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Human airway-derived extracellular matrix bioinks for the engineering of proximal airway-like constructs: A proof-of-feasibility study

Running title: Airway-derived ECM bioinks

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Abstract

Three-dimensional bioprinting offers a promising strategy for the fabrication of physiologically relevant airway constructs; however, generating hollow, anatomically accurate proximal airway structures using tissue-specific bioinks with sufficient printability and mechanical stability remains challenging. This study describes the development and characterization of biocompatible, polymer-blended human airway-derived decellularized extracellular matrix (AW-dECM) bioinks for extrusion bioprinting of structurally and mechanically relevant proximal airway-like hollow constructs. A formulation consisting of 30 mg/mL AW-dECM and nanofibrillar cellulose-alginate conjugated to RGD supported the fabrication of simple and complex hollow airway structures with elastic moduli of approximately 8-10 kPa, within the range measured for bulk proximal airway tissue. The bioinks also supported preliminary primary human airway epithelial cell viability and adhesion, as well as gene expression patterns consistent with differentiation toward mucociliary and secretory phenotypes during 28 days of air-liquid interface culture. Following subcutaneous implantation in immunocompetent rats, acellular AW-dECM polymer-blended bioinks maintained structural integrity over 30 days and were tolerated without evidence of infection or tissue necrosis, although a localized foreign body response was observed. Collectively, these findings demonstrate proof of concept for combining tissue-specific AW-dECM with commercially available printable polymers to fabricate mechanically relevant hollow airway-like constructs. This approach provides a basis for further development of advanced *ex vivo* airway models and regenerative airway engineering strategies.

Keywords: Airway-derived decellularized extracellular matrix; 3D bioprinting; Proximal airways, Polymer blended bioinks, Tissue engineering, Viscoelasticity

1. Introduction

Ex vivo bioengineering approaches for proximal airways remain challenging despite decades of research ¹⁻⁴. Emerging technologies like 3D bioprinting are now being applied to generate tracheal and airway constructs for congenital pediatric respiratory defects and for acute or chronic airway diseases or injury ⁵⁻⁸. These scaffolds, combined with airway-specific stem cells, have the potential to regenerate airway epithelium and restore tissue structure following injury. As current treatments for *congenital airway defects* (i.e., laryngeal clefts ⁹, tracheomalacia, bronchomalacia ¹⁰) or *respiratory diseases* (i.e., cystic fibrosis (CF) ¹¹ and primary ciliary dyskinesia (PCD) ¹²), fail to regenerate damaged or defective airway tissue, improved biofabrication strategies could significantly advance future therapeutic options.

An effective airway engineering strategy requires both appropriate cell sources and biomaterials capable of supporting tissue organization while maintaining printability and structural fidelity. Previously used cell types for proximal airway modeling and engineering applications include human primary bronchial epithelial cells (hBECs) or induced pluripotent stem cells (iPSCs) differentiated to basal epithelial progenitors that, when grown at air-liquid-interface, differentiate into a mature pseudostratified epithelium complete with functional mucociliary and secretory cells ¹³⁻¹⁶. However, commonly used substrates such as polyethylene terephthalate (PET) membranes coated with purified extracellular matrix (ECM) proteins including collagen or Matrigel TM ¹⁷, fail to recapitulate the stiffness, viscoelasticity and biochemical complexity of native airways. These limitations can result in altered cell morphology and transcriptional profiles, reducing the physiological relevance of engineered airway models. 3D bioprinting can overcome many of these limitations by enabling the fabrication of complex, physiologically relevant constructs that better mimic native airway mechanics and architecture while providing precise control over construct geometry.

Among candidate materials, extracellular matrix (ECM)-derived materials are particularly promising because they contain a complex composition of structural proteins, glycoproteins, proteoglycans and growth factors that regulate cell behavior and tissue homeostasis. Unlike synthetic materials that are biologically inert, ECM-derived materials have the potential to better support airway progenitor cell adhesion, growth, differentiation and ongoing physiological remodeling, making them particularly attractive for pediatric tissue engineering applications. In several studies, decellularized ECM (dECM)-derived materials have enhanced the differentiation and function of seeded or embedded iPSC-derived lung progenitors or primary airway or alveolar epithelial cells ¹⁸⁻²¹. To reduce immunogenicity, native tissues are processed using detergents such as Sodium Dodecyl Sulfate (SDS) or Sodium Deoxycholate (SDC) that remove all cellular and antigenic components while preserving the ECM ²². Compared with synthetic materials such as poly (lactic-co-glycolic) acid (PLGA) and polyethylene glycol (PEG), dECM retains tissue-specific biochemical constituents and mechanical properties that promote appropriate cell adhesion, proliferation and differentiation. Additionally, dECM-derived materials have the

potential to integrate with host tissue and under physiological remodeling over time ²³, which is important for pediatric patients whose airways continue to grow and develop ²⁴⁻²⁷.

