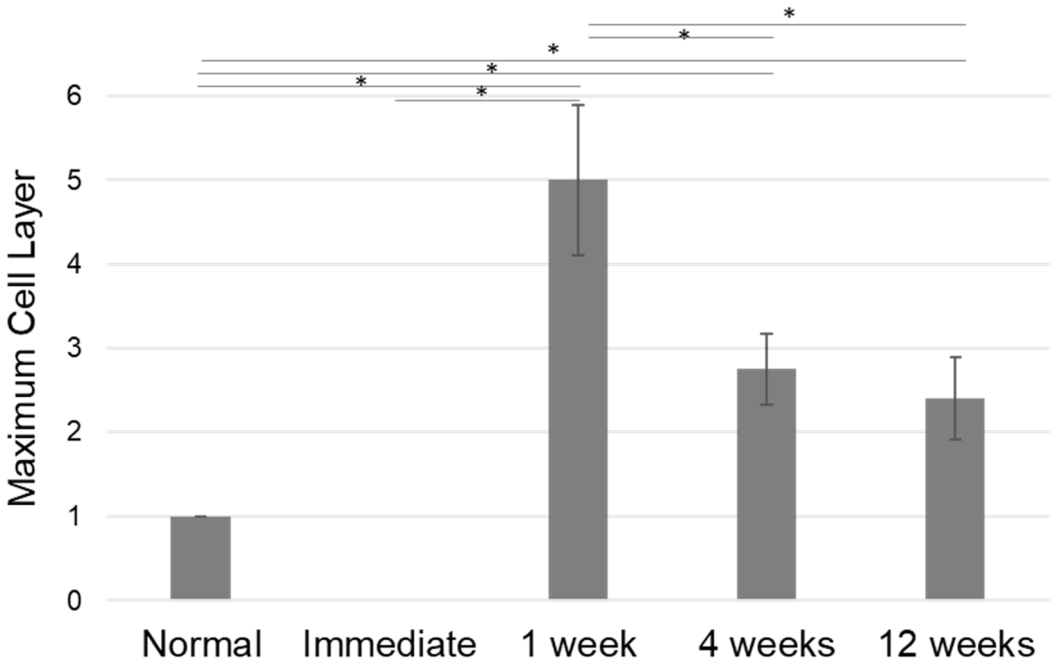


Figure 9. Serial changes of the of the maximum cell layer counts of the epithelium. * $p<0.05$.



Serial changes of submucosa after ETBD

Figure 10 shows the changes of the vascular structures in the submucosa. Immediately after ETBD, no vessels were could be identified. The average number of vessels per slide was 2.4 ± 1.4, 2.2 ± 1.3 and 2.4 ± 2.0 at 1-week, 4-weeks, and 12-weeks post-ETBD, respectively.

Figure 11 shows the changes of the depth of submucosa. No significant changes of depth were seen immediately after ETBD, but it was increased to 157±54.3 μm after 1 week. The depth of submucosa at 12 weeks was 105.2 ± 20.7 μm which was significantly thicker compared to the normal group (58.5 ± 21.3 μm, $p = 0.03$).

When the submucosa was compared by the area calculated in each histology sections, the area of submucosa immediately after ETBD and 1 week after ETBD was 59658.8 ± 11000.9 μm^2, 59565.4 ± 12127.2 μm^2 respectively, which was similar to the normal group (25029.3 ± 5766.9 μm^2). The area of submucosa was largest at 4 weeks (160532.6 ± 26206.8 μm^2) and it decreased to 74263.68 ± 20734.79 μm^2 at 12 weeks, which was significantly larger compared to the normal group ($p = 0.03$, Fig. 12).

When the proportion of the area of the submucosa (%area) was used for comparison, it was increased to 23.2 ± 1.3 % at 1 week, 24.4 ± 2.6 % at 4 weeks, and 25.9 ± 1.8 % at 12 weeks. The proportion was significantly larger at 12 weeks compared to the normal group (18.7 ± 2.4 %, $p = 0.03$, Fig. 13).

Figure 10. Serial changes of the vascular structures in the submucosa. * $p<0.05$.

