A 4-Axis Technique for Three-Dimensional Printing of an Artificial Trachea

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Abstract

BACKGROUND: Several types of three-dimensional (3D)-printed tracheal scaffolds have been reported. Nonetheless, most of these studies concentrated only on application of the final product to an *in vivo* animal study and could not show the effects of various 3D printing methods, materials, or parameters for creation of an optimal 3D-printed tracheal scaffold. The purpose of this study was to characterize polycaprolactone (PCL) tracheal scaffolds 3D-printed by the 4-axis fused deposition modeling (FDM) method and determine the differences in the scaffold depending on the additive manufacturing method.

METHODS: The standard 3D trachea model for FDM was applied to a 4-axis FDM scaffold and conventional FDM scaffold. The scaffold morphology, mechanical properties, porosity, and cytotoxicity were evaluated. Scaffolds were implanted into a 7 × 10-mm artificial tracheal defect in rabbits. Four and 8 weeks after the operation, the reconstructed sites were evaluated by bronchoscopic, radiological, and histological analyses.

RESULTS: The 4-axis FDM provided greater dimensional accuracy and was significantly closer to CAD software-based designs with a predefined pore size and pore interconnectivity as compared to the conventional scaffold. The 4-axis tracheal scaffold showed superior mechanical properties.

CONCLUSION: We suggest that the 4-axis FDM process is more suitable for the development of an accurate and mechanically superior trachea scaffold.

Keywords Three-dimensional printing · Trachea · Scaffold · 4-Axis · Fused deposition modeling

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1 Introduction

Tracheal tissue engineering is of particular importance because of the high mortality rates caused by tracheal stenosis or obstruction [1]. Several research groups have attempted to use various tissue-engineered artificial tracheal substitutes to repair segmental tracheal defects; however, none have yet proved satisfactory for clinical use owing to airway stenosis (overgrowth of granulation tissue from the anastomosis site), airway collapse (softening and flattening of the framework), and mucus impaction (because of a lack of a respiratory epithelium) [2].

Lately, three-dimensional (3D) printing is a fast-growing trend in tissue engineering owing to its ability to

fabricate patient-specific scaffolds with well-controlled porous architecture and the capability of printing cells in 3D configurations [3, 4]. A 3D-printed artificial tracheal scaffold has the advantage of fast and easy fabrication that reproduces the shape of a native trachea [3]. Therefore, recently, several types of tracheal scaffolds created by the 3D printing method have been reported [2, 3, 5–7]. Nonetheless, most of these studies concentrated only on application of the final product to an *in vivo* animal study and could not show the effects of various 3D printing methods, materials, or parameters for creating an optimal 3D-printed tracheal scaffold.

For successful tracheal reconstruction, tissue-engineered artificial tracheal grafts should meet several requirements, such as airtightness, prevention of airway collapse, host compatibility, moldability to fit physiological geometry, ease of implanting, compatibility with epithelial ingrowth, and little donor site morbidity [2, 5, 8]. These properties can be subdivided into three categories: (1) biocompatibility to facilitate resurfacing of the ciliated respiratory epithelium, (2) mechanical properties maintaining a proper trachea lumen during respiration and neck movements (rotation, flexion, and extension), (3) manufacturing and surgical requirements [5]. Biocompatibility and mechanical properties of 3D-printed scaffolds can be affected by the material used, printing conditions, and structure of the scaffolds. Therefore, the effects of various parameters such as pore size, shape, printing orientation, or layer thickness on the physical and mechanical properties of the scaffolds have been studied to better select the most suitable manufacturing parameters, especially for bone regeneration [9]. On the other hand, there has been no such study in the tracheal regeneration field.

