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ORIGINAL ARTICLE

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Development of novel layered polyglycolic acid sheet for regeneration of critical-size defect in rat trachea

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Key question

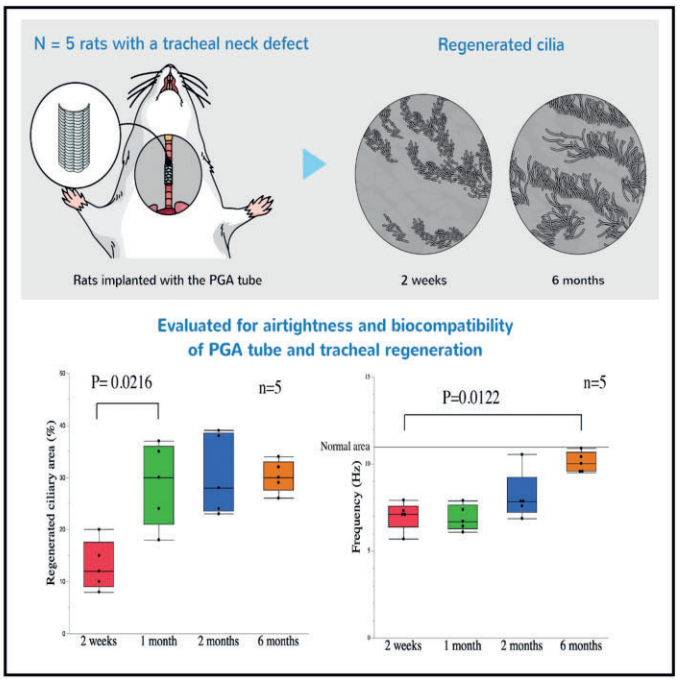
Is a novel layered PGA material effective in covering the central airway as a tracheal replacement?

Key finding(s)

A novel layered PGA material introduces tracheal regeneration both morphologically and functionally.

Take-home message

This PGA material appears to be a promising artificial trachea.



Abstract

OBJECTIVES: Polyglycolic acid (PGA) sheets are difficult to adapt to the central airway because of poor durability against high air pressure. Therefore, we developed a novel layered PGA material to cover the central airway and examined its morphologic traits and functional performance as a potential tracheal replacement.

METHODS: A critical-size defect in rat cervical tracheas was covered with the material. Morphologic changes were bronchoscopically and pathologically evaluated. Functional performance was evaluated by regenerated ciliary area, ciliary beat frequency and ciliary transport function determined by measuring the moving distance of microspheres dropped onto the trachea (μm/s). The evaluation time points were 2 weeks, 1 month, 2 months and 6 months after surgery (n = 5, respectively).

Presented at the 30th European Society of Thoracic Surgeons (ESTS) Meeting, The Hague, Netherlands, 21 June 2022.

RESULTS: Forty rats underwent implantation, and all survived. Histological examination confirmed ciliated epithelization on the luminal surface after 2 weeks. Neovascularization was observed after 1 month, tracheal glands after 2 months and chondrocyte regeneration after 6 months. Although the material was gradually replaced by self-organization, tracheomalacia was not bronchoscopically observed at any time point. The area of regenerated cilia significantly increased between 2 weeks and 1 month (12.0% vs 30.0%; $P = 0.0216$). The median ciliary beat frequency significantly improved between 2 weeks and 6 months (7.12 vs 10.04 Hz; $P = 0.0122$). The median ciliary transport function was significantly improved between 2 weeks and 2 months (5.16 vs 13.49 μm/s; $P = 0.0216$).

CONCLUSIONS: The novel PGA material showed excellent biocompatibility and tracheal regeneration both morphologically and functionally 6 months after tracheal implantation.

Keywords: Polyglycolic acid • Trachea • Regeneration

ABBREVIATIONS

CBF	Ciliary beat frequency
CTF	Ciliary transport function
DMEM	Dulbecco's modified Eagle's medium
PGA	Polyglycolic acid
SEM	Scanning electron microscopy

INTRODUCTION

Various materials have been investigated as alternatives to tracheal graft replacements, but the results have not been encouraging; thus, no biomaterial with satisfactory data for clinical use has been established [1]. Nonporous tracheal prostheses are now seldom used clinically, and the main reason is the lack of biocompatibility. Nonporous prostheses can reportedly cause the formation of granulation tissue due to insufficient vascularization or dehiscence at the anastomotic interface between the nonporous prosthesis and the native trachea [2]. From the viewpoint of *in situ* tissue engineering technology, external scaffold materials with good compatibility and adaptability is used to induce the self-repair of damaged parts and are replaced by autologous tissue.

Polyglycolic acid (PGA) sheets are a porous bioabsorbable material that functions as a good scaffold for tissue regeneration [3, 4]. PGA sheets have excellent biocompatibility and are often clinically used to treat pulmonary fistulas after lung resection in the field of general thoracic surgery [5, 6]. However, PGA sheets have inadequate durability against high air pressure in the central airway and therefore cannot be used to cover defects of the central airway.

To improve durability, we developed a novel layered PGA tube characterized by airtightness for the resistance of high central airway pressure and physical strength similar to that of the native trachea by changing the fibre thickness and braiding angle. The objective of this study was to confirm long-term survival after implantation of a novel PGA material and then verify the morphologic traits and functional performance of this novel PGA material as a potential tracheal replacement, which was reconstructed in critical-size rat tracheal defects.

MATERIALS AND METHODS

Ethics statement

All experimental protocols were approved by the Ethics Committee of the Graduate School of Medicine at Kyoto University (Medkyo21520, 5 March 2021).

Study design

The size of the critical tracheal defects was preliminarily determined using 20 rats. We then examined the long-term survival after implantation of the novel PGA material and the morphological characteristics and functional performance of the novel PGA material. According to our previous studies, the sample size was set at 5 rats per observational period: 2 weeks, 1 month, 2 months and 6 months after implantation [7]. However, because 2 rats were needed for the morphological and functional evaluation, the total sample size was set at 40 rats.

Animals

Male Wistar/ST rats aged 10–12 weeks and weighing 280–340 g were purchased from Japan SLC, Inc. (Hamamatsu, Japan). All animals received humane care in compliance with the Principles of Laboratory Animal Care formulated by the National Society for Medical Research.

Development of novel polyglycolic acid material

PGA fibers were made from PGA pellets (Kureha Corporation, Tokyo, Japan) using a melt spinning machine (Fibre Extrusion Technology, Leeds, UK). A PGA tube (braided angle, 45°; outer diameter, ∼3.0 mm) was fabricated from the fibers (Fig. 1A and B). The physical strength of the PGA tubes could be adjusted according to the PGA fibre thickness and the weave method.