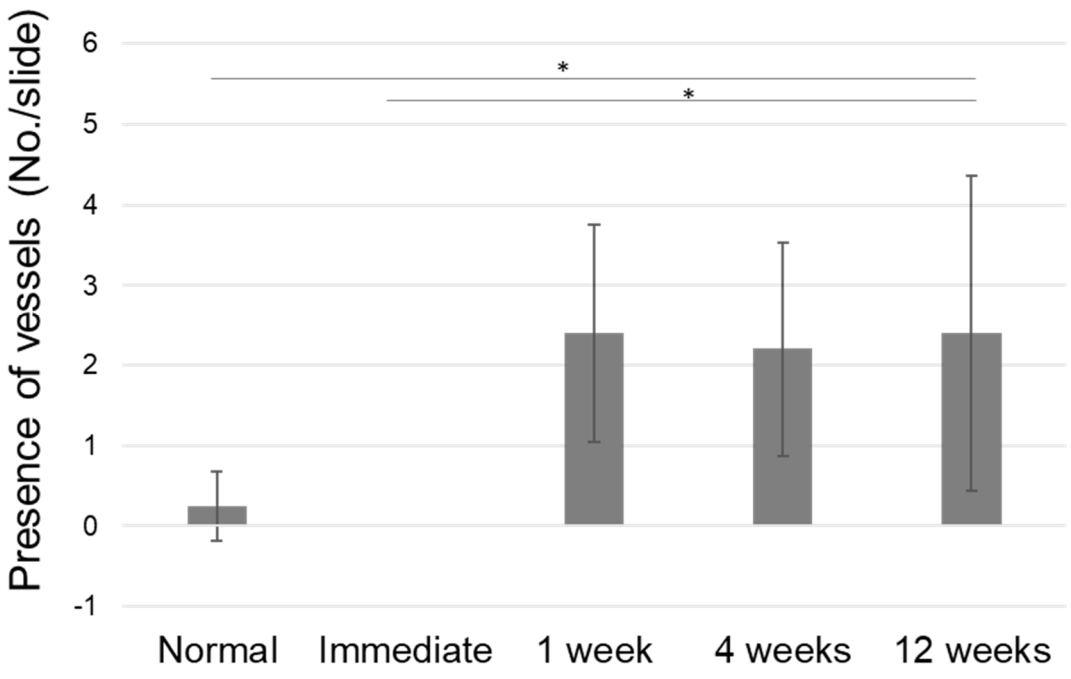


Figure 11. Serial changes of the of the depth of submucosa. * $p<0.05$.

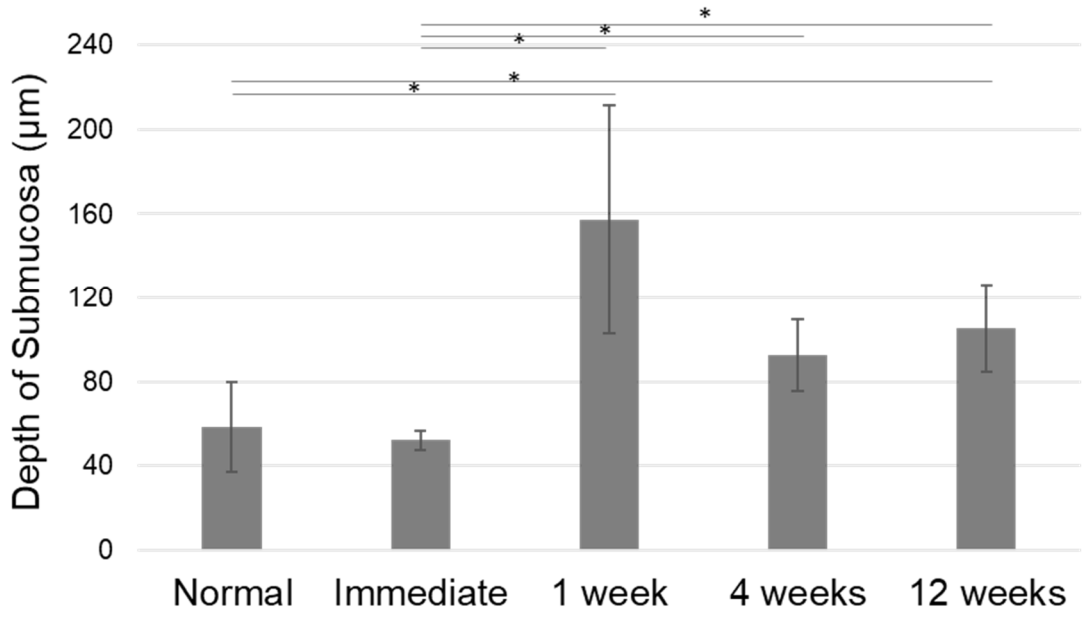


Figure 12. Serial changes of the area of submucosa. * $p<0.05$.

