

Tissue Engineered Trachea Using Decellularized Aorta

Ana C. Paz¹, Koji Kojima¹, Kiyotaka Iwasaki², Jason D. Ross¹, Jose A. Canseco¹, Mitsuo Umez² and Charles A Vacanti¹

¹Laboratory for Tissue Engineering and Regenerative Medicine, Brigham & Women's Hospital, Harvard Medical School

²Waseda Institute for Advanced Study, Center for Advanced Biomedical Sciences, Waseda University, Japan

Abstract

Background: Several approaches for the development of tracheal substitutes for the treatment of extensive tissue defects have been explored over the years. However, a completely satisfactory approach has not been achieved. Previously, we described a composite tissue engineered trachea (TET) using chondrocytes seeded onto a polyglycolic acid (PGA) fiber-mesh. This study was considered to improve the design and functionality of the TET by using a porcine decellularized aorta as the scaffold.

Methods: Chondrocytes were harvested from sheep tracheal cartilage and were suspended in medium. The chondrocytes were then seeded onto PGA and incubated in vitro for 1 week. A 3x4 cm piece was cut from a decellularized aorta and four 0.5x3 cm pieces were excised from one side in a comb like fashion. A silicon stent was inserted into this structure and the spaces were filled with chondrocyte seeded PGA. The three dimensional cell-polymer construct was then implanted into a subcutaneous pocket of a nude rat for 4 weeks. Both native and TET were analyzed for sulfated glycosaminoglycan (S-GAG) and hydroxyproline content, and stained for H&E, Safranin-O and Collagen Type-II antibody.

Results: The improved design of TET formed new cartilage rings in the structural configuration of native trachea. Furthermore, the decellularized aorta connected well to the cartilage, which provided an excellent support to the rings, as well as good flexibility to the engineered trachea. Histological evaluation of TET showed the presence of mature cartilage. S-GAG and hydroxyproline content was similar to native cartilage levels.

Conclusion: This study demonstrates the feasibility of engineering a trachea with defined cartilage rings and similar flexibility to the native trachea.

Keywords: Airway; Bioengineering; Cell biology/culture; Tissue Engineering; Trachea

Introduction

Experimental and clinical tracheal, bronchial repair or anastomosis began in the late 19th century [1]. Since then the technique of tracheal reconstruction by end-to-end anastomosis has been used in a variety of tracheal diseases, such as stenosis, tracheomalacia, tuberculosis, traumatic injury, and malignancies [2]. However, this established method is not appropriate for the repair of extensive lesions. Therefore, for these types of lesions other replacement methods, such as autografts [2,3], allografts [4,5], prosthetics materials [6-8] and tissue engineering [9-12] have been studied and developed. Some partial successes have been announced with some of these methods, but so far not one of them has archived a completely satisfactory long-term tracheal replacement for extensive lesions of the trachea.

Tissue engineering is an interdisciplinary field that applies the principles of engineering and life science toward the development of biological substitutes that restore, maintain, or improve tissue function [13]. The last decade of the 20th century saw an influx of new research concepts in terms of tissue engineering, which has had a revolutionary impact on the research of tracheal prosthesis [14]. In 1994 two of the first reports on the effort to engineer a trachea were made by Osada and colleagues [9] and Vacanti and colleagues [10]. With different approaches, both groups were trying to recreate the cylindrical shape of the trachea, combining material and cells to make a more biocompatible tracheal replacement. Our group believes that the requirements for an ideal TET are: (1) easy, less-invasive harvest procedure that yields sufficient cells in a relatively short time, (2) biocompatible and biodegradable scaffolding that avoids inflammatory responses, (3) proper flexibility and stiffness, (4) air tightness, (5) neovascularization,

(6) epithelial regeneration and (7) growth on the insert, which is especially important in the treatment of pediatric patients [15].

Scaffolds derived from xenogenic extracellular matrix have been used in numerous tissue-engineering applications [16]. These types of scaffolds are obtained by removing the cellular components of the tissues through different methods. The most effective method for the decellularization of tissues includes a combination of physical, chemical and enzymatic approaches [17]. In an optimal decellularization process, cells have to be completely removed from the tissue while preserving the components of the extracellular matrix [17,18]. Porcine extracellular matrix from different tissues, such as valves [18,19], urinary bladder [16] and small intestinal submucosa SIS [20,21], have yielded positive results when used as a scaffold for tissue repair.