Despite these biological advantages, dECM exhibits low printability because of its inherently reduced mechanical strength ²⁸. Our recent work has demonstrated that human airway-derived dECM exhibits a low stiffness (~100-300 Pa) ²⁹, making printed structures susceptible to gravity-induced deformation and limiting their ability to maintain complex geometries. Consequently, tissue-specific dECM is typically combined with mechanically supportive polymers such as alginate, gelatin or synthetic materials including polycaprolactone (PCL) or PLGA to provide structural support ²⁸. These blended formulations also increase shear-thinning behavior, a critical rheological property required for high fidelity extrusion bioprinting ³⁰. In parallel, support bath technologies such as freeform reversible embedding of suspended hydrogels (FRESH) have enabled fabrication of complex hollow tissues by providing temporary mechanical support during printing. Using this approach, bioinks are extruded into gelatin microparticle support baths containing ionic crosslinking agents that facilitate gelation and stabilization of intricate 3D architecture ³¹.

In the present study, we developed and characterized the suitability of regionally isolated human airway-derived decellularized extracellular matrix blended with alginate and nanofibrillar cellulose-RGD (Cellink-RGD) bioinks for extrusion bioprinting of hollow proximal airway-like constructs. AW-dECM + Cellink-RGD bioinks exhibited rheological and mechanical properties suitable for extrusion bioprinting, with elastic moduli within the range measured for native proximal airway tissue (~10-20 kPa) ³² and supported the fabrication of simplified and anatomically relevant hollow airway-like constructs with sufficient printability and shape fidelity. Primary human airway epithelial cells seeded on the bioinks remained viable and differentiated toward mucociliary and secretory phenotypes during 28 days of air-liquid interface culture. Following subcutaneous implantation in rats, bioinks maintained structural integrity over 30 days and were generally well tolerated despite a localized foreign-body response. Collectively, these findings demonstrate the feasibility of integrating tissue-specific AW-dECM with printable polymer blends to fabricate hollow airway-like constructs with biologically and mechanically relevant characteristics. This work provides a foundation for the continued development of physiologically relevant *ex vivo* airway models and future airway biofabrication strategies.

2. Materials and methods

2.1. Human lung decellularization and airway-specific isolation

Human lungs from normal, healthy patients (2 donors) with no known lung diseases or smoking history were obtained from the Autopsy Services at the University of Vermont (UVM), under appropriate institutional guidelines and HIPAA protections, and were decellularized using established protocols ^{33, 34}. Adequacy of decellularization was demonstrated by lack of intact cells or cell debris on hematoxylin and eosin-stained mounted 5 μ m paraformaldehyde-fixed sections, no intact bands on DNA gels, and < 50 ng DNA/ μ g protein ^{33, 34}. Airway-specific regions were then isolated from decellularized lungs using a previous protocol ³⁴. To produce the hydrogel,

airway preparations were cryomilled, lyophilized and digested under constant mixing over 72 h using 1 mg/mL pepsin (ThermoFisher Scientific) + 0.01 M HCl (Fisher)^{34,35}. Insoluble aggregates were removed by centrifuging the mixture at 600 rpm for 20 min. The resulting supernatant containing the digested lung tissue was removed, placed in a new conical tube, and pH balanced to 7.0 by adding 1:9 the volume of 0.1M NaOH to the mixture. Samples were then frozen at -80°C for 24 h and lyophilized for an additional 72 h. The decellularized lung tissues and subsequent hydrogel preparations used in this study have previously been extensively characterized for protein and glycosaminoglycan contents^{29,33}.

2.2. Preparation of AW-dECM polymer-blended bioinks for mechanical studies

Polymer-blended AW-dECM bioinks (30mg/mL) were prepared by solubilizing 45 mg lyophilized airway tissue in 1.5 mL Cellink-RGD bioinks (Cellink). Briefly, lyophilized lung tissue was added to a 3 mL syringe and mixed with Cellink-RGD. The bioink suspension was mixed using two 3 mL syringes connected via a female-female luer lock until a homogenous solution was obtained. Solubilized bioinks were then placed on ice for at least 1 h. For comparison, 45 mg lyophilized AW-dECM was also resuspended in 1.5 mL cold Dulbecco's Modified Eagle's Medium (DMEM) (Fisher) and placed on ice for solubilization over 1 h.

2.3. Preparation of AW-dECM polymer-blended bioinks for 3D bioprinting studies

Polymer-blended AW-dECM bioinks (30 mg/mL) were prepared by solubilizing 90 mg lyophilized decellularized airway tissue in 3 mL Cellink-RGD bioinks (Cellink). Samples were mixed using two 3 mL syringes connected via a female-female luer lock until a homogenous solution was obtained. Solubilized bioinks were then placed on ice for at least 1 h prior to printing, followed by loading into a 3 mL gas tight Hamilton syringe.

2.4. Oscillatory rheology measurements of AW-dECM Cellink-RGD bioinks

To assess the mechanical properties of 30 mg/mL AW-dECM alone and 30 mg/mL AW-dECM combined with Cellink-RGD bioink, a DHR-3 Discovery Rheometer (TA Instruments) was utilized. 600 μ L of each of crosslinked, previously swollen in PBS, hydrogel sample (n=6 per hydrogel type) was loaded onto a 12 mm parallel steel plate pre-warmed to 37°C. Gap height was set to 1000 μ m with 1% strain and 0.159 Hz frequency (1.0 rad/s). Oscillatory frequency sweeps were performed at 37°C on all materials and the storage modulus (G') and loss modulus (G'') were determined. To assess the shear thinning behavior (printability) or viscosity of AW-dECM + Cellink-RGD bioinks, flow sweep tests were performed on uncrosslinked samples (n=6) using a 12 mm parallel steel plate set to 25°C, gap height= 1200 μ m and shear rate = 0.001s⁻¹ to 200s⁻¹.