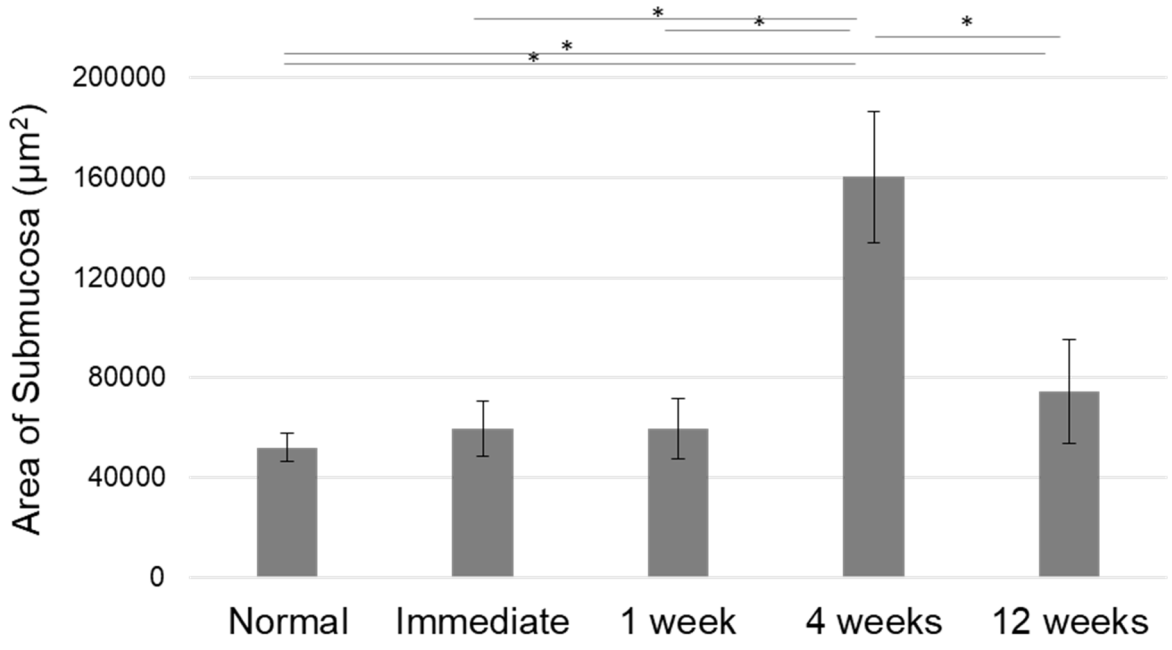
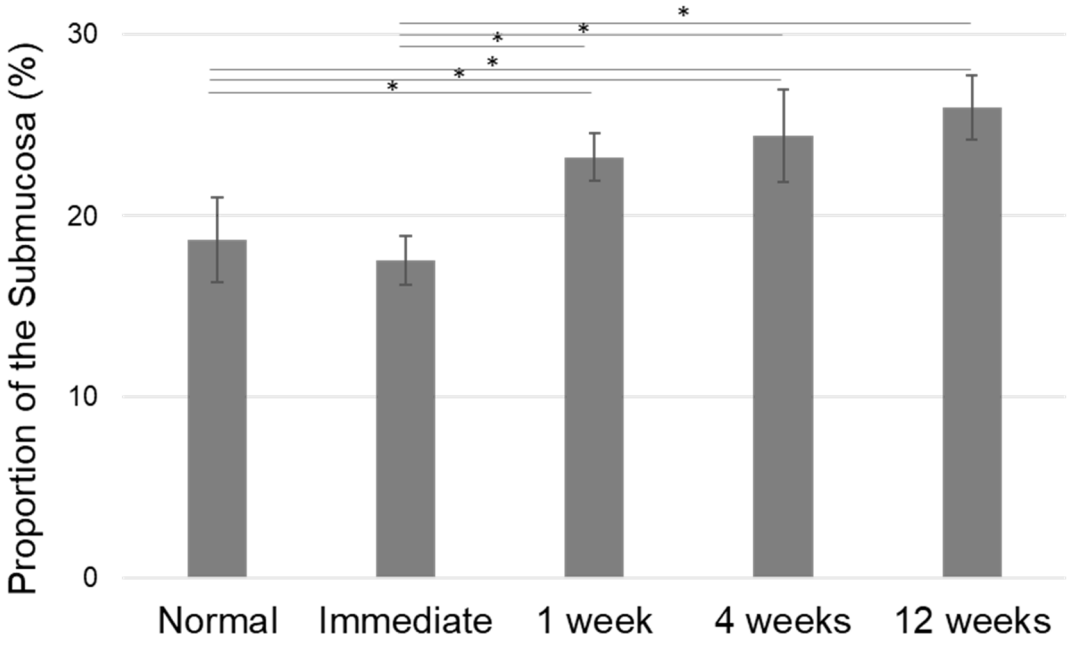


Figure 13. Serial changes of the % area of submucosa. * $p<0.05$.



Serial changes of tubal cartilage after ETBD

Out of the 20 ETs that were analyzed, 6 were without frank fracture lines. Of the remaining 14 ETs, a total of 21 fracture lines were identified. These fracture lines were found at 3 distinctive locations: at midpoint between the medial and lateral lamina of the tubal cartilage, and the lateral 2 points that divide the lateral lamina into thirds (Fig. 14). The frequency at which the fracture lines were identified were 48%, 19%, and 33% respectively, from the midpoint toward the far lateral. All of the cartilage fracture lines were observed immediately after ETBD.

The anterior-to-posterior diameter of the tubal cartilage, from the most medial point to the most lateral point showed no difference throughout 12 weeks (Fig. 15).

Figure 16 shows the serial changes of the area of the cartilage. Like the anterior-to-posterior diameter of the tubal cartilage, there were no serial changes regarding the area of cartilage.

Figure 17 shows the serial changes of the proportion of the area of the cartilage. The proportion of the area of the cartilage decreased after 4 weeks ($43.6 \pm 5.1\%$, $p = 0.02$) and after 12 weeks ($49.9 \pm 4.4\%$, $p = 0.03$) compared to the normal group ($57.7 \pm 2.5\%$).

Figure 14. Sites of cartilage fracture. The fracture lines were found at 3 distinctive locations: at midpoint between the medial and lateral lamina of the tubal cartilage (arrow), and the lateral 2 points that divide the lateral lamina into thirds (arrowheads).