The native tracheal strength of the rats was first measured to quantify the trachea's mechanical properties. To measure the mechanical strength of the native trachea, 10-mm-long specimens of the harvested trachea of 3 rats were placed on the table of an automatic compression tension tester, also known as a creepmeter (Yamaden, Tokyo, Japan), in the transverse axial direction (Fig. 1C). The load was continuously measured when the plunger contacted and compressed the trachea. The maximum load was measured when the plunger compressed the trachea by 1 mm. The load was measured several times for each sample, and these measurements were used to determine the strength required in the artificial trachea. The maximum load of the rat cervical trachea was 1.11 N and that of the PGA tube was 1.44 N (Fig. 1D). To test the airtightness of this PGA tube, the tube was submerged in a tank of saline solution kept at a temperature of 37°C and the air leakage pressure at the appearance of bubbles was measured. The pressure-resistant capacity was 25 cmH_2O.

Operative procedure

Determination of rat tracheal critical-size defect. The rats underwent general anaesthesia following intraperitoneal injections of

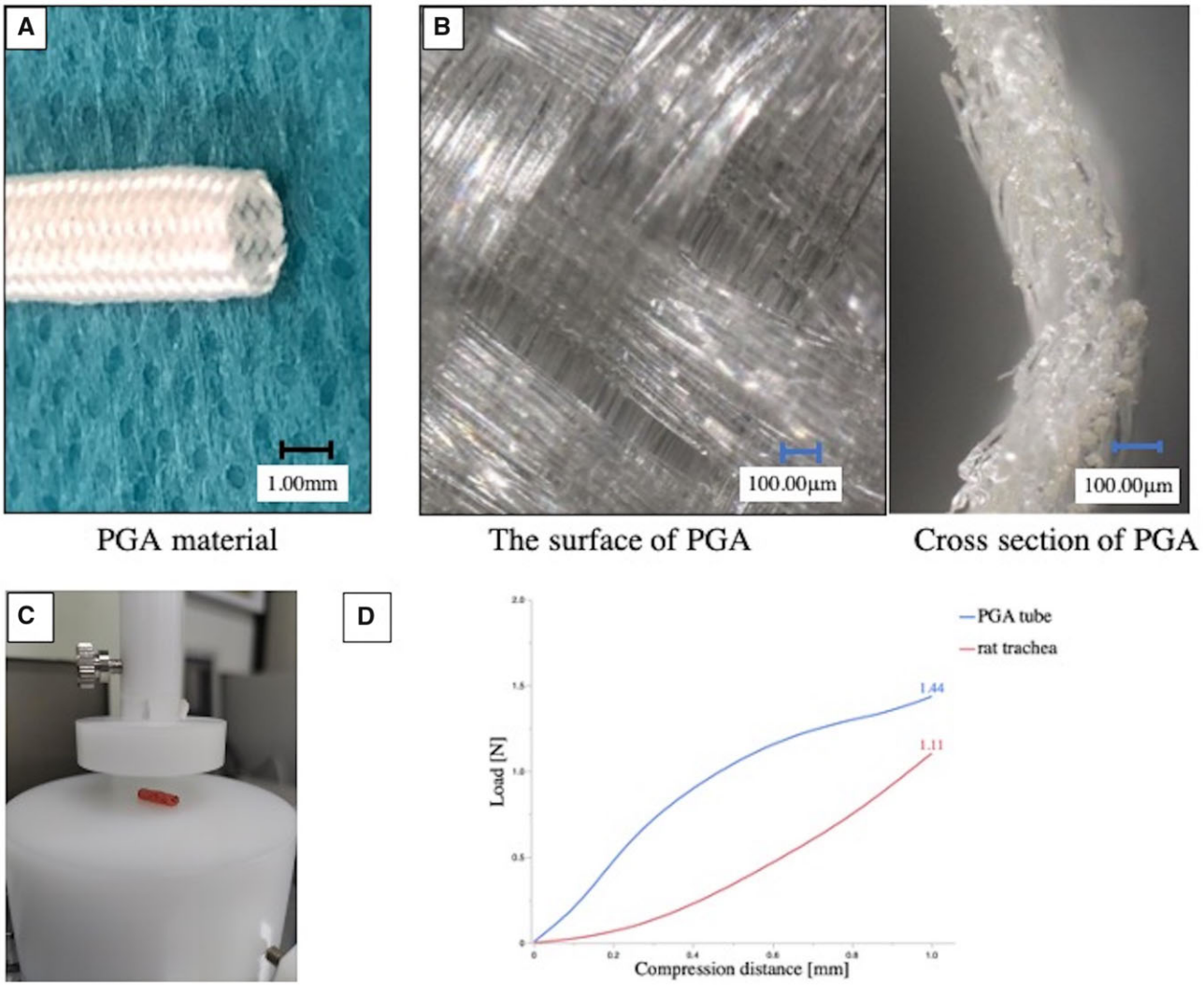


Figure 1: Novel PGA material. (**A**) The image shows a PGA material. (**B**) The surface and cross section of the PGA were evaluated with an electron microscope. (**C**) The maximum load of the artificial trachea was measured with an automatic compression tension tester. (**D**) The maximum load was found to be 1.44 N. PGA: polyglycolic acid.

medetomidine at 0.375 mg/kg (Nippon Zenyaku Kogyo, Fukushima, Japan), midazolam at 2.0 mg/kg (Astellas Pharma, Tokyo, Japan) and butorphanol tartrate at 2.5 mg/kg (Meiji Seika Pharma, Tokyo, Japan). The rats' cervical trachea was measured using a digital calliper (AS ONE Corporation, Osaka, Japan). Defects of 4-mm length (2 cartilage rings) and widths of 1, 2 or 3 mm (1/6, 1/3 and 1/2 of circumference, respectively) were created on the anterolateral wall of the cervical trachea using a scalpel (Fig. 2A–C). Muscle and skin were sutured with 5–0 Prolene, and survival was confirmed 2 weeks later.

Implantation of polyglycolic acid material in rat tracheal critical-size defect. A critical-size defect [semicircular tracheal defect of 4-mm length (2 cartilage rings) and 3-mm width] was created on the anterolateral tracheal wall of the rats and then covered with PGA (Fig. 4A). The tracheal defect and PGA were anastomosed with 4 stitches of 5–0 Prolene interrupted sutures. The airtightness, PGA biocompatibility and tracheal regeneration were evaluated with respect to 5 evaluation items: bronchoscopic examination, histological analysis, scanning electron microscopy (SEM), ciliary beat frequency (CBF) and ciliary transport function (CTF). The evaluation time points were 2 weeks, 1 month, 2 months and 6 months after surgery.

Evaluated outcomes

The primary outcome was the long-term survival after implantation of the novel PGA material, which was defined as the percentage of rats that were alive at the 4 observation points: 2 weeks, 1 month, 2 months and 6 months after implantation. The secondary outcomes were the morphological characteristics and functional performance of this novel PGA material as a candidate for tracheal replacement. To evaluate the morphological characteristics, we examined the bronchoscopic and histological findings. To verify functional performance, we analyzed the percentage of regrowth, CBF and CTF over the study period.