2.5. SEM analysis of bioink preparations

30 mg/mL AW-dECM alone or blended with Cellink-RGD bioink (n=3 per bioink type) were prepared for scanning electron microscopy analysis. Briefly, 30 mg/mL AW-dECM + Cellink-RGD was prepared as above and crosslinked using 0.1% CaCl₂ for 1.5 hours, followed by

rinsing with 1X PBS and incubation at 37 °C for 1 hour. 30 mg/mL AW-dECM alone was placed in a 3D printed polylactic acid (PLA)-based transwell (Matter Hackers) that was 8 mm x 2mm in size. Samples were then crosslinked for 1 hr at 37 °C and subsequently fixed with 2.5% Glutaraldehyde in 0.1 M cacodylate buffer at room temperature for additional 1 h. For post fixation steps, samples were submerged in 1% OxO4, 0.8% Ferricyanide in 0.1 M cacodylate buffer at room temperature for 1 h. Samples were then rinsed 3x with 0.1 M cacodylate buffer, followed by rinsing with distilled water (3x). Samples were dehydrated with ethanol for over 40 min and dried using 1:2 solution of hexamethyldisilazane (HMDS) and 100% ethanol, followed by a 2:1 solution of HMDS and ethanol for 20 min each. For final steps, samples were rinsed in 100% HMDS for 20 min (x2). Samples were then sputter coated with gold and imaged using a JEOL JSM-5900LV scanning electron microscope.

2.6. Fabrication of 3D bioprinter from commercially available FDM printer

A Flash Forge Adventurer 5M desktop printer (Flashforge) was modified into a 3D bioprinter based on a concept developed by researchers at Carnegie Mellon University and demonstrated at the “3D Bioprinting Open-Source Workshop”³⁶ (**Figure S1**). Briefly, Computer-Aided Design (CAD) files were uploaded and 3D printed to assemble the syringe pump (Replistruder) required for layer-by-layer deposition of bioinks (**Figure S1A**). The original parts were removed from the printer, and the 3D printed Replistruder parts were assembled (**Figures S1B + S1C**). Next, the original wifi board was removed and replaced with a Duet Wifi board following a previous protocol³⁶ (**Figure S1D**).

2.7. Conversion of pediatric airway CT scan into 3D printable file

Using an open-source online medical data repository (embodi3D), a CT scan of a pediatric airway was acquired and corresponding DICOM files were uploaded into the imaging platform Slicer 4.11. No HIPAA-protected information was obtained or utilized for the scan. Files were converted into .stl files and modified in Solidworks. The converted airway model was then uploaded into PRUSA software whereby it was modified and converted into G code for subsequent bioprinting (**Figure S2**). Final dimensions were 65 mm x 60 mm x 27 mm with 100% rectilinear infill.

2.8. FRESH support preparation

FRESH v2.0 gelatin microparticle support bath solutions were prepared using a complex coacervation method based on a previous protocol³⁷. Briefly, 125 mL distilled water was added to a 500 mL glass beaker and heated to 50°C, followed by the addition of 125 mL 100% ethanol (Fisher Scientific). To this solution, 3% Gelatin B (Fisher), 0.3% Gum Arabic, and 0.125% Pluronic were added stepwise. A stir bar was placed into the solution and the beaker covered with parafilm, followed by mixing at 350 rpm overnight. The following day, stirring was stopped to allow settling of the formed gelatin microparticles and after ~30 min, excess ethanol was removed. Gelatin microparticles were collected in a 50 mL conical tube and spun down at 750 xg for 3 min.

Excess solution was decanted followed by rinsing, mixing samples with distilled water and spinning at 1000 xg for 3 min (x2). For the final step, the support bath was mixed with 0.1% CaCl₂, spun at 1000 xg for 3 min and then set aside for subsequent printing. All support bath materials were used within a 2-h period to prevent material dehydration.

2.9. Extrusion-based 3D bioprinting of patients-specific airway and simplified hollow airway structure using AW-dECM polymer-blended bioinks and FRESH support

Simplified hollow airway structures (50 mm x 40 mm x 20 mm) were created using SolidWorks. Additionally, hollow cylinders (20 mm x 10 mm x 2 mm and 20 mm x 5 mm x 1 mm), were designed using Tinkercad. 3D models were uploaded into PRUSA software and converted into G-codes reflecting diameter of the syringe and needle used for bioprinting. These files were then uploaded into the custom built bioprinter. All prints were made using a 30-G needle (inner diameter: 0.160 mm, outer diameter: 0.310 mm) (Jensen Global) with a layer height set to 0.06 mm and 3 perimeters. For support bath setup, FRESH slurry was added to a 100 mm tissue culture dish and set up for bioprinting. Prepared bioinks were put into a 3 mL Hamilton gas tight syringe and loaded into the Replistruder syringe pump for FRESH bioprinting. Following extrusion of inks within the gelatin microparticle support bath containing the 0.1% CaCl₂ crosslinker, the airway scaffolds were released by incubating the print containers at 37°C for 1.5 h and rinsing with 1x PBS.