Previously, our group demonstrated the feasibility to engineer a trachea using tissue engineered cartilage and epithelium from different cells sources and with various biomaterials, such as PGA or Pluronic [11,22,23]. The present study is considered to have improved

***Corresponding author:** Koji Kojima, MD, PhD, Laboratory for Tissue Engineering and Regenerative Medicine, Department of Anesthesiology, Brigham & Women's Hospital, Harvard Medical School, 75 Francis St. Thorn 1327 Boston, MA, 02115, USA, Tel: 617 732 5863; Fax: 617 975 0884; E-mail: kojima@zeus.bwh.harvard.edu

Received December 01, 2011; **Accepted** December 08, 2011; **Published** December 09, 2011

Citation: Paz AC, Kojima K, Iwasaki K, Ross JD, Canseco JA, et al. (2011) Tissue Engineered Trachea Using Decellularized Aorta. J Bioengineer & Biomedical Sci S2:001. doi:10.4172/2155-9538.S2-001

Copyright: © 2011 Paz AC, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

the design and functionality of the tissue engineered trachea by using porcine decellularized aorta as a scaffold providing structural support, so as to create a tracheal tube with defined C-shape cartilage rings.

Materials and Methods

Chondrocytes isolation and culture

Samples of sheep tracheal cartilage (n=6), two to three tracheal rings, were obtained from 2 months-old sheep. To isolate chondrocyte cells, each sample was digested with 0.3% collagenase type II (Worthington Biochemical Corp. Freehold, NJ, USA) at 37°C for 5-7 hours while shaking. Chondrocytes were cultured in Ham's F-12 media (Gibco, Grand Island, NJ) containing 10% fetal calf serum (Gibco, Grand Island, NJ) with 292 µg/mL L-glutamine, 10,000 U/mL penicillin G, 10,000 U/mL streptomycin sulfate, 25 µg/mL amphotericin B, and 25 µg/mL ascorbic acid for 2 weeks. Culture medium was changed every 3 days. After 2 weeks, chondrocyte cells were trypsinized with 0.05% Trypsin EDTA (Gibco, Grand Island, NJ) at 37°C for 3 min. Isolated cells were counted using a hemacytometer and the viability was determined using the trypan blue (Sigma-Aldrich, Irvine, CA) exclusion method.

Cell seeding and implantation

After trypsinization, suspensions of approximately 100 x 10⁶

cell/ml was obtained. Each suspension was seeded onto 60 x 50 x 2 mm matrix of non-woven PGA fiber mesh polymer. The cell-polymer constructs were incubated in-vitro for 1 week. Subsequently, 6 pieces of 3x4 cm were cut from a porcine decellularized aorta (Figure 1A) and four 0.5x3cm pieces were excised from one side in a comb like fashion from each piece (Figure 1B). A 4 cm silicon tube stent was inserted into each of the decellularized aortas (Figure 1C) and the spaces were filled with chondrocyte-seeded PGA to create the tracheal rings (Figure 1D). Three-dimensional cell-polymer-aorta constructs were then implanted into a subcutaneous pocket of 6 nude rats for 4 weeks.

Porcine aorta decellularization

Porcine aortas were decellularized using a unique device for tissue-decellularization (Figure 2A). The device was developed by Dr. Iwasaki and his colleagues at the Waseda Institute of Advanced Study, in Tokyo, Japan [19]. The device consists of a microwave irradiation unit and pulsatile circulation system. A porcine aorta with a length of 5 cm and a diameter of 16 mm was connected to a supporting chamber at both ends and incorporated into a pulsatile circulation system. Deoxycholic acid detergent with a concentration of 1wt% was circulated. In parallel, a microwave at the frequency of 2.45GHz was irradiated. In order to prevent degeneration of extracellular matrix by heat generated by the microwaves, a cooling system for the working