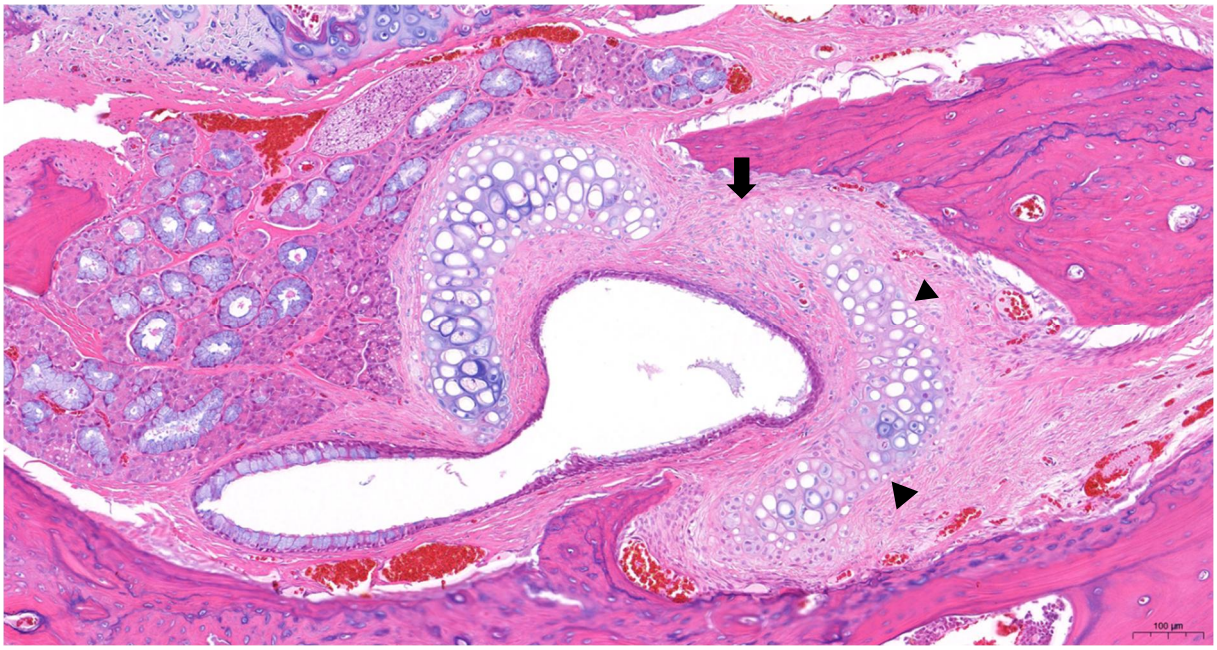


Figure 15. Serial changes of the anterior-to-posterior diameter of tubal cartilage. * $p<0.05$.

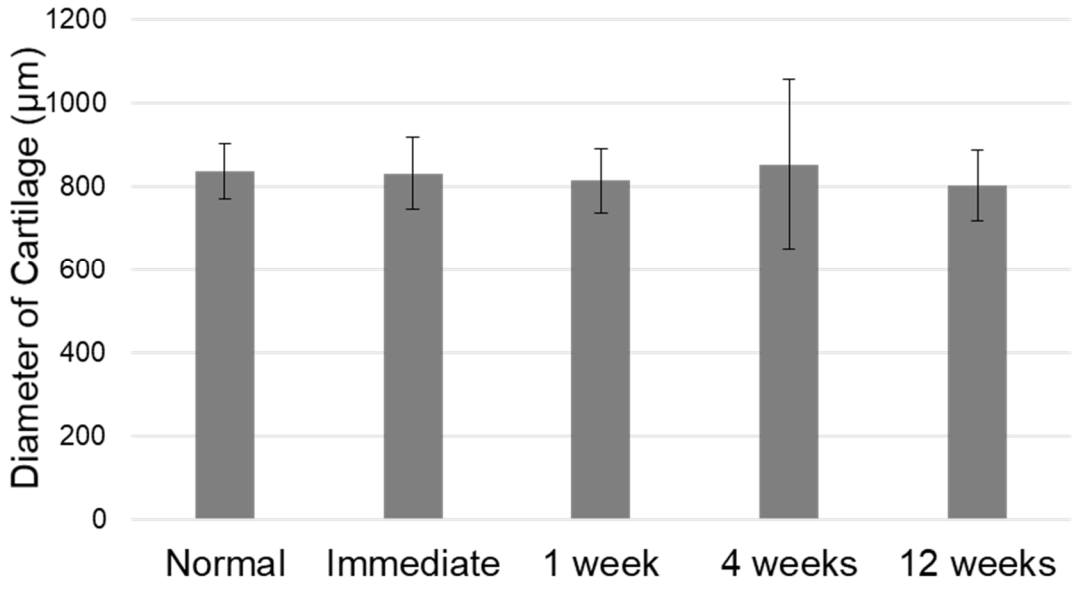


Figure 16. Serial changes of the area of tubal cartilage. * $p<0.05$.