Evaluation

Bronchoscopic examinations. All rats were anesthetized with intraperitoneal injections and examined with a bronchoscope of 1.9-mm diameter and 10-cm length (HOPKINS® Telescope 0°;

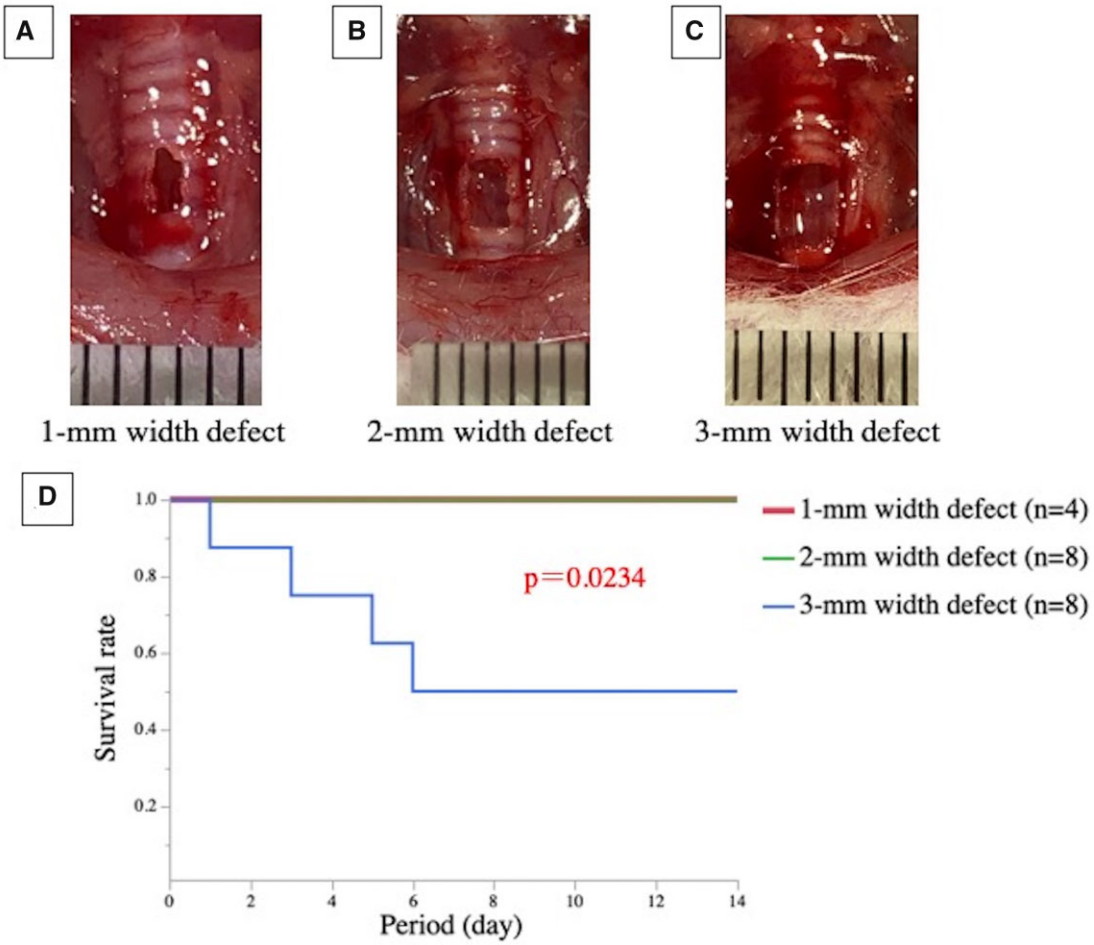


Figure 2: Determination of critical-size rat tracheal defect. Defects with a length of 4 mm (2 cartilage rings) and a width of (**A**) 1 mm, (**B**) 2 mm or (**C**) 3 mm (1/6, 1/3 or 1/2 of circumference, respectively) were created on the anterolateral wall of the cervical trachea using a scalpel. (**D**) A defect with a length of 4 mm and a width of 1 mm was created in 4 rats, and a defect with a length of 4 mm and a width of 2 or 3 mm was created in 8 rats. All rats with a 1- or 2-mm-wide defect survived, but 50% of the rats with a 3-mm-wide defect died within 7 days after surgery.

KARL STORZ Endoscopy Japan K.K., Tokyo, Japan) under spontaneous breathing. The airtightness of the material, breathing pattern of the rats (tracheomalacia-like dyskinesia) and intraluminal details of the trachea were recorded.

Histological analysis. After the bronchoscopic examinations, the animals were sacrificed. The trachea was then removed, fixed in 10% formalin and embedded in paraffin. Embedded tissues were subsequently sliced transversely at the centre of the PGA material. The deparaffinized tissue sections were stained with haematoxylin and eosin and examined by another thoracic surgeon and a pathologic specialist.

Scanning electron microscopy. The trachea was removed and fixed in 2.5% glutaraldehyde (Muto Co., Ltd., Tokyo, Japan) for 2 days and then fixed in 1% osmium tetroxide (Nacalai Tesque, Inc., Kyoto, Japan). The specimens were then dehydrated by sequential immersion in increasing concentrations of ethanol up to 100% followed by immersion in tertbutyl alcohol. Next, the samples were freeze-dried and coated with platinum ions under a vacuum, and the cilia of the luminal surfaces were observed using a scanning electron microscope (JSM-7900F; JEOL, Tokyo, Japan). Because ciliated cells appear darker than other epithelial cells in SEM images, the regenerated ciliary area was quantified by measuring the dark portions of the central 520 μm × 370-μm transplant area (Fig. 6A). The regenerated ciliary area was assessed with a microscope (BZ-X800; Keyence, Osaka, Japan).

The SEM observation area was defined as the implantation site divided into 3 parts, with the central third defined as the centre of the PGA material and the 2 edges as the periphery of the PGA material. The CBF and CTF were similarly evaluated at the centre of the PGA material.

Analysis of CBF. The trachea was removed, and the exposed luminal surface was immersed in Dulbecco's modified Eagle's medium (DMEM) (Nacalai Tesque) containing 4 μg/ml of fluorescein isothiocyanate-conjugated wheat germ agglutinin (Vector Laboratories, Burlingame, CA, USA) for 1 h. After being washed in DMEM, the stained tracheal cilia were laid in DMEM/nutrient mixture F-12 (DMEM/F-12; Nacalai Tesque), and the luminal surfaces were then observed under a microscope (BX-51N; Olympus Optical Co. Ltd., Tokyo, Japan) equipped with a stage-heater (37°C; Blast, Tokyo, Japan). Motile cilia of the regenerated epithelia were recorded at 125 frames/s using a high-speed camera

(FASTCAM Mini UX50; Photron, Tokyo, Japan), and the mean CBF in the region was analyzed using ImageJ (National Institutes of Health, Bethesda, MD, USA).

Analysis of CTF. Fluorescent microspheres (Polysciences Inc., Warminster, PA, USA) in DMEM/F-12 were dropped onto the luminal tracheal surfaces, and orally directed movements of the microspheres at the centre of the transplant areas were recorded at 50 frames/s using a FASTCAM Mini UX50 camera connected to a BX-51 microscope. Vector data for microsphere transport velocities between sequential frames were generated using DiaTrack software, and the speed of oral directional transport along the lung–oral axis was calculated.