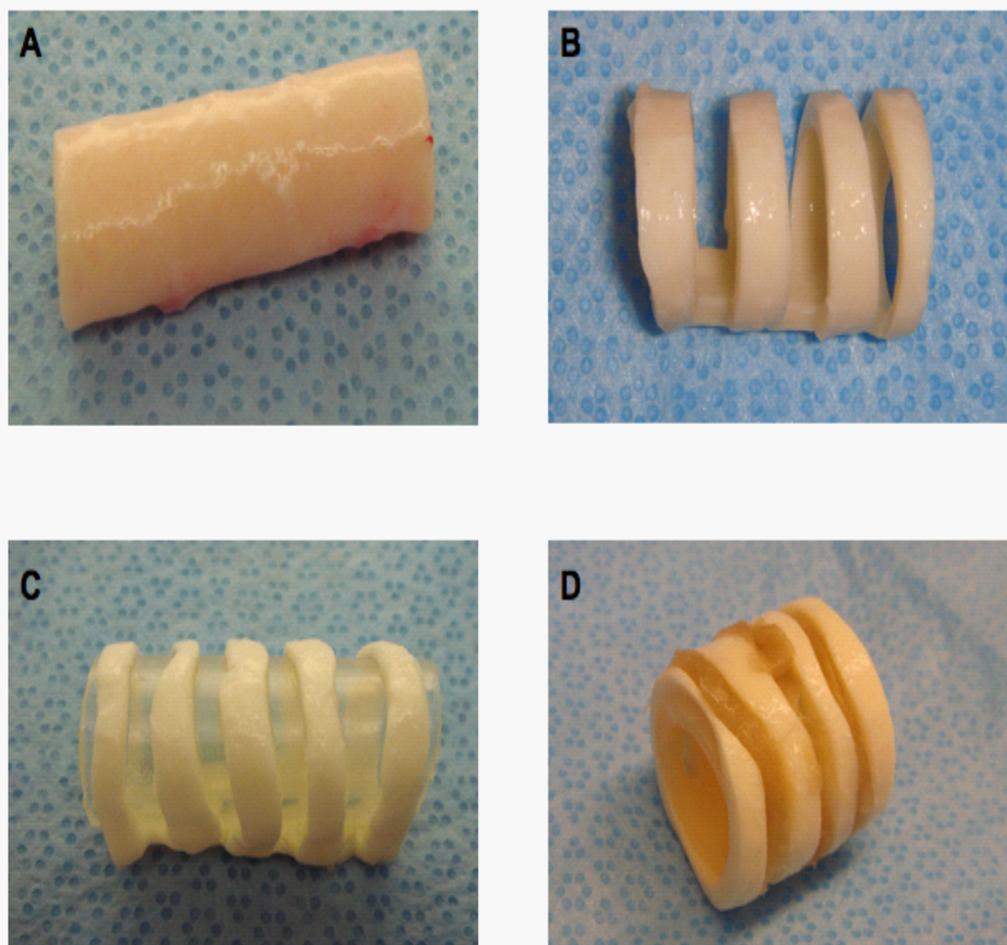


Figure 1: Appearance of the decellularized porcine aorta (A). Sections of the decellularized aorta were excised in a comb like fashion (B). Silicon stents were placed inside the sectioned decellularized aorta (C). The spaces were filled with chondrocyte seeded PGA (D).

fluid was equipped in the system. The temperature of the circulating detergent was monitored and regulated below 37°C using a feedback control mechanism throughout the period of decellularization. Porcine aortic tissue was aseptically harvested. The six aorta pieces were treated with the microwaves for a total of 24 hours in the pulsatile pressure and flow environment (Figure 2B).

Histological examination

Samples of TET and native trachea were fixed in 10% phosphate-buffered formalin, embedded in paraffin and sectioned. Sections were stained with H&E, Safranin-O and anti-Collagen Type II antibody.

Fluorescent immunohistochemistry or Immunofluorescence of collagen Type II antibody

Paraffin sections of TET and native trachea were heated at 60°C for 10 min and rehydrated with Xylene and decreasing concentrations of Ethanol in water. Subsequently, tissue was permeabilized in 1mg/ml of Pepsin (Sigma, St Louis, MO) in 10mM Tris HCL for 1.5 hours at 37°C and blocked with 10% rabbit serum (Jackson Immunoresearch, West Grove, PA). Incubation with the first antibody, anti-collagen type II (1/50, Chemicon, Temecula, CA), was carried overnight. Control slides were not exposed to the first antibody; instead they were incubated overnight with just the blocking solution. The visualization of the first antibody was made with anti-mouse IgG Texas Red (1/100, Jackson Immunoresearch, West Grove, PA). Cell nucleus was counter stained with 4', 6-Diamidino-2-phenylindole, dilactate, DAPI (Sigma, St Louis, MO). Finally, tissue was dehydrated with different concentrations of Ethanol and Xylene and covered with Xylene based media.

Biochemical assay

Due to the fact that proteoglycans and collagen type II are the main components of the cartilage extracellular matrix (ECM), it is important that the TET and the native tracheal rings have similar amounts of these components. The amount of proteoglycans was calculated measuring

the amount of S-GAG, which form chain(s) that are attached through covalent bonding to a core protein to form proteoglycans. Additionally, the amount of collagen type II was calculated measuring the amount of Hydroxyproline, which is a major component of the collagen type II protein.

Biochemical analyses were performed on harvested tissue engineered (n=6) and native trachea cartilage (n=6) to quantify and compare the level of cartilage specific ECM components. First, around 30 mg of each sample was measured and placed to dry out for 24 hours in a Vacuum Desiccator using Drierite (W.A. HAMMOND DRIERITE CO.LTD., Xenia, OH) as a drying agent. Then, samples were weighted again and were digested for 16 hours at 60°C in 1ml of Papain solution, which contained 100 mM sodium phosphate (Na₂HPO₄), 10 mM sodium EDTA (Na₂EDTA), 10 mM cysteine hydrochloride (Sigma, St Louis, MO), 5 mM EDTA, and 125 mg/mL papain (Sigma, StLouis, MO). Finally, the digested samples were stored at -20°C. S-GAG content of the digested samples was quantified with the Blycan Assay kit (Biocolor Ltd., Belfast, North Ireland). Briefly, 1 ml of Blycan Dye Reagent, composed of 1,9-dymethyl-methylene blue in an inorganic buffer, was added to each digested sample. After 30 min the Blycan Dye Reagent bonded to S-GAG. Samples were centrifuged at > 10000 g for 10 min and the supernatant was removed. 1 ml of Blycan Dissociation Reagent, which dissociates the S-GAG-dye complex, was added to each sample. Absorbance was measured at 656 nm with a spectrophotometer. The S-GAG content of each sample was determined by reading the concentration in the standard curve. Hydroxyproline content of the digested samples was determined by the procedure of Stegemann and Stadler [24]. Briefly, the digested samples were hydrolyzed with equal volumes of 6N HCL at 115°C for 16 hours. Chloramine T and p-dimethyl amino-benzaldehyde were added to the hydrolyzed samples; absorbance was measured at 560 nm with a spectrophotometer immediately after addition of the dye. The hydroxyproline content of each sample was determined by reading the concentration in the standard curve. Means and standard deviations