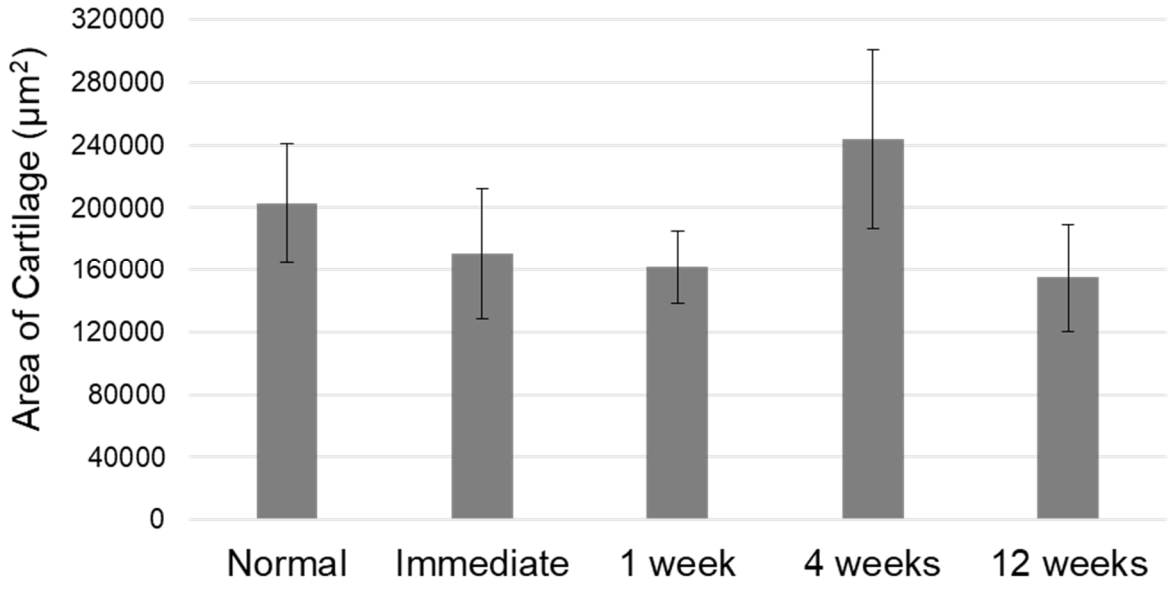
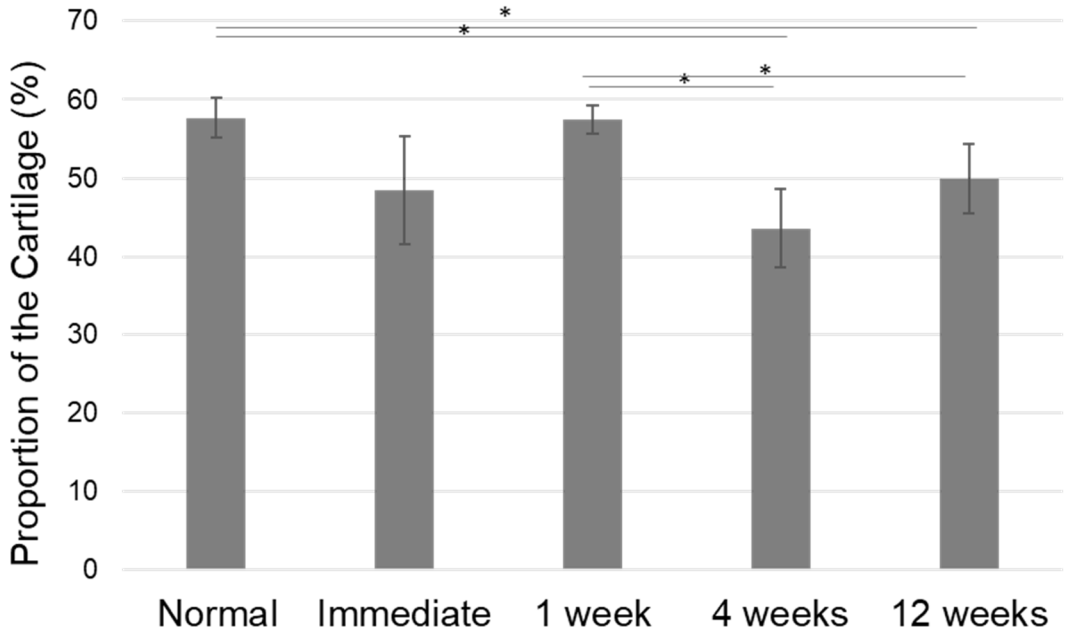


Figure 17. Serial changes of the % area of the tubal cartilage. * p<0.05.

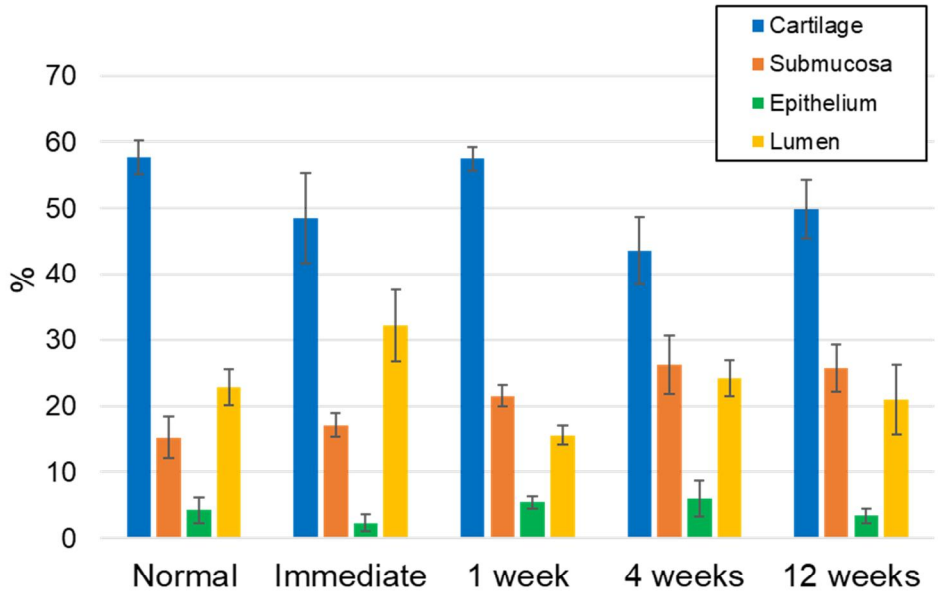


Summary of overall changes in the proportion of area

Figures 18 shows the overall changes in the proportion of area of cartilage, submucosa, epithelium, and lumen. The % area of cartilage is reduced at 4 weeks and 12 weeks post-ETBD as compared to the normal group. The % area of submucosa is increased at 1 week, 4 weeks and 12 weeks post-ETBD as compared to the normal group.