There are several 3D printing processes including fused deposition modeling (FDM), stereolithography (SLA), digital light processing (DLP), and selective laser sintering (SLS). The main differences among the processes are in the way layers are deposited to create parts and in the materials that are used. The FDM method applies extrusion of material out of nozzles, which can move horizontally along the X- and Y-axes. The extruded filament is deposited onto a platform, which can move vertically [7]. Lately, several versions of the FDM process were introduced by modification of the platform, nozzle, or G-code file [6, 10, 11].

Here, we developed a polycaprolactone (PCL) 3D-printed tracheal scaffold using a 4-axis FDM process. The 4-axis FDM builds the structure on the rotating printing plate and has been suggested as a feasible manufacturing technique for vascular stents [10]. We hypothesized that the properties of the scaffold made by same material could be changed depending on the way the material was deposited, and 4-axis FDM technique is more feasible for creating an elaborate tracheal scaffold because the trachea

is a hollow cylindrical organ that is similar to a vascular stent.

Among various biodegradable synthetic materials, PCL is an FDA-approved polymer that has superior mechanical strength and durability with comparable biocompatibility. Additionally, PCL is water-soluble and does not require the use of organic solvents that are incompatible with the current 3D-printing system [1, 3].

The purpose of the present study was to characterize PCL tracheal scaffolds 3D-printed by the 4-axis FDM method. The scaffold morphology, mechanical properties, porosity, water uptake, swelling ratio, and cytotoxicity were evaluated and an animal experiment on these scaffolds was conducted. These properties were compared with those of a PCL trachea scaffold 3D-printed by the conventional FDM method.

2 Materials and methods

2.1 Preparation of a 3D-printed tracheal graft

A standard 3D trachea model for FDM was generated using commercial CAD software (CADian3D®, version 2015, Intelli Korea, South Korea) at a size of 18 mm (inner diameter), 22 mm (outer diameter), 2 mm (wall thickness), and 25 mm (height) that mimics the structure of a native trachea. The standard 3D trachea model had a cylinder shape with rectangular convolutions (Fig. 1A–C). This standard 3D trachea model was applied to the 4-axis FDM scaffold and conventional FDM scaffold. Processes of generation of the 4-axis scaffolds were as follows [10]. The PCL (Purasorb® PC 12, CorbionPurac) was placed in a heating jacket heated up to 140 °C and extruded through a ceramic nozzle using a lab-made 3D bio-printer (Korea Institute of Machinery and Materials, Korea). The inner diameter of the nozzle is 0.2 mm. PCL is printed on the rotating metal rod with an outer diameter of 22 mm. The thickness of each layer is 0.2 mm, and the final height of the tracheal scaffold is 25 mm (Fig. 1D). Conventional FDM scaffolds was generated as follows. The STL file of the standard 3D model was processed by a slicer (Simplify3D®, layer height 0.2 mm, printing speed 3 mm/s, fill density 60%) to convert the model into a series of thin layers and produce a G-code file. Biodegradable PCL (Purasorb® PC 12, CorbionPurac) was ejected through a dispensing stainless-steel nozzle, and the strand of PCL was plotted layer by layer using a double-head 3D printer (BT-3000, NBR-Tech Co., Ltd., Chuncheon, Korea) (temperature 140 °C, nozzle size 0.2 mm; Fig. 1E).

Fig. 1 CAD modeling images of the trachea scaffold (A–C), and the schematic process of fabricating a 3D-printed tracheal scaffold (D, E). **A** Top view. **B** Front side view. **C** 3D reconstructed view. **D** 4-axis technique. **E** Conventional technique

2.2 Scanning electron microscopy (SEM) and energy dispersive spectrometer (EDS)

Low-vacuum scanning electron microscope (S-350N, Hitachi, Tokyo, Japan) located at the Korea Basic Science Institute, Chuncheon, Korea was used to exam the morphology of the scaffolds. The samples of the each scaffold were coated with a thin 10-nm layer of gold/palladium for 30 s at a 15-mA discharge current with an Ion Sputter (1010, Hitachi, Tokyo, Japan) [12]. The micrographs were taken at an accelerating voltage of 2 kV. For EDS analysis, a variable pressure field emission SEM (FE-SEM) (EVOVR LS10 equipped with EDX manufactured by Carl Zeiss SMT) was used.