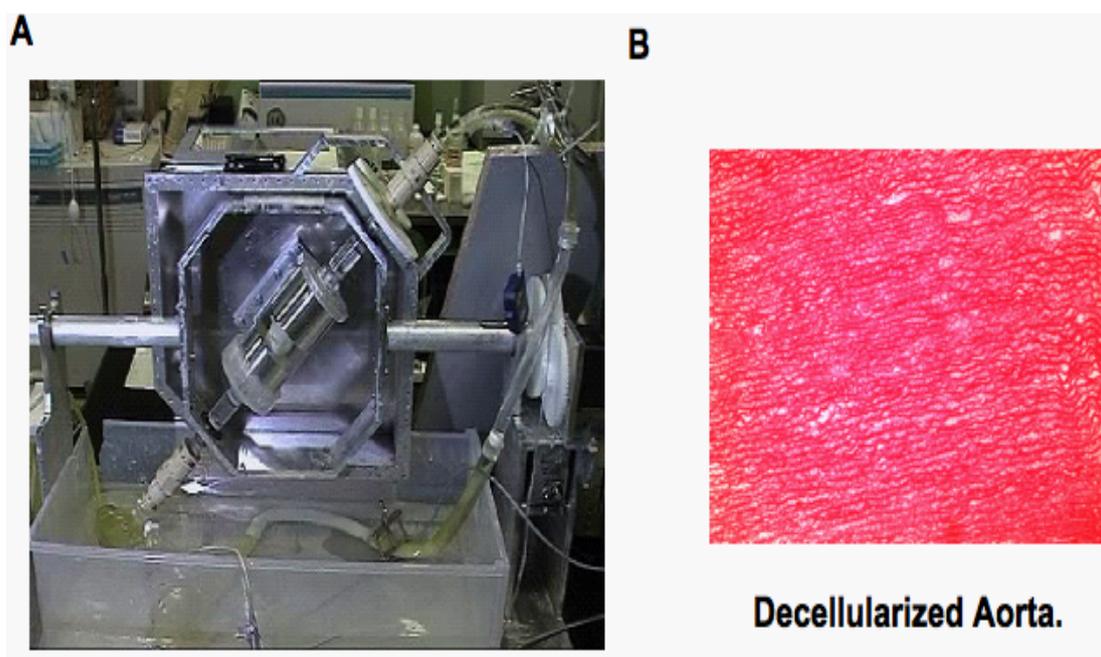


Figure 2: Novel device used to decellularize the sections of porcine aortas (A). H and Estaining of decellularized porcine aorta (B).