Statistical analysis

Continuous data are presented as mean (standard deviation) or median with interquartile range. The survival rates of rats with critical-size tracheal defects were calculated using the Kaplan–Meier method, and the groups were compared using the log-rank test. The primary outcome was the percentage of rats that were still alive at each observation period, and the 95% confidence interval of each percentage was calculated. Data regarding the regenerated ciliary area, CBF and speed of orally directed microsphere transport were obtained from 5 rats of each group and compared using the Wilcoxon test. All statistical analyses were performed using JMP Pro 16 software, and *P*-values <0.05 were considered statistically significant.

RESULTS

Critical-size defect

In total, 20 rats underwent the experiment. The mean tracheal diameter in the 20 rats was 3.05 (standard deviation: 0.12) mm, and the median was 3.00 (interquartile range: 2.95–3.15) mm. A defect of 4-mm length and 1-mm width (1/6 of circumference) was created in 4 rats. A defect of 4-mm length and 2- or 3-mm width (1/3 or 1/2 of circumference, respectively) was created in 8 rats.

All 4 rats with a defect of 4-mm length and 1-mm width (1/6 of circumference) survived for 2 weeks. Two weeks after surgery, the defect area was covered with fibrotic tissue, and bronchoscopic and histological findings showed slight narrowing at the defect area.

All 8 rats with a 2-mm-wide defect survived, but 50% (4/8) of the rats with a 3-mm-wide defect died within 7 days after surgery (Fig. 2D). The bronchoscopic and histological findings showed more granulation and stenosis in rats with a 3- than 2-mm-wide defect (Fig. 3A and B).

Thus, the size of this semicircular defect was determined to be the critical defect size, and it was used as a model for the PGA tube implantation experiments.

Implantation of PGA material in rat tracheal critical-size defect

Primary outcome

Forty rats underwent implantation of the PGA material and were equally divided into 4 groups. The rats that were still alive at each time point were sacrificed for evaluation. All 10 rats survived until sacrifice at each evaluation time point. The percentage of survival was 100% (95% confidence interval: 0.72–1.00, each period).

The sample size was determined based on our previous experiments; no statistical sample size calculation was conducted. However, when the sample size was 40, the expected survival percentage was 100% and the half-width of the confidence interval was 28%, and when alpha was 5%, the probability of obtaining a confidence interval half-width of less than or equal to the value was >99%. Therefore, the number of rats in this study was considered acceptable to evaluate long-term survival after implantation of novel PGA material.

Secondary outcome: morphological characteristics

Bronchoscopic findings. Bronchoscopic examinations 2 weeks after PGA material implantation showed that the luminal surface was covered with new epithelium. Neovascularization across the material was bronchoscopically observed after 2 months and clearly observed after 6 months (Fig. 4B). Stenosis, granulation and dyskinesia of the portion of the trachea covered with the material were not observed at any time point. All rats were observed under anaesthesia with spontaneous breathing and showed no tracheomalacia-like findings in the implantation area. Subcutaneous emphysema did not appear in the neck at any time points, and the airtightness of the trachea was also maintained.

Histological findings. Macroscopic examination revealed only slight adhesions around the PGA at all time points. No tracheal stenosis was present at the anastomosis site, and the luminal surface of the artificial trachea was smooth. The PGA material was macroscopically confirmed after 2 weeks and 1 month. After 2 months, the PGA material could not be distinguished from the surrounding tissue (Fig. 4A).

Histological examination confirmed collagen fibrosis and ciliated epithelization on the PGA material after 2 weeks (Fig. 5A). Neovascularization was observed after 1 month (Fig. 5B) and tracheal glands after 2 months (Fig. 5C). After 6 months, the PGA had been completely replaced by autologous tissue, and chondrocyte regeneration was observed in the centre of the PGA in 60% (3/5) of cases (Fig. 5D). The other cases (2/5) exhibited chondrocyte regeneration at the periphery of the PGA material. Slight inflammation around the PGA material was observed after 2 weeks, but no inflammatory reaction was observed after 1 month. All pathological findings were recorded with full agreement between 2 observers.

Secondary outcome: functional performance

Evaluation of regenerated ciliary area. The regenerated ciliary area was measured at the centre of the PGA at 2 weeks, 1 month, 2 months and 6 months after surgery and compared among the time points. After 2 weeks, the regenerated ciliary area in the centre of the PGA was significantly smaller than that at the periphery of the PGA [12% (9.0%–17.5%) vs 38% (32.0%–40.5%), respectively; $P = 0.00122$]. The regenerated ciliary area in the centre tended to be smaller than that at the periphery at each time point.

The regenerated ciliary area in the centre of the PGA was 12.0% (9.0%–17.5%), 30.0% (21.0%–36.0%), 28% (23.5%–38.5%) and 30% (27.5%–33.0%) at each respective time point (Fig. 6B).