2.10. Culture and seeding of primary human airway epithelial stem cells on 3D bioprinted AW-dECM + Cellink-RGD bioinks

Lung tissues for primary human airway epithelial cell isolation (one donor) were deidentified and obtained from the University of Iowa Cells and Tissues core in the Precision Medicine Center for Cystic Fibrosis with approval of the University of Iowa Institutional Review Board (ID #: 199507432). Isolated primary human airway epithelial cells derived from these tissues and used in this study were graciously provided by the Ryan Lab at the University of Iowa State. 500k cells were cultured and expanded in T75 flasks (Fisher) using Pneumacult Ex Medium (Stemcell Technologies) + supplements (A0830, DH1, Hydrocortisone, Rock Inhibitor). Airway cells (P2) were then seeded at 50k/bioprinted discs (6 mm x 1 mm thick, crosslinked for 1.5 h in 0.1% CaCl₂ and rinsed 2x with PBS and incubated at 37°C for 1 h in transwell inserts and cultured in Pneumacult-EX medium over 4 days. Cells were then air lifted and differentiation medium (Pneumacult ALI-Stemcell Technologies) + supplements (Hydrocortisone, Heparin) were added to the basal compartment of the wells (n=16 wells). The experiment was replicated twice to confirm reproducibility of findings. Cells were then harvested on Day 28 for qPCR analysis.

2.11. *In vitro* assessment of airway epithelial cell viability on 3D bioprinted AW-dECM + Cellink-RGD bioinks

To assess biocompatibility of airway cells seeded on 30 mg/mL AW-dECM + Cellink-RGD bioinks, samples from above were incubated with Calcein AM/Ethidium Bromide staining (Fisher)

at 37°C for 10 min on days 1, 3, 7 and 14 (n=3/day). Samples were evaluated using a Zeiss LSM 880 microscope. The experiment was replicated twice to confirm reproducibility of findings

2.12. qPCR analysis of airway cells seeded on 3D bioprinted AW-dECM + Cellink-RGD bioinks

On Day 28, airway cells seeded on bioinks (n=4 constructs, biological replicates=2) were treated with 1 mL Trizol/well per manufacturer's protocol. Following cell lysis, 200 uL chloroform was added to each sample, vortexed and incubated at room temperature for 2-3 min. Samples were then spun down at 12,000 xg for 15 min at 4°C. Following centrifugation, the top phase was removed and 500 uL isopropanol was added and mixed. Samples were incubated at room temperature for 10 min and then centrifuged as above for an additional 10 min to precipitate isolated RNA. Following centrifugation, the supernatant was removed and 350 uL RLT lysis buffer was added to the RNA pellet. 350 uL 70% ethanol was added to the sample, mixed and dispensed into a column for RNA purification (RNeasy Minikit-Qiagen). RNA concentration was determined using a Nanodrop spectrometer (Thermofisher). Housekeeping genes *B2M* and *YWHAZ* were used for normalization of gene expression.

2.13. Subcutaneous implantation of acellular 3D bioprinted AW-dECM + Cellink-RGD bioinks in rats

To assess biocompatibility and degradation of bioinks, bioprinted samples (Cellink-RGD alone-**control** or 30 mg/mL AW-dECM + Cellink-RGD- **treatment**; 8 mm x 3 mm discs) were implanted subcutaneously (**control**- left side, **treatment**- right side) in the lateral dorsal region of Sprague Dawley rats, weighing 300-500 g (16 weeks of age, n=24; 14 females, 10 males). All animal work was previously approved by the Uconn Health Institutional Animal Care and Use Committee (Protocol #: AP-210081-1126). No randomization or predefined exclusion criteria were applied. Males and females were divided equally among the different groups. Prior to surgery, rats were kept in a temperature-controlled environment (22°C) with 50% relative humidity and 12-12-hour light-dark cycles, with access to water and food as needed. Anesthesia was administered using 4% Isoflurane via a nose cone. Two 3-cm incisions were made, one on the left side and one on the right side of the lateral dorsum in each rat under sterile conditions. Each animal received one sample of Cellink-RGD bioink and one sample of 30 mg/mL AW-dECM + Cellink-RGD bioink circular discs. Bioinks were implanted in a subcutaneous pocket between the skin and muscle of each rat and closed with 3-0 Vicryl suture. Post-operatively, all animals received Ketoprofen (Zoetis) SQ once a day over 3 days for pain management. On Days 3, 14 and 30, eight rats per timepoint were euthanized via CO2 overdose to evaluate biocompatibility (fibrosis, inflammation and presence of granulation tissue), angiogenesis and degradation. Four animals from the Day 30 group were used for mechanical and degradation assessments of the implanted constructs, while the remaining four animals were used for evaluation of cellular infiltration and fibrosis.