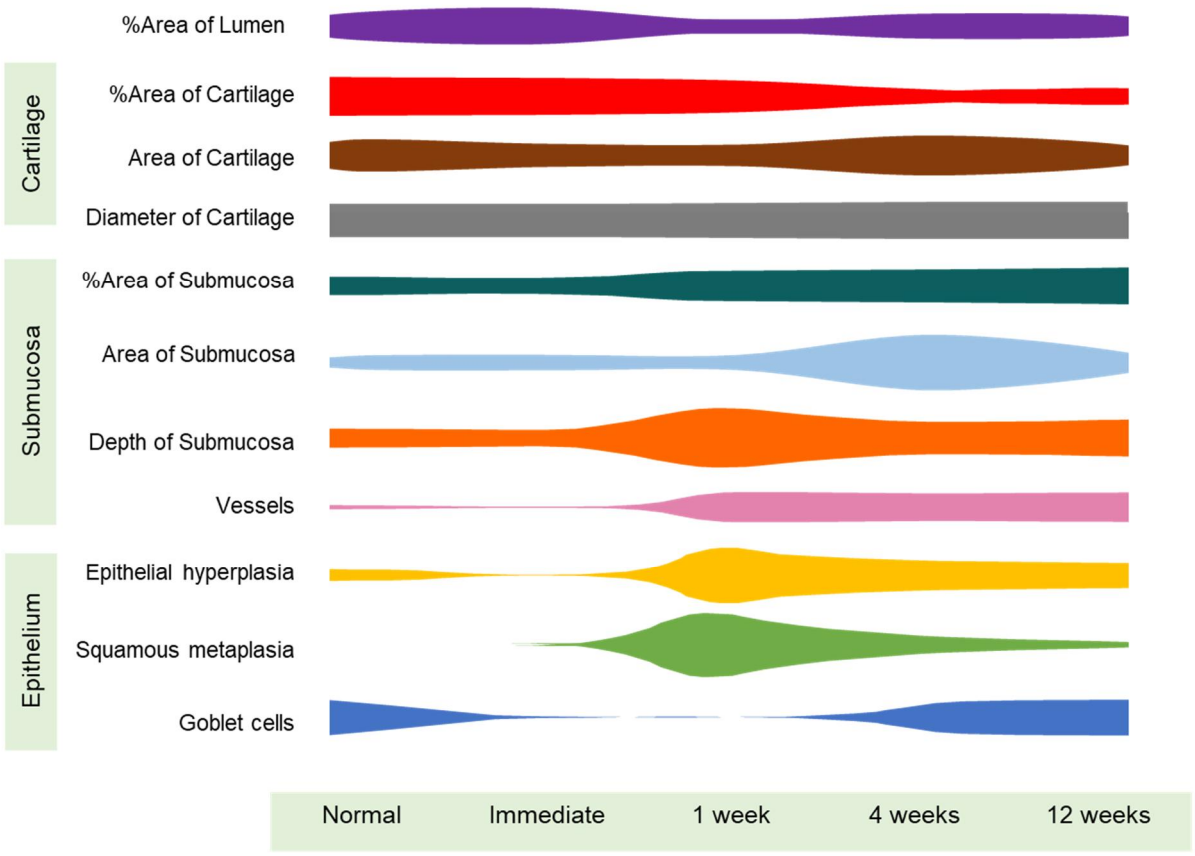
Figure 19 shows the summany of all the changes identified in this study regarding variables included in the epithelium, submucosa and cartilage.

Figure 18. Overall changes in the proportion of area of cartilage, submucosa, epithelium, and lumen. The % area of cartilage is reduced at 4 weeks and 12 weeks post-ETBD as compared to the normal group. The % area of submucosa is increased at 1 week, 4 weeks and 12 weeks post-ETBD as compared to the normal group. P values are shown in the area below the *x* axis. C; cartilage, S; submucosa, E; Epithelium, L; lumen.



	Normal	Immediate	1 week	4 weeks	12 weeks
Normal		C 0.114 S 0.343 E 0.200 L 0.057	C 0.905 S 0.032 E 0.413 L 0.016	C 0.016 S 0.032 E 0.286 L 0.730	C 0.032 S 0.032 E 0.730 L 0.556
Immediate			C 0.111 S 0.032 E 0.032 L 0.016	C 0.190 S 0.032 E 0.032 L 0.032	C 0.905 S 0.032 E 0.286 L 0.032
1 week				C 0.008 S 0.151 E 1.000 L 0.008	C 0.016 S 0.095 E 0.032 L 0.151
4 weeks					C 0.056 S 0.690 E 0.095 L 0.310

Figure 19. Summary of changes of variables included in the epithelium, submucosa and cartilage.