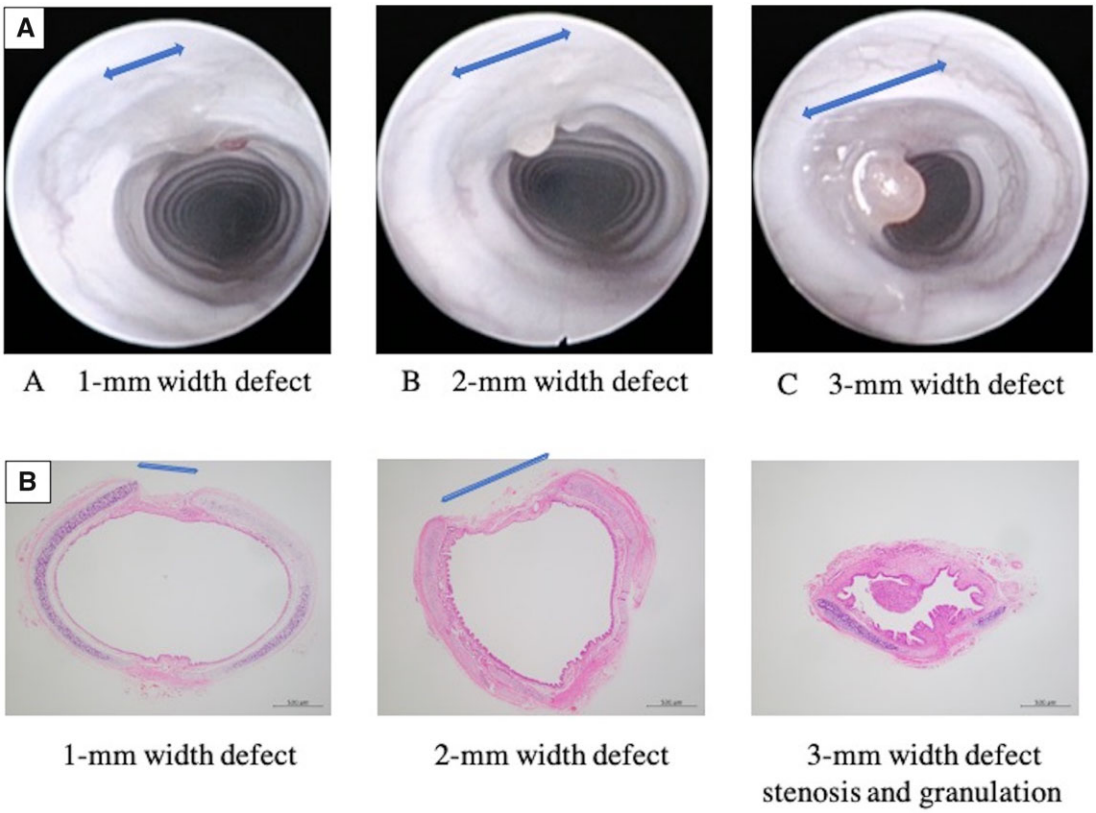


Figure 3: Bronchoscopic and pathologic findings 2 weeks after creation of tracheal defects of each size. (**A**) Postoperative bronchoscopic findings with a 1-, 2- and 3-mm-wide defect at 2 weeks. (**B**) Postoperative histological findings with a 1-, 2- and 3-mm-wide defect at 2 weeks. The bronchoscopic and histological findings showed more granulation and stenosis in rats with a 3-mm-wide defect. The arrows indicate the defect area.

The regenerated ciliary area significantly increased between 2 weeks and 1 month ($P = 0.0216$) and remained the same thereafter. The regenerated ciliary area increased on a logarithmic scale [$y = 22.900312 + 6.0268974 \log(x)$] (Fig. 6B).

Outcome of ciliary beat frequency. The CBF of the normal area was approximately 11 Hz. The CBF in the centre and periphery of the regenerated regions of the PGA did not differ significantly after 2 weeks [7.12 (6.39–7.56) vs 6.77 (6.29–7.84) Hz, respectively; $P = 1.00$]. The CBF in the centre and periphery of the PGA was almost the same at each time point.

The CBF in the centre of the PGA at each time point was 7.12 (6.39–7.56), 6.71 (6.26–7.64), 7.85 (7.23–9.21) and 10.04 (9.58–10.66) Hz, respectively (Video 1A and B). The CBF significantly improved between 2 weeks and 6 months ($P = 0.0122$) and increased on a quadratic scale ($y = 6.461 + 0.8405x - 0.03866x^2$) (Fig. 7A).

Outcome of ciliary beat frequency. The CTF of the normal area was ∼25 μm/s. The speed of orally directed transport along the lung–oral axis was slower in the centre of the regenerated regions of the PGA than in the periphery after 2 weeks [5.16 (2.43–9.13) vs 10.56 (9.67–12.14) μm/s, respectively; $P = 0.0947$]. The CTF tended to decrease towards the centre in each period. The CTF in the centre of the PGA was 5.16 (2.43–9.13), 13.9 (11.08–17.93), 13.49 (10.96–23.69) and 22.53 (16.37–24.67) μm/s, respectively (Video 1C and D). The CTF significantly improved between 2 weeks and 2 months ($P = 0.0216$) and increased on a logarithmic scale [$y = 11.818139 + 5.7220022 \log(x)$] (Fig. 7B).

DISCUSSION

Our experiments indicated that the novel PGA has excellent biocompatibility with chondrocyte regeneration until 6 months after implantation; additionally, the cilia on the PGA regenerated both morphologically and functionally. These findings suggest that our material may be a promising candidate for an artificial trachea.

Previous studies using PGA as an artificial trachea did not achieve long-term survival because material absorption varies from 2 to 4 months and typically depends on the response of the recipient tissue [8, 9]. To induce more rapid and complete regeneration, several challenges of tracheal replacement using PGA with the addition of chondrocytes and growth factors have recently been conducted [10]. Three-dimensional bioprinted artificial trachea has sufficient strength and can prevent collapse of the implanted artificial trachea until a sufficient blood supply is obtained [11]. However, it has some disadvantages including high cost and a long preparation time for implantation.

With respect to the relationship between the absorption period of the developed PGA material and the functional strength required to retain the tracheal lumen, the histological findings showed that the PGA material remained 2 months after surgery and was completely replaced by autologous tissue 6 months after surgery. However, bronchoscopy at 6 months after surgery revealed no tracheomalacia-like changes or flattening of the trachea in the regenerated area of the defect. A similar phenomenon has been observed clinically; Oue *et al.* [12] reported the histopathological changes that occurred after tracheobronchial reconstruction with costal cartilage grafts in 6 infants with congenital tracheal stenosis.