2.3 Fourier transform infrared (FTIR) spectroscopy

Frontier Optica (Perkin Elmer Inc., U.K.) was used to analyze the attenuated total reflection (ATR)-FTIR spectra of the scaffolds to confirm the secondary chemical transition of the PCL-3D printed scaffold due to heating and

foreign body during the 3D printing process. The spectra were recorded from 8300 to 350 cm^{-1} with 0.4 cm^{-1} resolution and 32 scans.

2.4 Mechanical test

Tensile and uniaxial compressive properties of the printed tracheal scaffolds were measured using a universal testing machine (QM100S, qmesys, Kyounggi, Korea) with a 200-kN capacity load cell and a cross-head loading rate of 0.5 mm min^{-1} . The force required to pull the specimen apart and the magnitude of sample strength before breaking were measured. Three samples of each scaffold were used for this analysis.

2.5 Porosity

Liquid porosity of the scaffolds was measured by a method described elsewhere [13, 14]. Ethanol was used as the displacement liquid. The scaffold was immersed in a known volume (V_1) of ethanol in a measuring cylinder for

10 min. The total volume of ethanol and of the ethanol-impregnated scaffolds was regarded as (V_2). The ethanol-impregnated scaffold was then removed from the cylinder and the residual ethanol volume was recorded as (V_3). The porosity (P) of the scaffolds was calculated as follows: $P (\%) = [(V_1 - V_3)/(V_2 - V_3)] \times 100$ [12]. Three samples of each scaffold were used for these measurements. The results were expressed as mean $\pm$ standard deviation.

2.6 Cell viability in a CCK-8 assay

To test cell viability, NIH3T3 fibroblasts were cultured in the presence of the scaffolds, and the CCK-8 kit was used to assess enzymatic activity of the cells. NIH3T3 fibroblasts were revived from liquid nitrogen storage after 2 subcultures in DMEM. From this cell suspension, 200 μ l containing 2×10^4 cells was seeded on each scaffold. These cells were resuspended in a culture plate and incubated at 37 °C and 5% CO₂. The contents of wells were removed by suction after 1 and 2 days of incubation. The CCK-8: culture medium mixture in a ratio of 1:9 was added to each scaffold, and incubated for 2 h. Then, a 96-well plate was lowered into a 100- μ l ELISA microplate reader (Molecular Devices, SpectraMax® Plus 384, Sunnyvale, CA, USA) and was analyzed by measuring absorbance at 450 nm. The cell proliferation was determined by comparing the absorbance of sample solutions to that of the control well containing only the CCK-8 solution [12].

2.7 Creation of an animal model and implantation of the scaffold

Four male New Zealand rabbits, approximately 3 months old and weighing 3.0–3.5 kg, were used in the animal experiment. This study was approved by the institutional review board of Hallym University (IRB 2016-64), Chuncheon, Korea. The animals were anesthetized by intramuscular injection of a mixture of 5 ml of Zoletil® (Tiletamine and zolazepam, Virbac Korea, Seoul, South Korea) and 2.5 ml of Rumpun® (xylazine chloride, Bayer Korea, Seoul, South Korea) at 0.5 ml kg⁻¹ in the thigh region. Each rabbit was placed in a supine position with the neck slightly extended. A vertical skin incision was made at the midline of the neck, and the strap muscle was dissected to the anterior tracheal wall and larynx. A 7 $\times$ 10-mm piece of tracheal cartilage was removed from the fourth to sixth tracheal rings. The defect was covered with the 3D-printed scaffold, and sutured with Ethilon® 5-0 (Johnson & Johnson, New Brunswick, NJ, USA). The strap muscles were closed with 4-0 Vicryl® (Johnson & Johnson), and the skin incision was closed with 4-0 Nylon® (Johnson & Johnson) (Fig. 2).