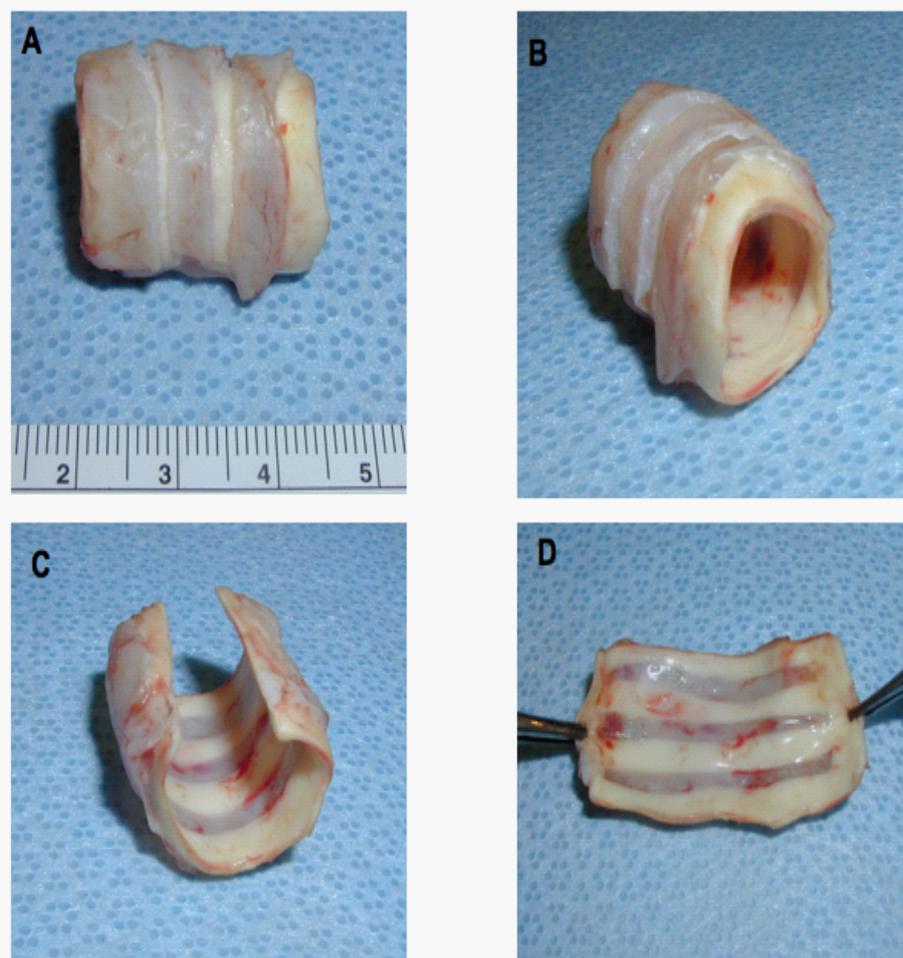


Figure 3: Appearance of tissue engineered trachea following 4 week harvest, Lateral view (A), frontal view (B) and inside view (C,D).

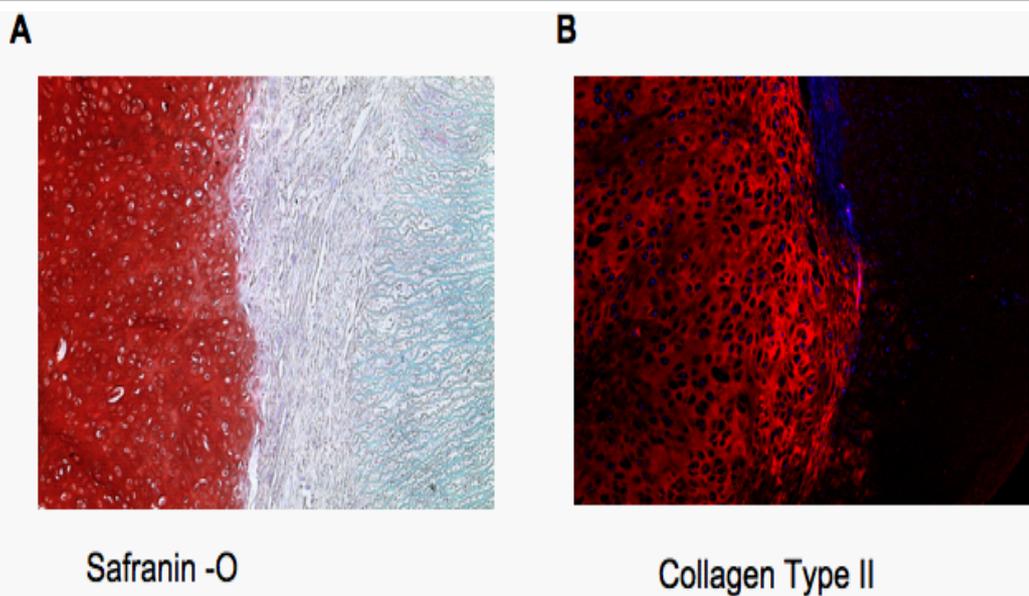


Figure 4: Safranin-O stains demonstrating were deeply positive and indicate of abundant protepglycan production (A). Immunostaining with Collagen type II antibody reveled its existence in the TET cartilage (B).

were calculated for each assay. Statistical significance was determined with a Welch's t-test.

Results

Gross morphology

After 4 weeks, the three-dimensional cell-polymer-aorta constructs formed a TET with defined C-shape cartilage rings, which visibly recreated the structure of a normal trachea (Figure 3 A,B). The decellularized aorta appeared intact and well integrated to the de novocartilage, thus the entire construct felt firm and stable to the touch, and seemed to have adequate stiffness (Figure 3 C,D). Finally, the decellularized aorta appeared to provide appropriate support to the tracheal cartilage.

Histology assay

Histological examination of the specimens showed evidence of the formation of mature cartilage in the TET cartilage, and the decellularized aorta connected well with the de novo cartilage. Hematoxyline and Eosin (H&E) staining revealed a resemblance of the microscopic morphology in TET cartilage to that of normal hyaline cartilage, and showed the decellularized aorta without infiltration of any type of cells. Safranin-O staining demonstrated the present of proteoglycans in the ECM of both TET and native tracheal cartilage (Figure 4A). Finally, immunostaining for Collagen type II antibody in TET and native tracheal cartilage was predominant in both ECMs and also showed the de novocartilage did not invade the decellularized aorta (Figure 4B).