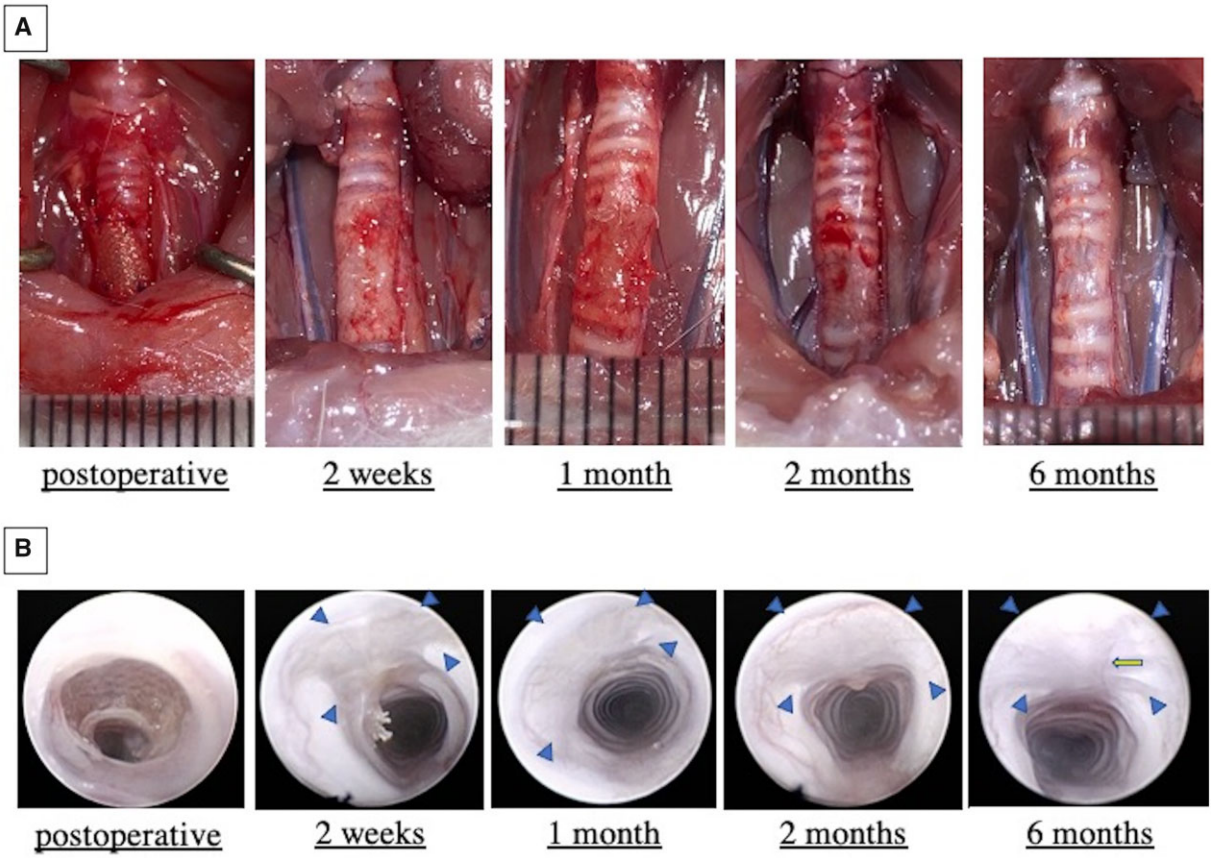


Figure 4: Findings of surface and tracheal lumen at PGA implantation site. (**A**) The semicircular tracheal defect (critical-size defect) was covered with the PGA material. Images were obtained at 2 weeks, 1 month, 2 months and 6 months after fixing the PGA. (**B**) Bronchoscopic examination findings are shown for each postoperative time point. The blue arrows indicate the PGA implantation sites. Two weeks after prosthesis implantation, the luminal surface was covered with new tissue. Neovascularization across the material was bronchoscopically observed after 2 months and clearly observed after 6 months (arrows). Stenosis and granulation were not observed at any time point. PGA: polyglycolic acid.

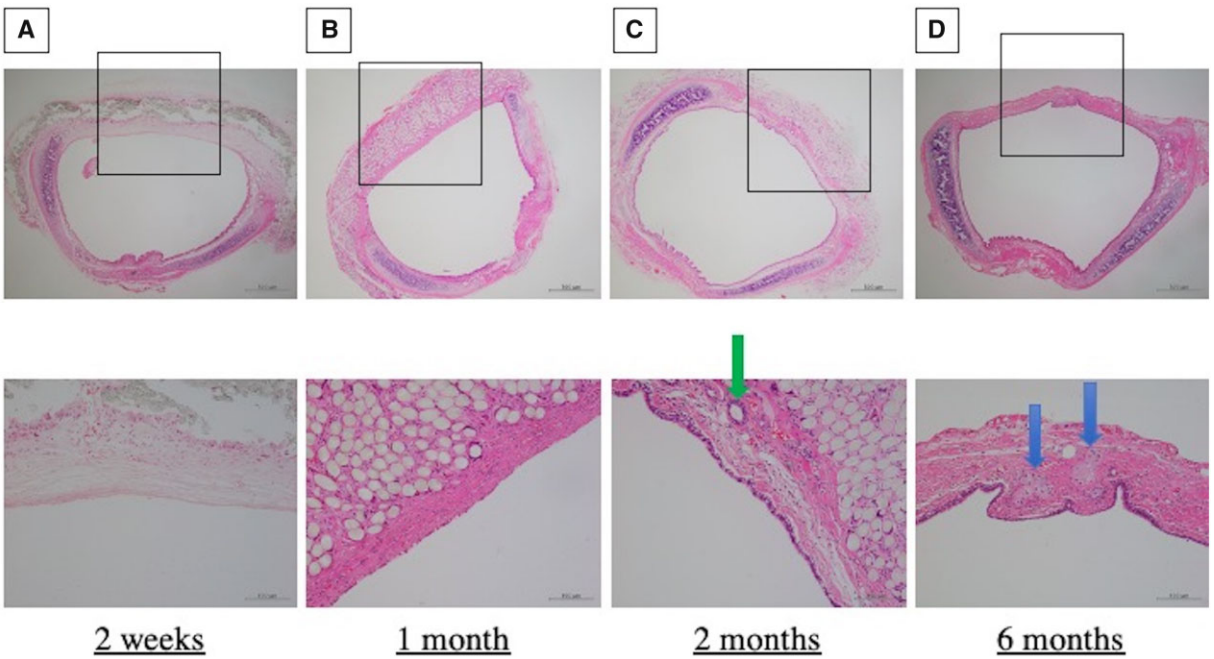


Figure 5: Haematoxylin and eosin staining (original magnification: 500× and 100×). The bottom images are magnifications of the top images (black square). (**A**–**D**) Representative histologic sections of the trachea at 2 weeks, 1 month, 2 months and 6 months after surgery. (**A**) Collagen fibrosis and ciliated epithelization was confirmed on the PGA material after 2 weeks. (**B**) Neovascularization was observed after 1 month and (**C**) tracheal glands were observed after 2 months (arrows). (**D**) Chondrocyte regeneration was observed in the centre of the PGA after 6 months (arrows). No inflammatory reaction around the PGA material was observed. PGA: polyglycolic acid.