2.8 Bronchoscopic examinations

Bronchoscopy was performed on all surviving animals at 1, 2, 3, 4, and 8 weeks after implantation. A rigid 4-mm 0° endoscope (Karl Storz, Tuttlingen, Germany) was used. The inner surface of the scaffold-grafted areas was examined and we determined the following: (1) narrowing of the lumen, (2) re-epithelization, (3) formation of granulation tissue around the scaffold-grafted area, (4) integration of the implanted scaffolds. Degrees of stenosis were classified on a four-point scale: 0–10%, no narrowing is noted; 11–50%, narrowing noted as mild; 51–70%, narrowing noted as moderate; and over 71%, severe narrowing.

2.9 Computed tomographic examination

To determine tracheal airway narrowing, computed tomography (CT) was carried out in all surviving animals 4 and 8 weeks after the surgical operation.

2.10 Histological examination

The animals were euthanized in compliance with the Animal Experiment Guidelines of the Hallym University Medical Research Institute. The specimens were retrieved at 8 weeks after implantation. One animal was used for each time point. The specimens were embedded in paraffin blocks and sectioned into 5- μ m-thick slices for slides. The slices were stained with hematoxylin–eosin (H&E).

2.11 Statistical analysis

All the data are presented as a mean $\pm$ standard deviation in each experimental group. A *t* test was used to compare the groups in GraphPad Prism 6 (GraphPad Software, San Diego, CA). Data with a *P* value less than 0.05 were considered statistically significant.

3 Results

3.1 Gross morphology and SEM data

Figure 3 shows the external appearance of the scaffolds. The measured inner diameter of 4-axis scaffolds was 18 ± 0.3 mm and that of conventional scaffolds was 18 ± 0.8 mm. The outer diameter was 22 ± 0.2 mm in the 4-axis scaffolds and 22 ± 1.2 mm in the conventional scaffolds. The height was 25 ± 0.7 mm in the 4-axis scaffolds and 25 ± 1.1 mm in the conventional FDM scaffolds. The wall thickness was 2.0 ± 2.1 mm in the 4-axis scaffolds and 2.5 ± 2.1 mm in the conventional scaffolds. Three different samples of each type were tested.

Fig. 2 The surgical procedure for the tracheal scaffold implantation in a New Zealand white rabbit. **A** Exposure of a trachea. **B** A 7×10 -mm piece of tracheal cartilage was removed (arrowhead). **C** 4-axis PCL scaffold was implanted into the defect region. **D** Conventional PCL scaffold was implanted into the defect region in the same manner

Fig. 3 Gross findings and SEM images of the 3D-printed artificial trachea scaffolds. **A** 4-axis PCL scaffold. **B** Conventional PCL scaffold

These results revealed that the 4-axis FDM scaffold had greater dimensional accuracy and was significantly closer to CAD software-based design. SEM images showed that 4-axis scaffolds had more uniform PCL fiber, pore size,

and pore shape as compared to those of conventional scaffolds. The conventional scaffolds showed flattened PCL fiber with a junction between fibers deformed and fused together in some part of the scaffold which were

strongly associated with un-uniformed structure of the scaffold.

3.2 FTIR spectroscopy

FTIR analysis was performed to identify any changes in the chemical structure that could have occurred during the 3D printing process. The FTIR spectra of the PCL pallet before 3D printing and each scaffold after 3D printing showed no significant difference in the functional groups (Fig. 4). These results indicated that there was no structural transition or contamination with impurities in the scaffold during the 3D printing process.

3.3 Mechanical properties

Tensile strength was 0.319 ± 0.083 MPa in the 4-axis scaffolds and 0.067 ± 0.023 MPa in the conventional scaffolds ($n = 3$; $P < 0.05$). Compressive strength was 0.0575 ± 0.009 MPa in the 4-axis scaffolds and 0.023 ± 0.004 MPa in the conventional scaffolds ($n = 3$; $P < 0.05$). These outcomes indicated that the 4-axis PCL scaffold had significantly superior tensile and compressive strength compared to those of the conventional scaffolds (Fig. 5).