Biochemical assay

S-GAG and Hydroxyproline content in TET were not statistically significantly different to native tracheal cartilage levels. S-GAG content of the TET cartilage was $33.31 \mu\text{g}/\text{mg} \pm 6.26 \mu\text{g}/\text{mg}$ and in native tracheal cartilage was $37.67 \mu\text{g}/\text{mg} \pm 3.14 \mu\text{g}/\text{mg}$ (Figure 5A). The Hydroxyproline content of the TET cartilage was $1.66 \mu\text{g}/\text{mg} \pm 0.54 \mu\text{g}/\text{mg}$ and in the native trachea cartilage was $2.19 \mu\text{g}/\text{mg} \pm 0.34 \mu\text{g}/\text{m}$. S-GAG and Hydroxyproline content in TET cartilage was 88% ($p=0.17$) and 75% ($p=0.08$) of the native trachea cartilage respectively (Figure 5B).

Discussion

Our group has previously demonstrated the feasibility of engineering a trachea using PGA, a thermo reversible hydrogel (pluronic F127) [22] and a thermo reversible gelation polymer (TGP) [26] alone. These past results showed mature and adequate engineered cartilage in a tracheal shape in an animal model with a T cell deficiency. When we moved on to creating an autologous model in vivo in an immunocompetent host, the chondrocytes seeded on PGA, Pluronic F127 or TGP were sufficient for creating healthy cartilage. However, the tissue did not hold a proper tracheal shape due to the lack of sufficient glycosaminoglycan and collagen content. This was likely due to the development of a strong inflammatory response or perhaps because the gelatinous consistency of Pluronic F127 and TGP is poor at maintaining a proper shape. To attempt to circumvent the effects of inflammation, we experimented with the use of an anti-inflammatory drug during the implantation period, but the results showed no significant differences to those without the use of anti-inflammatory agents. Moreover, the tissue generated in the autologous immunocompetent model did not demonstrate sufficient engineered tracheal cartilage using either, PGA, Pluronic F127 and TGP. Thus, we hypothesized that using an alternative material that led to a reduced or absent inflammatory response would generate improved tracheal cartilage. This belief guided our group to focus on using aorta because of its flexibility, and similar size and tubular shape to the human trachea. However, as shown by Tsukada and colleagues [26], using an aortic allograft alone for tracheal replacement leads to necrosis with calcifications or connective tissue replacement of the allograft. Furthermore, the allograft showed no signs of angiogenesis or even evidence of tracheal cartilage regeneration. Finally, the 8 cm aortic allografts shortened up to 87.5% when used for tracheal replacement.

Therefore, based on our previous results, our group concluded that a combination of cell and polymer with a decellularized aortic scaffold would be able to prevent the generation of a strong inflammatory response, avoid necrosis, calcification and contraction, improve overall tracheal cartilage regeneration and structure, and enhance the angiogenic response.

Current decellularization protocols that have become standard are based on 0.1% sodium dodecyl sulfate (SDS) or 3% Triton X-100

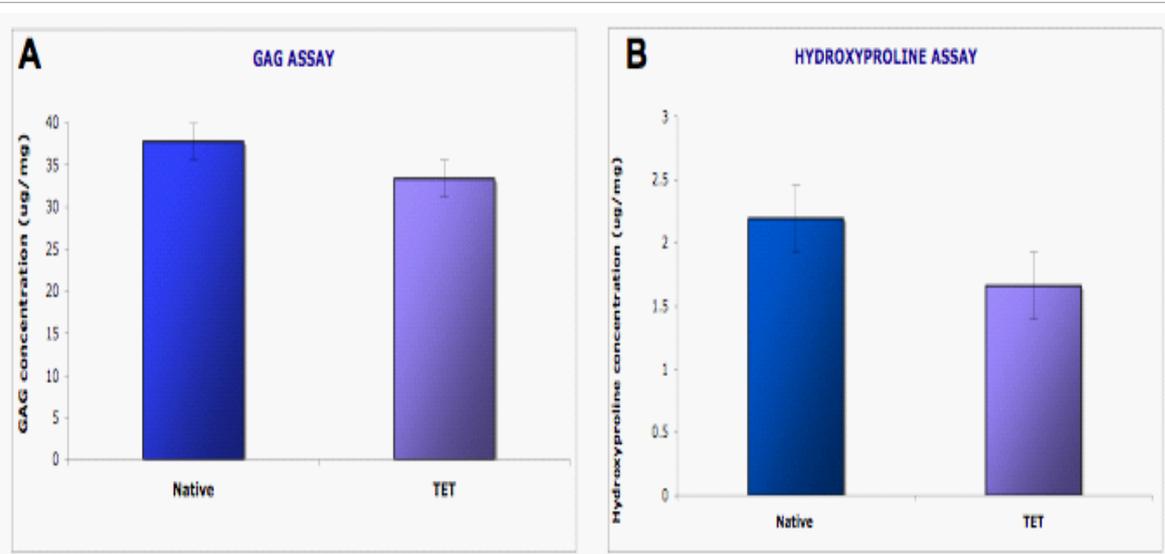


Figure 5: Biochemical assays. GAG (A) and Hydroxyproline (B) content in TET. No statistically significantly different was determined by a Welch's t-test.