2.14. Histological analysis

Tissue samples were fixed in 10% Formalin (Fisher Scientific) for 24 h. Samples were then embedded in paraffin and 5 μm or 10 μm sections were cut using a microtome. For histological assessments, samples were mounted on positively charged slides (Fisher) and stained with hematoxylin-eosin (H & E) and Masson's trichrome (MT) stain to evaluate cellular infiltration/fibrosis. Investigator blinding was performed for all microscopy image analyses. Six fields per scaffold were scanned and quantitative analysis of cellular infiltration (6 images/sample, 10 measurements/image) was performed using ImageJ software.

2.15. Statistical analysis

For analysis of capsule thickness/cellular infiltrate using H & E, measurements obtained from multiple images within each implant were averaged to generate one value per implant. Because each animal received one Cellink-RGD implant and one AW-dECM + Cellink-RGD implant, material measurements were treated as paired observations within each animal. Different groups of animals were euthanized at each implantation time point; therefore, material was analyzed as a within-animal repeated factor and implantation time as a between-animal factor. Data were analyzed using a two-way Anova and Šídák's multiple-comparisons test was used to compare materials within each time point. The animal was considered the biological experimental unit.

Statistical significance of qPCR data obtained from airway cells seeded on 30 mg/mL AW-dECM compared to 30 mg/mL AW-dECM + alginate-RGD blended bioinks were assessed using One-way Anova with Tukey's Multiple Comparisons (GraphPad Prism). Housekeeping genes used for normalization were *YWHAZ* and *B2M*. Mean $\pm$ SD. $p < 0.05$ *; $p < 0.01$ **; $p < 0.001$ ***; $p < 0.0001$ ****, $n = 4$ technical replicates, $N = 2$ biological replicates (study repeated twice using same donor cells). Data calculated using delta-delta Ct method. Statistical analysis performed on fold gene expression values ($2^{-(\Delta\Delta Ct)}$).

3. Results

3.1. AW-dECM polymer-blended RGD bioinks exhibit enhanced mechanical properties compared to AW-dECM alone

The topography and mechanical properties of 30 mg/mL AW-dECM + Cellink-RGD bioink samples were assessed using scanning electron microscopy (SEM) and oscillatory rheometry (**Figure 1**). Analysis of bioink topography using SEM showed that AW-dECM + Cellink-RGD bioinks had a smooth and less porous surface compared to 30 mg/mL AW-dECM alone (**Figure 1A + 1B**). Collagen fibers were evident in both samples (**Figure 1B**). Flow sweeps performed on AW-dECM + Cellink-RGD bioinks demonstrated excellent shear thinning behavior as indicated by a decrease in viscosity with increasing shear rate ($\sim 15 \text{ kPa}\cdot\text{s}$ at 0.01 s^{-1} compared to $\sim 1.0 \text{ Pa}\cdot\text{s}$ at 200 s^{-1}) (**Figure 1C**). Frequency sweeps also revealed that both materials exhibited viscoelastic properties. 30 mg/mL AW-dECM + Cellink-RGD and Cellink-RGD alone exhibited a statistically

significant higher storage modulus (~ 8 kPa) compared to 30 mg/mL AW-dECM alone (~ 100 Pa) (Figure 1D + 1E).

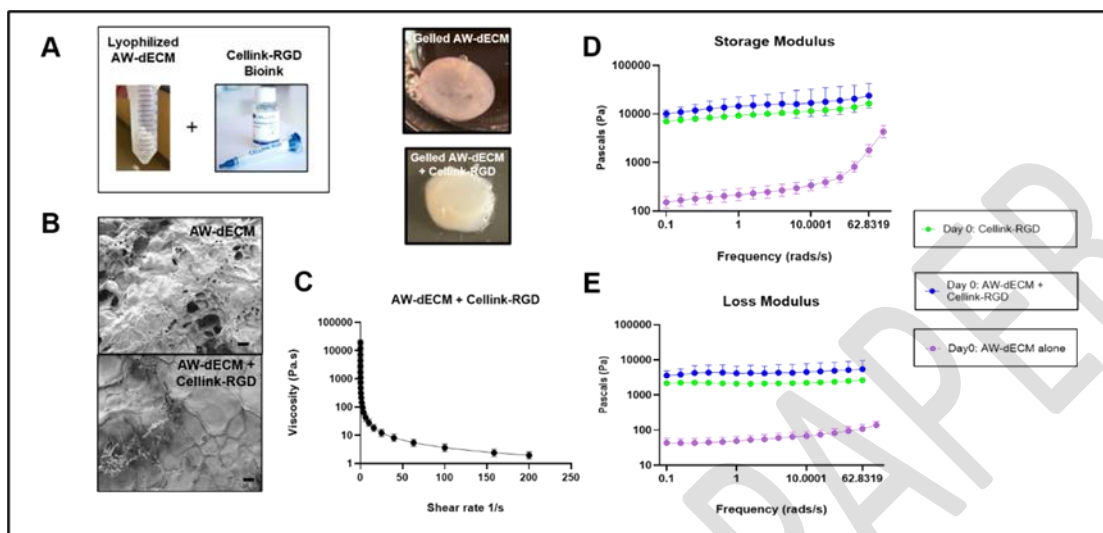


Figure 1: Mechanical and Physical Characterization of AW-dECM ± Cellink-RGD bioinks.