3.4 Porosity

Figure 6A shows porosity of the scaffolds according to the liquid displacement method. The 4-axis scaffold showed higher porosity than the conventional scaffold did ($28.72 \pm 9.88\%$ vs. $27.21 \pm 3.65\%$), but there was no statistically significant difference. This result was consistent with cross-sectional SEM images of the scaffolds.

Fig. 4 FTIR analysis. **A** PCL pallet before 3D printing. **B** 4-axis PCL scaffold. **C** Conventional PCL scaffold

3.5 Cell viability according to the CCK-8 assay

Figure 6B shows the results of the CCK-8 assay after 1 and 2 days of incubation. The NIH 3T3 fibroblasts gradually increased their cell proliferation with time, indicating that the environment provided by each scaffold was favorable. In this figure, we can clearly see that there is no toxic effect attributable to each scaffold. The 4-axis scaffolds showed lower absorbance than conventional scaffolds did on day 2. Nonetheless, there were no statistically significant differences among the groups.

3.6 The animal experiment

Four animals survived without severe complications until the designated time points. Signs of severe respiratory distress such as stridor or a wheezing sound were not observed in any animal. The outcomes from the animal experiment are summarized in Table 1. No animals showed severe narrowing. The 4-axis scaffold group showed less inflammation and better mucosal regeneration on the luminal surface as compared to that of the conventional scaffold group.

3.7 Bronchoscopy and CT analysis

To evaluate the implanted scaffold region, the bronchoscopic examination was performed at 1, 2, 3, 4, and 8 weeks after implantation. No animals showed severe stenosis or mucus pooling at the designated time point. In the 4-axis scaffold group, mild inflammation was seen at the scaffold grafting site at 1 and 2 weeks postoperatively. Nonetheless, these phenomena gradually decreased with time, and completely disappeared by the postoperative 8 weeks (endoscopic examination). Nevertheless, we could see mild inflammation around the scaffold-grafted site continued until postoperative 8 weeks in the conventional scaffold group (Fig. 7A). Inner surfaces of the reconstructed region were fully covered with the mucosa 8 weeks after implantation in the 4-axis scaffold group. The conventional scaffold group showed incomplete regeneration of the mucosa, with inflammation and granulation at postoperative 8 weeks. There were no signs of detachment of the scaffold from the trachea or granulation formation toward the tracheal lumen. The axial and 3D reconstructed CT scan images also revealed no signs of stenosis in both groups (Fig. 7B).

3.8 Histological examination

Histological examination at postoperative 8 weeks revealed that both scaffolds successfully maintained the original shape and luminal contour of the trachea. During

Fig. 5 Mechanical properties of 3D-printed PCL tracheal scaffolds. **A** Compressive strength. **B** Tensile strength

Fig. 6 **A** Results on porosity. **B** Cell viability of the preprinted PCL pallet and 3D-printed PCL scaffolds. *NS* not significant

retrieval of the specimen, we observed severe adhesion between the scaffolds and surrounding soft tissues in the

conventional scaffold group. In contrast, the 4-axis scaffolds were biologically integrated with normal surrounding

Table 1 A summary of animal experiments

No.	Scaffold type	Time point (week)	Bronchoscopic and CT examination				Dyspnea
			Narrowing ^a	Epithelization	Granulation	Integration	
I-1	4-axis	4	None	++	++	+++	–
I-2	4-axis	8	None	+++	+++	+++	–
II-1	Conventional	4	None	+	++	++	–
II-2	Conventional	8	None	++	++	++	–

^aNarrowing scores. 0–10% narrowing: none, 11–50% narrowing: mild, 51–70% narrowing: moderate, over 71% narrowing: severe; +++ good, ++ moderate, + poor

tissues without severe adhesion 8 weeks after implantation. Cell ingrowth into the porous region of the 4-axis scaffold was observed (Fig. 8B). In the luminal region, a newly formed epithelium completely covered the inner surface of the scaffolds, and ingrowth of the ciliary respiratory epithelium from a normal region was observed. On the other hand, neocartilage regeneration was not observed (Fig. 8).