(tert-octylphenylpolyoxyethylene). However, this detergent-based methods sacrifice the mechanical integrity of the underlying ECM. It has been reported that ECM damage is due to collagen fibers becoming more loosely packed following decellularization due to the loss of glycosaminoglycans, thus there populated decellularized material does not have the same mechanical strength in vivo [28-30].

Our new decellularization method consists of a microwave irradiation unit along with apulsatile circulation system. It treats aortas with microwave irradiation enhancing the immersion of detergent into the thick aortic tissue by its resonance effect on water molecules. This then leads to the cell membranes being dissolved, causing less damage to collagen fibers. As a result, the unique decellularization method used in this study has advantages in preserving mechanical integrity while dissolving cell membranes. A previous study on this decellularization method demonstrated that it allows the preservation of the ultimate strength and elastic modulus of decellularized aortic valve leaflets and aorta, both results comparable to untreated controls [19].

Based on the success of this novel decellularization method, our group conceived the idea of using a decellularized aortic graft as a flexible support structure for our engineered cartilage created from a PGA scaffold. Our results from this study show that we engineered mature rigid cartilage tissue in between the decellularized aortic graft, generating a well-connected TET with no obstructions. Thus, by creating a cell-polymer decellularized aortic graft construct we were able of engineering a construct with enhanced structural support.

Of note, in this initial study in a nude rat model we were unable to reconstitute the pseudostratified columnar, ciliated epithelium native to the trachea. However, our group has demonstrated that wrapping of an injured deepithelialized trachea with an external epithelial/endothelial cell sheet provided the tissue with similar or enhanced benefits as a native luminal epithelial layer [31]. Thus, when moving to an autologous immunocompetent model, we plan to wrap the engineered tissue and decellularized graft construct with an epithelial and/or endothelial cell sheet to provide the construct with a means to control injury and fibrous response, and preserve tissue perfusion, leading to an enhanced overall reparative response.

In conclusion, our study has demonstrated the feasibility of creating a structurally sound tissue engineered tracheal replacement by combining chondrocytes seeded on a PGA scaffold with a decellularized aortic scaffold. By using the aorta as a backbone, our group was able to improve the structural stability of the regenerated cartilage, and generate tissue strikingly similar to native cartilage. The next step is to repeat the study in an autologous immunocompetent animal model, and examine the tissue histologically as well as biomechanically. The ultimate goal is to optimize our novel technique with the hopes of providing a new therapy for patients with extensive tracheal disease.