1A: Macroscopic images of gelled AW-dECM alone and gelled AW-dECM + Cellink-RGD prior to SEM analysis. **1B:** SEM images of AW-dECM vs. AW-dECM + Cellink-RGD, scale bars= 200 μ m. Black arrows indicate presence of collagen fibers. **1C:** Flow sweep evaluating viscosity of AW-dECM + Cellink-RGD bioinks in Pa.s, n=6. **1D and 1E:** Frequency sweep evaluating storage and loss modulus in Pascals (Pa) of AW-dECM alone vs. Cellink-RGD alone vs. AW-dECM + Cellink-RGD. Data represented as mean $\pm$ SD, n=6 per material type tested.

3.2. AW-dECM polymer-blended bioinks can be successfully used to bioprint simplified and CT-derived hollow airway structures

To assess printability and shape fidelity of 30 mg/mL AW-dECM + Cellink-RGD bioinks, hollow cylinders (10 mm diameter x 2 mm height x 2 mm wall thickness) were bioprinted using the FRESH approach. After preparing the gelatin microparticle slurry bath (Figure 2A-2D), extrusion multiplier rate settings ranging from 0.55-1.3 were modified using PrusaSlicer 2.8.0 software prior to printing to determine optimal rate for filament integrity and strength. Five hollow cylinders, each bioprinted using a different multiplier rate, were extruded into FRESH support baths and removed by placing the printed cylinders at 37 °C for 1.5 h (Figure 2E). Based on subsequent print height, rate of bioink shrinkage after 2 hours at room temperature and structural integrity as verified by bright field microscopy, it was determined that an extrusion multiplier rate of 1.3 resulted in the most mechanically stable filament structure (Figure 2E-2G). Using these settings, hollow cylinders representing the thickness of a child's upper airway (20 mm x 5 mm x 1 mm and 20 mm x 10 mm x 2 mm in size), were successfully bioprinted and removed from the bath following incubation at 37°C for 1.5 h. Measurement of the cylinders' diameter and length

confirmed that they matched the dimensions of the original CAD files and were able to undergo manipulation without collapsing (**Figure 2H-2L**). Measurement of the cylinder portrayed in **Figure 2K + 2L** demonstrated a wall thickness = 1 mm (**Figure 2M + 2N**). Next, we evaluated the ability to bioprint more complex hollow airway structures using AW-dECM + Cellink-RGD bioinks (**Figure 3A**), which included two simplified hollow airway structures (**Figure 3B + 3C**) and a CT-derived pediatric airway construct (**Figure 3D**). Each bioprinted airway construct was successfully printed a total of three times. (*Note: quantitative measurements of wall thickness were not taken at this time). For each construct, the intended hollow geometries were accurately bioprinted with high fidelity and resolution into FRESH support baths using a 30g needle and rectilinear infill pattern set at 100%. Following printing, the airway constructs were successfully removed by incubating the bath at 37° C for 1.5 h.