4 Discussion

The trachea is a cylindrical organ that is composed of 15–20 C-shaped hyaline cartilages and a ciliated respiratory epithelium on the luminal surface. The cartilages provide enough rigidity to support the airway framework to prevent collapse of the tracheal lumen during respiration. In addition, they enable proper flexibility to ensure stability of the trachea during neck movements (rotation, flexion, and extension), and coughing [1, 3, 5, 6, 8]. The respiratory epithelium is composed of numerous cilia and submucosal glands for mucociliary clearance and mucosa defense. Several tissue-engineered artificial tracheal substitutes have been used to reconstruct tracheal defects. Lately, the tracheal scaffolds created by the 3D printing technology have been reported [1–3, 5–7]. Park et al. created a poly-(L-lactic acid-co-ε-caprolactone) (PLCL)/gelatin tracheal scaffold by an indirect 3D-printing technique, in which a sacrificial mold is printed using a 3D printing system, and the desired biomaterial is cast in the cavity of the mold [6]. Other researchers have printed a PCL, poly-(L-lactic acid) (PLA), or polyurethane (PU) artificial trachea by the direct FDM 3D printing method [1–3, 5, 7].

FDM was developed and implemented for the first time by Scott Crump in the 1980s and was commercialized in 1990 [11]. The FDM printer builds structures layer by layer from the bottom up by heating and extruding a thermoplastic filament such as PLA and PCL. This technique is the most widespread 3D printing process today because of its popularity, simplicity, and availability in the open

source community. Nonetheless, the FDM technique still has some weaknesses including insufficient accuracy, its inability to print overhanging structures without support, the weak bonding force between layers, shifting of layers, and shrinking of the lower parts caused by weight of the upper [11, 15]. The accuracy can be improved by lowering the layer height and using a nozzle of a smaller diameter. Even though the accuracy is improved by these approaches, there is still a surface deviation or surface roughness problem for printing surfaces that are not parallel to the printer plate. The multi-axis technique involving rotation of either the nozzle or the printed part has been introduced to solve the surface problem of the FDM method [11].

This is the first study to compare several versions of tracheal scaffolds created by different 3D-printing methods to determine the optimal 3D printing process. To develop the biomimetic tracheal scaffold, we mainly focused on three categories: (1) biocompatibility, (2) proper mechanical support, and (3) manufacturing and surgical requirements. As the first step to fabricate the 3D-printed trachea scaffold, we selected a proper material and type of the 3D printing process that could fulfill these requirements. PCL is a synthetic absorbable polymer that is widely used in tissue engineering. Biodegradable polymeric materials have the advantages of biocompatibility, but it is a controversial topic whether the implanted engineered tracheal tissue construct can provide proper biomechanical properties for the functional repair during the remodeling process. The degradation rate of the scaffold material should exceed the speed of cell proliferation and tissue regeneration to ensure sufficient mechanical support throughout the remodeling process [5, 7]. Therefore, a nonbiodegradable polyurethane 3D-printed tracheal scaffold was suggested in another study [5]. Nonetheless, long-term follow-up should be considered for verifying lifelong complications. PCL is proposed as a tracheal scaffold material in many studies because it has been shown to maintain its shape and molecular weight up to 2 years *in vitro* and in rats [16]. At least 1.5–2 years is needed to allow for sufficient airway growth to overcome collapse upon exhalation, and