References

1. Grillo HC (2002) Tracheal replacement: a critical review. *Ann Thorac Surg* 73: 1995-2004.
2. Martinod E, Seguin A, Pfeuty K, Fornes P, Kambouchner M, et al. (2003) Long-term evaluation of the replacement of the trachea with an autologous aortic graft. *Ann Thorac Surg*: 75: 1572-1578.
3. Cavadas PC, Bonanad E, Baena-Montilla P, Vera-Sempere FJ (1996) Prefabrication of a free flap for tracheal reconstruction: an experimental study. Preliminary report. *Plast Reconstr Surg* 98: 1052-1062.
4. Carbonegani P, Spaggiari L, Solli P, Corradi A, Cantoni AM, et al. (1999) Experimental tracheal transplantation using a cryopreserved aortic allograft. *Eur Surg Res* 31: 210-215.
5. Conconi MT, De Coppi P, Di Liddo R, Vigolo S, Zanon GF, et al. (2005) Tracheal matrices, obtained by a detergent-enzymatic method, support in vitro the adhesion of chondrocytes and tracheal epithelial cells. *Transpl Int* 18: 727-734.
6. Neville WE (1982) Reconstruction of the trachea and stem bronchi with Neville prosthesis. *Int Surg* 67: 229-234.
7. Tsukada H, Osada H (2004) Experimental study of a new tracheal prosthesis: pored Dacron tube. *J Thorac Cardiovasc Surg* 127: 877-884.
8. Schultz P, Vautier D, Charpiot A, Lavalle P, Debry C (2007) Development of tracheal prostheses made of porous titanium: a study on sheep. *Eur Arch Otorhinolaryngol* 264: 433-438.
9. Osada H, Takeuchi S, Kojima K, Yamate N (1994) The first step of experimental study on hybrid trachea: use of cultured fibroblasts with artificial matrix. *J Cardiovasc Surg (Torino)* 35: 165-168.
10. Vacanti CA, Paige KT, Kim WS, Sakata J, Upton J, et al. (1994) Experimental tracheal replacement using tissue-engineered cartilage. *J Pediatr Surg* 29: 201-204.
11. Kojima K, Bonassar LJ, Roy AK, Vacanti CA, Cortiella J (2002) Autologous tissue-engineered trachea with sheep nasal chondrocytes. *J Thorac Cardiovasc Surg* 123: 1177-1184.
12. Weidenbecher M, Tucker HM, Awadallah A, Dennis JE (2008) Fabrication of a neotrachea using engineered cartilage. *Laryngoscope* 118: 593-598.
13. Langer R, Vacanti JP (2003) Tissue engineering. *Science*: 260: 920-926.
14. Kochneva ZV, Vabishchevich AV (1999) The anesthesiological aspects of the support for endoscopic manipulations on the tracheobronchial tree. *Anesteziol Reanimatol* 48-50.
15. Kojima K, Vacanti CA (2004) Generation of a tissue-engineered tracheal equivalent. *Biotechnol Appl Biochem* 39: 257-262.
16. Badylak SF (2004) Xenogeneic extracellular matrix as a scaffold for tissue reconstruction. *Transpl Immunol* 12: 367-377.
17. Gilbert TW, Sellaro TL, Badylak SF (2006) Decellularization of tissues and organs. *Biomaterials*: 27: 3675-3683.
18. Schenke-Layland K, Vasilevski O, Opitz F, Konig K, Riemann I, et al. (2003) Impact of decellularization of xenogeneic tissue on extracellular matrix integrity for tissue engineering of heart valves. *J Struct Biol* 143: 201-208.
19. Iwasaki K, Ozaki S, Kawai T, Yamaguchi S, Eto M, et al. (2005): Innovative bio-reactor technologies produced a completely decellularized and pre-endothelialized functional aortic valve, Proceedings of the 12th International Conference on Biomedical Engineering, 1A2-07, CD-ROM, ISSN: 1727-1983, ISBN:981-05-4572-X.
20. Brown B, Lindberg K, Reing J, Stolz DB, Badylak SF (2006) The basement membrane component of biologic scaffolds derived from extracellular matrix. *Tissue Eng* 12: 519-526.
21. Huber JE, Spievack A, Simmons-Byrd A, Ringel RL, Badylak S (2003) Extracellular matrix as a scaffold for laryngeal reconstruction. *Ann Otol Rhinol Laryngol* 112: 428-433.
22. Kojima K, Bonassar LJ, Roy AK, Mizuno H, Cortiella J, et al. (2003) A composite tissue-engineered trachea using sheep nasal chondrocyte and epithelial cells. *FASEB J* 17: 823-828.
23. Kojima K, Ignatz RA, Kushibiki T, Tinsley KW, Tabata Y, et al. (2004) Tissue-engineered trachea from sheep marrow stromal cells with transforming growth factor beta2 released from biodegradable microspheres in a nude rat recipient. *J Thorac Cardiovasc Surg* 128: 147-153.
24. Stegemann H, Stalder K (1967) Determination of hydroxyproline. *Clin Chim Acta*: 18: 267-273.
25. Kheir E, Stapleton T, Shaw D, Jin Z, Fisher J, et al. (2011) Development and characterization of an acellular porcine cartilage bone matrix for use in tissue engineering. *J Biomed Mater Res A* 99: 283-294.
26. Yasuda A, Kojima K, Tinsley KW, Yoshioka H, Mori Y, et al. (2006) In vitro culture of chondrocytes in a novel thermoreversible gelation polymer scaffold containing growth factors. *Tissue Eng* 12: 1237-1245.
27. Tsukada H, Ernst A, Gangadharan S, Ashiku S, Garland R, et al. (2010) Tracheal Replacement with a silicone-stented, fresh aortic allograft in sheep. *Ann Thorac Surg*: 89: 253-258.

-
28. Kheir E, Stapleton T, Shaw D, Jin Z, Fisher J, et al. (2011) Development and characterization of an acellular porcine cartilage bone matrix for use in tissue engineering. J Biomed Mater Res A 99: 283-294.
29. Remlinger NT, Czajka CA, Juhas ME, Vorp DA, Stolz DB, et al. (2010) Hydrated xenogeneic decellularized tracheal matrix as a scaffold for tracheal reconstruction. Biomaterials 31: 3520-3526.
30. Zou Y, Zhang Y (2011) Mechanical Evaluation of Decellularized Porcine Thoracic Aorta. J Surg Res .
31. BG, Kojima K, Vacanti CA, Edelman ER (2008) Tissue-engineered endothelial and epithelial implants differentially and synergistically regulate airway repair. Proc Natl Acad Sci USA 105: 7046-7051.

This article was originally published in a special issue, **Regenerative Medicine** handled by Editor(s). Dr. Lisa M Larkin, University of Michigan, USA.