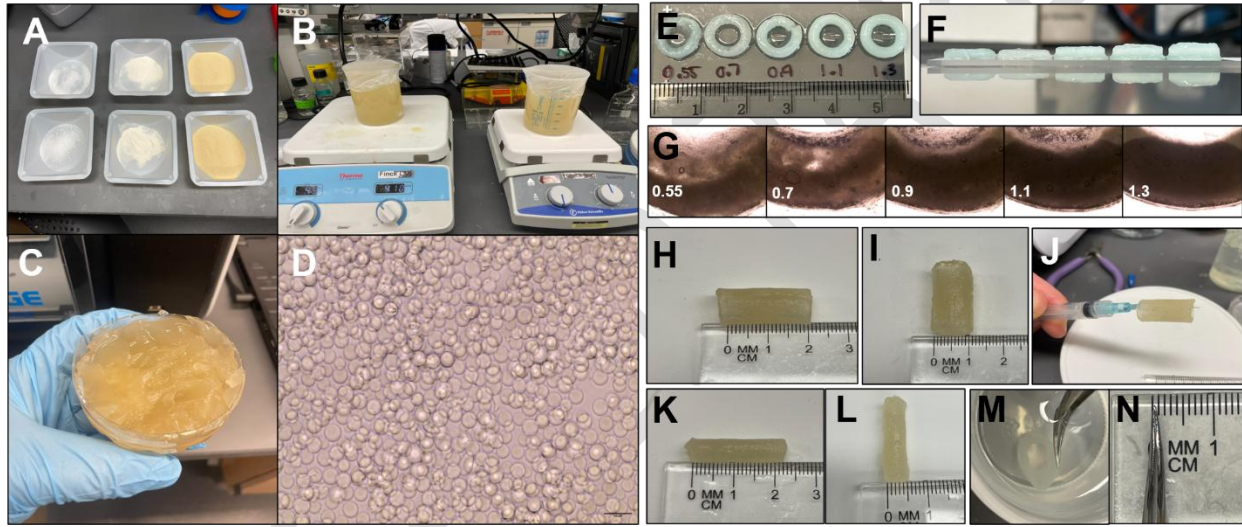


Figure 2: FRESH support preparation and proof of concept for 3D bioprinting hollow cylinders using AW-dECM polymer, blended bioink. 2A-2D: Preparation of FRESH support and bright field image of uniform gelatin microparticles. Scale bar = 50 μ m. **2E+2F:** Testing of different extrusion flow rates for optimal filament density and construct height/thickness. **2G:** Bright field images of hollow, cylinders bioprinted using different extrusion flow rates. **2H-2J:** 20 mm x 10 mm x 2 mm hollow cylinder bioprinted using FRESH approach + 30 mg/mL AW-dECM + Cellink-RGD bioink. **2K+2L:** 20 mm x 5 mm x 1 mm hollow cylinder bioprinted using FRESH approach + 30 mg/mL AW-dECM + Cellink-RGD bioink. **2M+2N:** Wall thickness of bioprinted cylinder (20 mm x 5 mm x 1 mm) in mm.

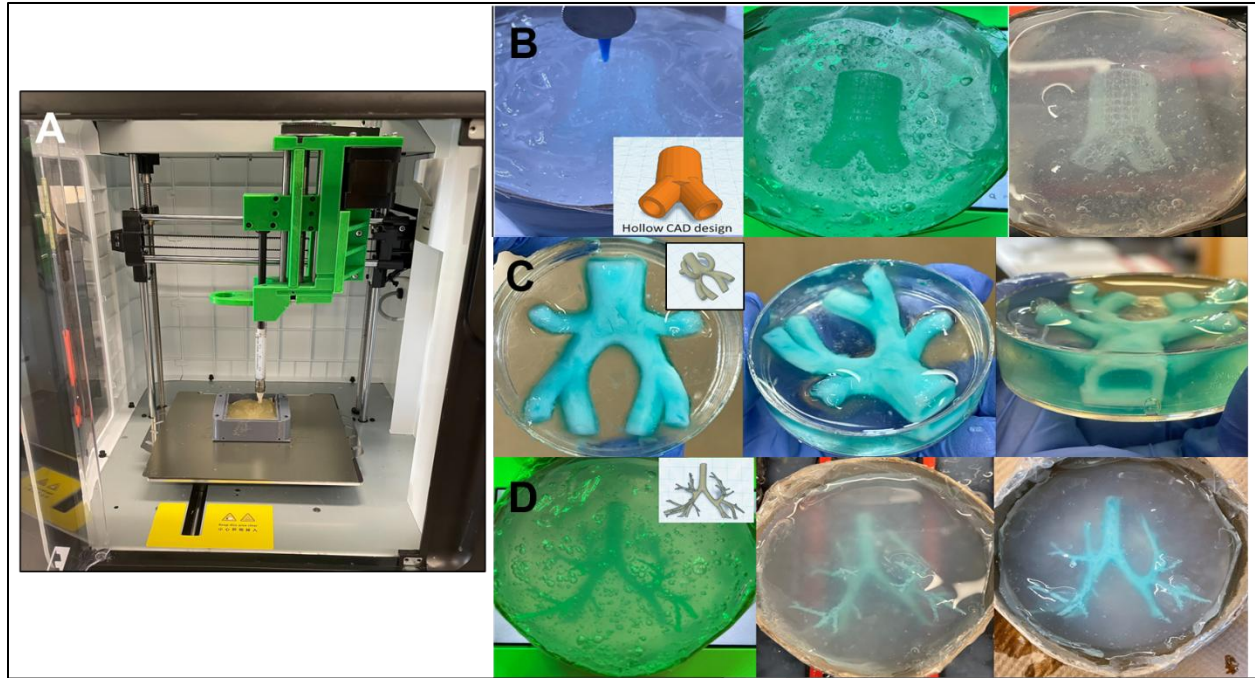


Figure 3: Simplified and CT-derived hollow airway structures 3D bioprinted using AW-dECM + Cellink-RGD bioink and the FRESH approach. 3A: Image of FlashForge Adventurer 3D printer converted into 3D bioprinter. **3B:** 3D bioprinted airway-like structure printed into support bath. **3C:** 3D bioprinted simplified, hollow airway structure. Blue food dye added for visualization. **3D:** 3D bioprinted airway structure derived from a pediatric CT-scan. Blue food dye added for visualization.

3.3. Primary airway epithelial cells exhibit enhanced viability, adhesion and spreading on AW-dECM Cellink-RGD bioinks *in vitro*

To assess *in vitro* biocompatibility, Cellink bioinks conjugated with RGD peptides were combined with 30 mg/mL AW-dECM as described previously. Human primary bronchial epithelial cells successfully adhered to bioinks and exhibited excellent spreading and viability by Day 3 (**Figure 4A**). Additionally, growth and spreading were further enhanced by Day 14 as indicated by Calcein AM/Ethidium bromide staining, demonstrating the *in vitro* cytocompatibility of these bioinks with primary human airway epithelial stem cells (**Figure 4A**).

3.4. AW-dECM polymer-blended bioinks conjugated with RGD support the differentiation of seeded human airway epithelial cells to mucociliary and secretory phenotypes over 28 Days

qPCR analysis revealed the differentiation of primary human airway cells seeded on bioinks to mucociliary and secretory phenotypes over 28 Days (**Figure 4B + 4C**). Results were compared to Day 0 cells and airway cells seeded on 30 mg/mL AW-dECM alone for 28 days.