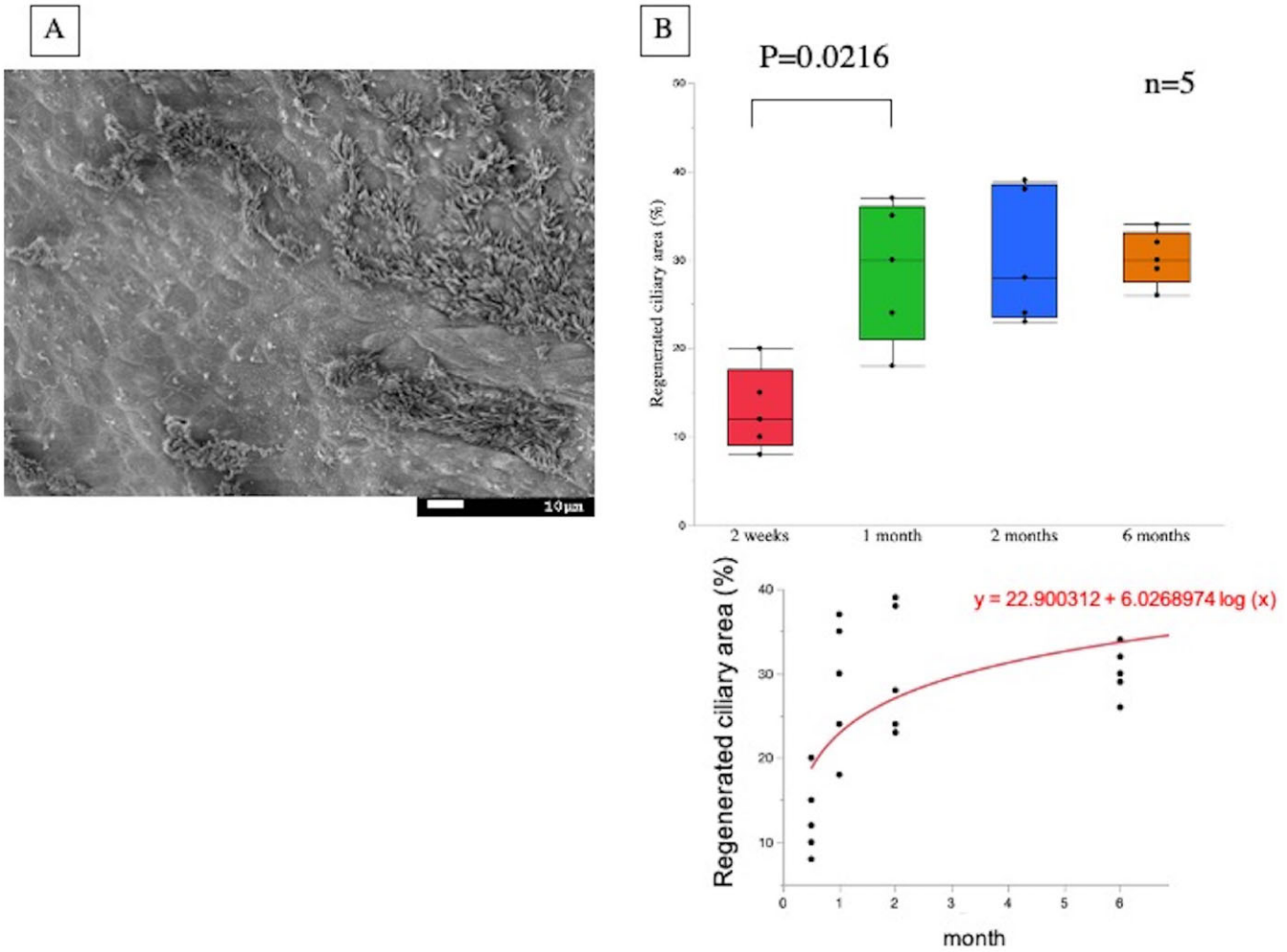
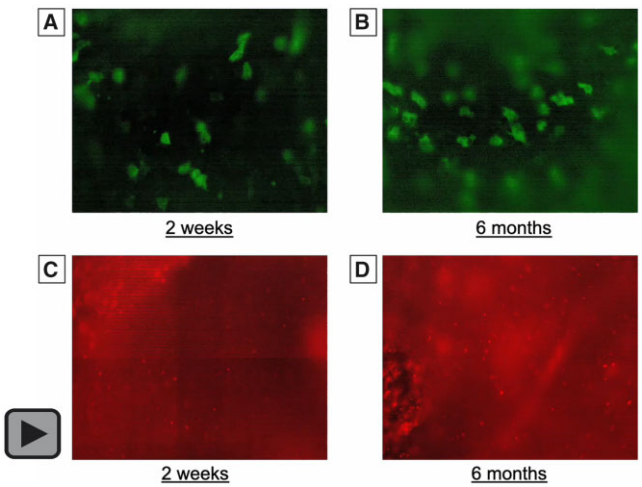


Figure 6: Scanning electron microscope analysis of regenerated ciliary area at the centre of PGA at each time point. (**A**) Representative image shows the luminal surface of the PGA after 1 month. (**B**) The graph demonstrates the change in the regenerated ciliary area from 2 weeks to 6 months (n = 5). The regenerated ciliary area significantly increased from 2 weeks to 1 month (P = 0.0216) and remained the same thereafter. The regenerated ciliary area increased on a logarithmic scale. PGA: polyglycolic acid.



Video 1: Results of CBF and CTF analysis at 2 weeks and 6 months. (**A** and **B**) The video shows the CBF of wheat germ agglutinin-stained cells at 2 weeks and 6 months. (**C** and **D**) The video shows oral directional movement of microspheres dropped onto the tracheal lumen at 2 weeks and 6 months. The CBF and CTF in the implantation site significantly improved from 2 weeks to 6 months. CBF: ciliary beat frequency; CTF: ciliary transport function.

The stenosis was repaired by replacement of a section of rib cartilage as an augmentation patch into the anterior surface of the trachea, which had been incised through the entire length of the stenosis. The graft gradually decreased in size after the operation and was completely replaced by mature scar tissue 2 years after the operation. However, the external diameter of the reconstructed site was the same or increased, and the patent inner layer was reserved in every case [12]. Regarding chondrocyte regeneration in the implanted biomaterial, no reports to date have described cartilage regeneration 6 months after artificial tracheal implantation alone. However, whether regenerated cartilage has the ability to maintain the original lumen of the tracheal cartilage remains unclear.

In this study, although the regenerated ciliary area significantly increased by up to 30% in the centre of the PGA material for up to 1 month, the CBF and CTF towards the oral side improved until 6 months. This may mean that the cilia regrowth matrix and the direction of ciliary movement are completed early after PGA implantation. Regarding the importance of the direction of ciliary movement, Thomas *et al.* [13] reported that epithelial ultrastructural abnormalities may increase the risk of allograft colonization by pathogenic organisms despite a normal CBF, suggesting that not