Fig. 7 **A** Representative bronchoscopic images of the 3D-printed PCL scaffolds. **B** Axial and 3D reconstructed CT images of the animal at postoperative 4 and 8 weeks. The black arrowhead indicates the implanted scaffolds

degradability plays a key role in biocompatibility [4]. One resorbable material whose degradation profile fulfills this requirement is PCL. For these reasons, we chose PCL as the scaffold material. Lastly, we have utilized the FDM 3D printing technology for easy fabrication of the tracheal scaffolds that are amenable to simple and customized production. To overcome limitations of the conventional FDM technique—such as surface roughness, a weak bonding force between layers, and shrinking of the lower part—we utilized the 4-axis FDM technology in this study. The 4-axis FDM that is used in the present study has been

suggested as a feasible manufacturing technique for vascular stents [10]. We hypothesized that because the trachea is a hollow cylindrical organ, which is similar to a vascular stent, building of the structure on a rotating printing plate is more suitable for creation of an elaborate tracheal scaffold.

During the examination of gross morphology, the 4-axis FDM scaffold showed greater dimensional accuracy and was significantly closer to CAD software-based designs with a predefined pore size and pore interconnectivity as compared to the conventional scaffold. Dimensional accuracy is the most critical factor to ensure dimensional

Fig. 8 H&E staining of the 3D-printed trachea 8 weeks after implantation. **A–D** 4-axis specimen. **E–H** Conventional specimen after 8 weeks. **C** cartilage, **L** lumen, **P** 3D-printed PCL scaffold, **RE** respiratory epithelium

repeatability of a manufactured scaffold [9]. Additionally, according to the SEM results, we clearly saw that the 4-axis scaffold showed more uniform PCL fiber, pore size, and pore shape as compared to the conventional scaffold which showed irregular structure caused by flattened PCL fiber with a junction between fibers deformed and fused together in the part of the scaffold. This result indicates that the 4-axis FDM technique can overcome limitations of the conventional FDM technique such as shrinkage of the lower part of the scaffold by gravity, which is strongly associated with printing accuracy.

In this study, chemical properties from the FTIR analysis of the 4-axis scaffolds revealed no significant changes in the functional groups before and after a 3D printing process. Any changes in the chemical structure during the 3D printing process are some of the most important factors associated with safety of the implantable scaffolds. No matter how safe the materials used for creating a scaffold are, safety cannot be guaranteed if any chemical structural changes occur during the manufacturing process.

Mechanical properties of the implanted tracheal scaffold should be similar to those of native tracheal tissue. High-pressure changes occur in a tracheal lumen during respiration; therefore, sufficient rigidity to support the airway framework is necessary in the tracheal scaffold. We compared tensile and compressive strength of 4-axis and conventional PCL tracheal scaffolds. These results suggest that the 4-axis FDM scaffold has significantly superior mechanical properties as compared to those of the conventional scaffold. These results may be influenced by the more uniform scaffold structure of the 4-axis scaffold than the conventional scaffold. The effects of the printing orientation and layer thickness on the physical and

mechanical properties of the 3D-printed scaffold have been studied, and these parameters are reported to have a significant effect on compressive strength of scaffolds [9]. Mechanical tests in the present study revealed that 4-axis FDM could improve mechanical performance of a 3D-printed trachea scaffold compared to a conventional FDM scaffold.

In conclusion, the 4-axis FDM can make more uniform structure of the scaffold which attribute to high porosity and mechanical property. This study has significance in confirming that physical properties of scaffold can be changed depending on the method of depositing material. As 3D-printed medical devices have increased, detailed description of manufacturing method and mechanical properties of the scaffold are strongly recommended these days [17]. The 4-axis FDM technique in which the printing surface is parallel to the printer plate is a more suitable technique for fabrication of an accurate and mechanically superior 3D printed trachea scaffold in contrast to the conventional FDM method.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This study was approved by the institutional review board of Hallym University (IRB 2016-64), Chuncheon, Korea.

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