DISCUSSION

The present study investigated the serial histopathologic changes of the rat ET after balloon dilation up to 12 weeks postdilation. Immediately after ETBD, we observed desquamation of nearly all epithelial cells. There were no significant changes in the submucosa. Four out of 5 tubal cartilages were fractured. At 1-week, there was partial recovery of ciliated epithelia cells along with squamous metaplasia and epithelial hyperplasia. Goblet cells were not recovered at this time point. The depth of the submucosa had increased; however, this was not significant when the area was used for comparison. Vascular structures increased in the submucosa. There were no changes in the thickness or the area of cartilage. By 4-weeks post-ETBD, goblet cells were re-encountered, squamous metaplasia and epithelial hyperplasia decreased. There was persistent thickened submucosa, which was statistically significant, as measured both by depth and area. There were no changes in the thickness or the area of cartilage. At 12-weeks post-ETBD, the proportion of goblet cells, squamous metaplasia and epithelial hyperplasia were similar to the normal group. There was persistent thickened submucosa, which was statistically significant. The surrounding cartilage was healed and no changes in the thickness or the area of cartilage were identified also. Increased submucosal fibrosis and maintaining of the cartilage framework despite of the cartilage fractures were the major findings after ETBD in the current study. The clinical implication of our finding is that the increased submucosal fibrosis might be related to the recovery of the ET patency.

With the introduction of the ETBD, chronic ETD can be surgically managed but only 36-80% of the patients are responsive.[21,25,31,32] This means that despite the superiority of ETBD to conventional medical management, there is still a population of patients with unmet needs, necessitating a further management option. The difficulties in developing further managements lie in the lack of histology studies. Therefore, a thorough investigation of the histologic changes after balloon dilation might provide new insights to the possible options.

Due to practical issues, histopathologic studies of the human ET can only be carried out at the nasopharyngeal orifice. Kivekas et al. obtained pre- and postoperative biopsy specimens in 13 patients

who underwent ETBD. They suggested the crushing effect of the balloon on lymphocytes and lymphocytic follicles that were later replaced with thinner fibrous scar as a possible mechanism of therapeutic effect of the ETBD.[33] However, the whole length of the ET needs to be investigated to evaluate the effect of balloon dilation, especially as the cartilage surrounding the ET is considered a crucial structure in maintaining normal ET function. A proper histopathological study of the whole ET requires an appropriate animal model. Generally, sheep and pigs are considered to be good models of the human middle ear.[34] However, a smaller animal model would allow large scale and high throughput studies, making it cost-efficient, particularly as we intended to see tissue reactions after balloon dilation.[35] In this study, we were able to dilate the ET of rats using commercially available micro balloon catheters. Table 1 summarized the findings from the study by Kivekas et al. and the current study.

Table 1. Comparison of key findings of Eustachian tube histology in human (Kivekas et al.) and rats (current study).

Variables	Kivekas et al.	Current study
N	Preballoon (n=13)	Normal control (n=20)
	Immediate postballoon (n=13)	Immediate postballoon (n=5)
	5-12 weeks postballoon (n=3)	1-week postballoon (n=5)
		4-weeks postballoon (n=5)
		12-weeks postballoon (n=5)
Location of examineed mucosa	Nasopharyngeal mucosa	Mucosa in the cartilaginous ET
Epithelium	Epithelium & cilia quality, basal layer integrity: relatively preserved	Epithelial loss immediately after ETBD
		Squamous metaplasia, epithelial hyperplasia: initial increased at 1 week post-ETBD, then gradual decrease until 12 weeks post-ETBD
		Goblet cells: returned to normal level at 4 weeks post-ETBD
Submucosa	Submucosal lymphocytes, lymphoid follicles: decreased	Lymphocytes could not be analyzed
	Seromucinous glands: similar	
	Collagen quantity/fibrosis: similar	Increased in depth from 1 week post-ETBD
		Increased in area from 4 weeks post-ETBD
Cartilage	Cannot be analyzed because the ET mucosa was biopsied near to the nasopharynx	Fracture & healing
		No change in dimensions

The effect of ETBD seems to be related to the fibrosis of the submucosal connective tissue. Looking to histopathologic exams of balloon dilation in other tissues, Modi et al. performed balloon dilation of the rabbit subglottis.[36] The similarity between the subglottis and ET is that both structures have a cartilaginous framework lined by respiratory epithelium and mucosa. Thirty days after dilation, the authors found regeneration of a normal epithelial lining, with submucosal fibrosis, particularly where larger balloon sizes had been used. Similarly, submucosal fibrosis after ETBD, could possibly stiffen the ET.

In the urethra, another non vascular structure formed by the urothelium, submucosal connective tissue and smooth muscle, formation of strictures has been related to collagen rich connective tissue with few fibroblasts and smooth muscle fibers.[37] Excess type 3 collagen in comparison with type 1 collagen has been detected in cases of ureteral strictures and is considered to be the culprit behind ureteral fibrosis and stenosis of the lumen.[38-41]

The epithelial damage in our study was observed immediately after the dilation; however, the mucosal lining was regenerated at 1 week post-ETBD, goblet cells were recovered by 4 weeks post-ETBD and the squamous metaplasia and epithelial hyperplasia decreased to normal levels at 12 weeks post-ETBD. The epithelium is thought to recover between 4 and 12 weeks. It can be argued that regenerated healthy mucosa will be thinner, facilitating ET opening, and the possibly recovered function of cilia and mucus production may aid the clearance of secretions from the middle ear.[42] In addition, ET mucosa is known to generate a surfactant that reduces surface tension, which would be reduced in OME,[43,44] and the restored healthy mucosa may secrete surfactant, thus aiding ET opening.