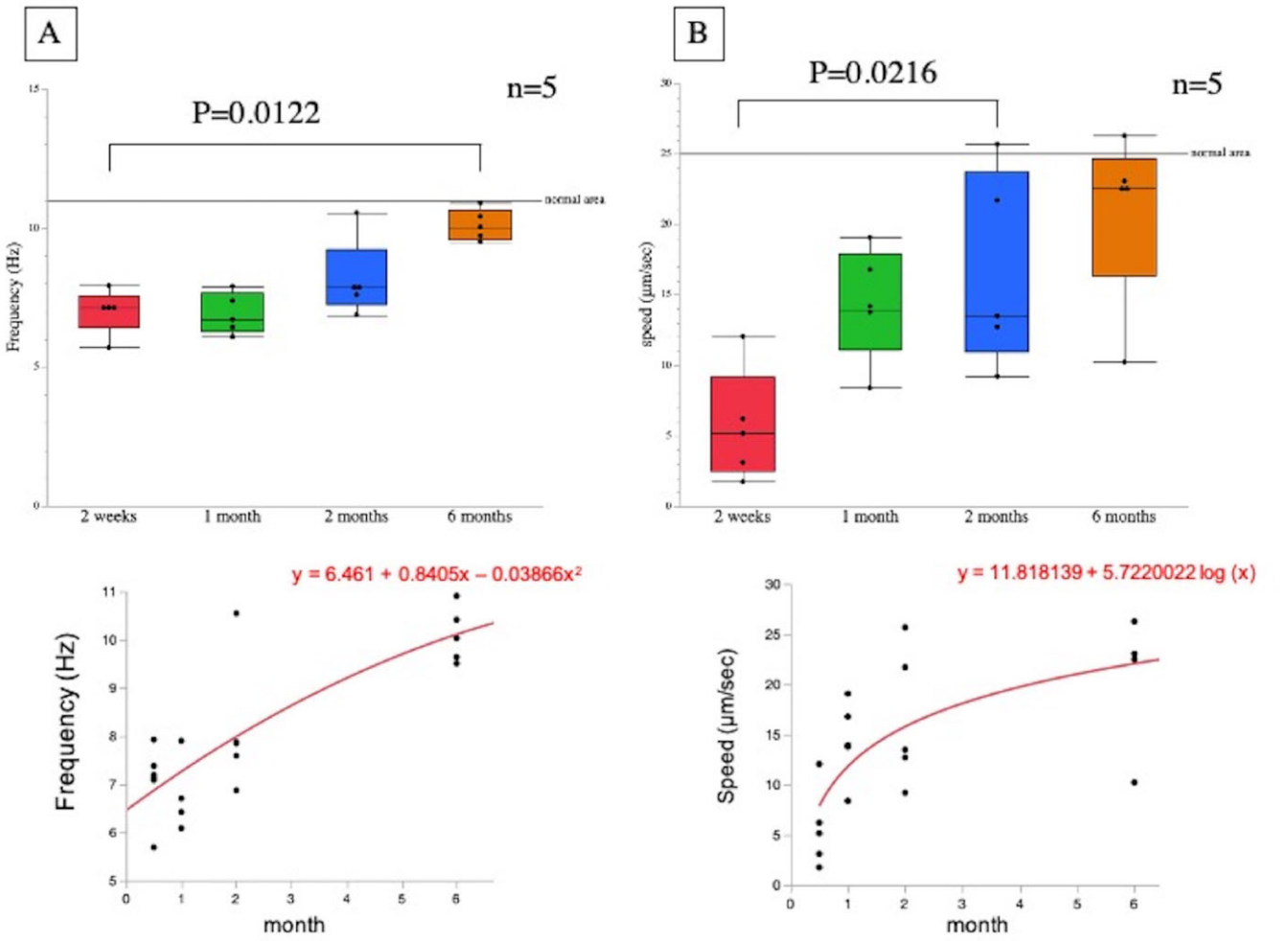


Figure 7: Results of ciliary beat frequency and ciliary transport function analysis at each time point from 2 weeks to 6 months. (**A**) The graph shows the relationship between the frequency and the time point. The line represents the frequency of the normal area (11 Hz). The frequency in the implantation site gradually increased and improved to nearly normal values at 6 months (P = 0.0122). The frequency increased on a quadratic scale. (**B**) The vertical axis of the graph represents the speed of the microspheres. The speed gradually increased and improved to near normal values (25 μm/s) as indicated by the line at 6 months (P = 0.0216). The speed increased on a logarithmic scale.

only the CBF but also the direction of ciliary movement (such as the CTF, which is the calculated speed of oral directional transport along the lung–oral axis) may be more important for preventing infection. Although there is currently no method to unify the movement of cilia, the regulation of planar cell polarity might be essential to unify the direction of the airway epithelial cilia [7, 14, 15].

Limitations

Two limitations of this study should be acknowledged. First, the study involved a relatively small sample and limited time period. We did not conduct a statistically detailed sample size calculation, and the sample size cannot be considered sufficient. The novel PGA material did not result in airway collapse because of complete self-organization 6 months after surgery, but further long-term observation is needed to examine the increase in chondrocyte regeneration and the pressure resistance of the PGA material. Second, although we demonstrated excellent biocompatibility by covering critical-size tracheal defects in a rat model, the defects were small and the novel PGA material needs to be optimized and validated in a large animal tracheal defect model in the future.

CONCLUSION

We developed a novel layered PGA material. Using a rat cervical tracheal defect model, we confirmed that the novel PGA material has excellent biocompatibility and effectively facilitates tracheal regeneration both morphologically and functionally 6 months after surgery.

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DATA AVAILABILITY

The data underlying this article will be shared on reasonable request to the corresponding author.

Author contributions

Yoshitake Murata: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Writing—original draft. **Yojiro Yutaka:** Conceptualization; Methodology; Project administration; Supervision; Writing—review & editing. **Rieko Hirata:** Data curation; Formal analysis; Methodology; Resources; Writing—review & editing. **Masatsugu Hamaji:** Methodology; Project administration; Writing—review & editing. **Akihiro Yoshizawa:** Formal analysis; Methodology; Writing—review & editing. **Yo Kishimoto:** Methodology; Writing—review & editing. **Koichi Omori:** Methodology; Project administration; Writing—review & editing. **Hiroshi Date:** Methodology; Project administration; Supervision; Writing—review & editing.

Reviewer information

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REFERENCES

[1] Grillo HC. Tracheal replacement: a critical review. Ann Thorac Surg 2002;73:1995–2004.
[2] Grillo HC. The history of tracheal surgery. Chest Surg Clin N Am 2003; 13:175–89.
[3] Nakamura T, Inada Y, Shigeno K. Artificial sensory organs: latest progress. J Artif Organs 2018;21:17–22.
[4] Nakamura T, Suzuki K, Mochizuki T, Ohde Y, Kobayashi H, Toyoda F. An evaluation of the surgical morbidity of polyglycolic acid felt in pulmonary resections. Surg Today 2010;40:734–7.
[5] Yano T, Haro A, Shikada Y, Okamoto T, Maruyama R, Maehara Y. A unique method for repairing intraoperative pulmonary air leakage with both polyglycolic acid sheets and fibrin glue. World J Surg 2012;36:463–7.
[6] Ueda K, Tanaka T, Li TS, Tanaka N, Hamano K. Sutureless pneumostasis using bioabsorbable mesh and glue during major lung resection for cancer: who are the best candidates? J Thorac Cardiovasc Surg 2010;139:600–5.
[7] Nakamura R, Katsuno T, Tsuji T, Oyagi S, Kishimoto Y, Suehiro A *et al.* Airway ciliated cells regenerated on collagen sponge implants acquire planar polarities towards nearby edges of implanted areas. J Tissue Eng Regen Med 2021;15:712–21.
[8] Tsukada H, Matsuda S, Inoue H, Ikada Y, Osada H. Comparison of bioabsorbable materials for use in artificial tracheal grafts. Interact CardioVasc Thorac Surg 2009;8:225–9.
[9] Ikada Y. Challenges in tissue engineering. J R Soc Interface 2006;3:589–601.
[10] Komura M, Komura H, Kanamori Y, Tanaka Y, Suzuki K, Sugiyama M *et al.* An animal model study for tissue-engineered trachea fabricated from a biodegradable scaffold using chondrocytes to augment repair of tracheal stenosis. J Pediatr Surg 2008;43:2141–6.
[11] Taniguchi D, Matsumoto K, Tsuchiya T, Machino R, Takeoka Y, Elgalad A *et al.* Scaffold-free trachea regeneration by tissue engineering with bio-3D printing. Interact CardioVasc Thorac Surg 2018;26:745–52.
[12] Oue T, Kamata S, Usui N, Okuyama H, Nose K, Okada A. Histopathologic changes after tracheobronchial reconstruction with costal cartilage graft for congenital tracheal stenosis. J Pediatr Surg 2001;36:329–33.
[13] Thomas B, Aurora P, Spencer H, Elliott M, Rutman A, Hirst RA *et al.* Persistent disruption of ciliated epithelium following paediatric lung transplantation. Eur Respir J 2012;40:1245–52.
[14] Wallingford JB. Planar cell polarity signaling, cilia and polarized ciliary beating. Curr Opin Cell Biol 2010;22:597–604.
[15] Marshall WF. Cilia self-organize in response to planar cell polarity and flow. Nat Cell Biol 2010;12:314–5.