The dimensions of the tubal cartilage did not change throughout the study period. Cartilage healing process after fracture in general is an equilibrium between deposition of type I collagen (scar tissue) and expression of type II collagen (repair). Small full-thickness cartilage defects are mainly replaced by fibrocartilage, whereas partial-thickness defects heal by deposition of fibrous scar tissue.[45] Full-thickness cartilage fractures were induced in the current study. Furthermore, for cartilaginous structures,

there is a tendency to return to its original shape unless the deformation is maintained for several months.[46] These factors could account for the non-existing changes in the cartilage framework. The fact that the dimensions of the cartilage did not change could suggest the safety of ETBD. If the thickness of the cartilage did change, we cannot exclude the possibility of cartilage contracture leading to worsened luminal stricture.

The main limitation of the current study was that no functional assessment of the ET could be done. The ET is a dynamic organ that remains closed at rest and opens during certain movement such as swallowing or yawning. The compliance of the ET is a known factor of its proper function.[47] Therefore we cannot ascertain whether fibrosis (low compliance) induced by balloon dilation would positively affect the ET function. Another limitation is that this study was carried out on rats with normal ET. In the normal rat ET, we cannot analyze lymphocytic involvement. Also, ETBD induced thicker submucosa but no frank changes in the lumen. In a chronically inflamed ET, the lumen would be narrower therefore a rat model of otitis media and ETD would provide better knowledge of the histologic changes of ETBD.

CONCLUSION

Major histopathologic changes of the ET after ETBD were de-epithelialization followed by squamous metaplasia and re-epithelialization. Goblet cells were recovered after 4 weeks. Vascular structures increased. There was persistent thickened submucosa. Cartilage fractures healed with no change in its dimensions. The effect of ETBD might be related to the fibrosis of the submucosal connective tissue. This study is the first report describing the serial histological changes after ETBD and would be helpful for understanding the histological changes after ETBD and planning future animal studies for histological examination after placing various types of stents which could be used for intractable ET dysfunction.

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국문 요약

배경: 이관기능장애 환자에서 풍선이관성형술은 유망한 결과를 보이지만, 아직 풍선이관성형술 후 이관에 어떠한 조직학적 변화가 생기는지 어떠한 기전으로 이관기능이 호전되는지 아직 알려지지 않았다. 본 연구에서는 백서 모델에서 풍선이관성형술 후 이관에 시간에 따른 어떤 조직병리학적 변화가 생기는지 연구하고자 한다.

방법: 20 마리의 수컷 Wistar rat (10주 령)의 왼쪽 이관에 직경 1mm, 길이 5mm의 풍선 카테터를 삽입하여 확장하였다. Wistar rat는 각각 5마리씩 4개 그룹에 무작위로 배정되었다. 풍선 확장 직후 5마리, 1주 후 5마리, 4주 후 5 마리, 12주 후 5 마리를 희생시켰다. 조직 평가에는 상피 세포의 변화, 점막하의 깊이 변화 (섬유화 변화 정도) 및 이관을 둘러싸는 연골의 변화를 포함하였다.

결과: 풍선이관성형술 직후 상피층은 탈락하고 1주차에 다시 회복되기 시작하였다. 술잔세포는4주차, 12 주차에 정상 이관과 비슷한 분포를 보였다. 1주차에 편평상피화생이 관찰되었고 4주차, 12주차에는 감소하여 정상 수준과 차이 없게 되었다. 상피 증식 또한 1주차에 가장 많았고 4주, 12주차에 감소된 양상이었다. 그러나 정상과 비교하였을 때 12주차에 상피 증식은 유의미하게 증가되어 있었다. 점막하층의 혈관 구조물은 1주차부터 증가되어 12주차에 유의미하게 증가된 양상을 보였다. 점막하층의 두께와 면적은 1주차

부터 지속적으로 증가되어 12주차까지 증가된 상태로 유지되었다. 이관 연골은 48%가 중간 지점에서, 52%가 외측에서 발생하였다. 내측 외측 두께는 풍선이관성형술 후 변화가 없었다. 연골의 면적 변화 또한 정상과 비교하여 1, 4, 12주차 시점에 변화가 없었다.

결론: 풍선이관성형술 후 이관의 주요 조직 병리학적 변화는 상피층의 탈락 후 재생, 점막 하층의 섬유층 증가이다. 연골은 골절 후 섬유화로 인한 치유가 되었으나 두께나 면적의 변화는 관찰되지 않았다. 본연구는 풍선이솬확장술 이후 발생한 이관의 변화를 확인한 첫 보고로 풍선확장술이후의 조직의 변화를 이해하는데 도움이 될 것으로 생각되면, 향후 스텐트 등 추가적인 치료법에 대한 연구에 기초자료로서 사용될 수 있을 것으로 생각된다.