Results revealed a statistically significant increase in *FOXJ1* expression in primary airway cells seeded on AW-dECM alone and AW-dECM + Cellink-RGD compared to Day 0 ($p \leq 0.0001$ and $p \leq 0.001$, respectively) (**Figure 4C**). There was also a statistically significant increase in *MUC5AC* and *MUC5B* expression across both conditions compared to Day 0. For *MUC5AC* expression, there was a statistically significant increase in AW-dECM + Cellink-RGD vs Day 0 samples ($p \leq 0.05$) and AW-dECM alone vs. Day 0 samples ($p \leq 0.0001$) (**Figure 4C**). For *MUC5B* expression, there was a statistically significant increase in AW-dECM + Cellink-RGD vs. Day 0 samples ($p \leq 0.05$) and AW-dECM alone vs. Day 0 samples ($p \leq 0.001$) (**Figure 4C**). In terms of *SCGB1A1* expression, there was no significant difference in Day 0 vs. AW-dECM + Cellink-RGD samples, however there was a statistically significant increase in samples seeded on AW-dECM alone vs. Day 0 and AW-dECM + Cellink-RGD ($p \leq 0.0001$) (**Figure 4C**). There was also a statistically significant increase in basal cell expression (*KRT5*) in cells seeded on AW-dECM alone compared to Day 0 samples ($p \leq 0.05$), however no difference in expression was seen between cells at Day 0 and cells seeded on AW-dECM + Cellink-RGD indicating basal cell phenotype was maintained over the 28 day period (**Figure 4C**). Overall, *TJP1* expression was elevated on AW-dECM alone compared to Day 0 and AW-dECM + Cellink-RGD samples.

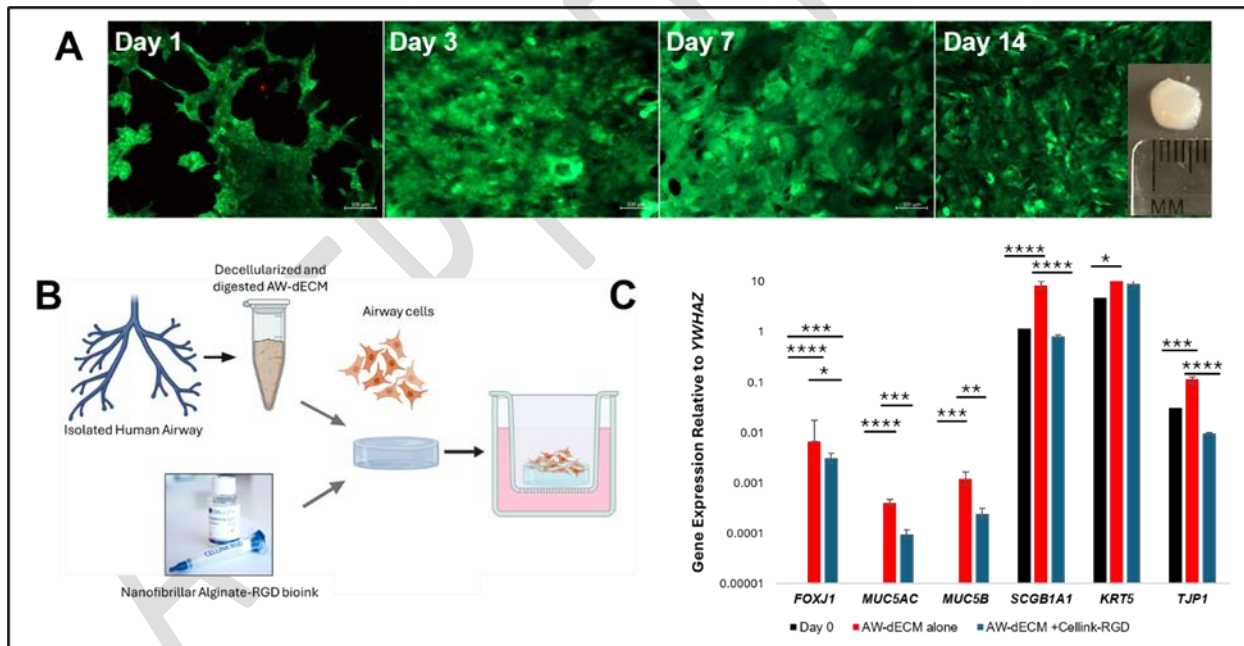


Figure 4: Viability and characterization of primary human airway epithelial cells seeded on AW-dECM + Cellink-RGD bioinks cultured at Air Liquid-Interface over 28 Days. 4A: Live/dead analysis of primary human airway epithelial cells seeded on top of 30 mg/mL AW-dECM + Cellink-RGD bioink over 14 Days, scale bars= 100 μ m. **4B:** Schematic of cell seeding experiment. **4C:** qPCR analysis of *FOXJ1*, *MUC5AC*, *MUC5B*, *SCGB1A1*, *KRT5* and *TJP1* expression in primary human airway epithelial cells (P2) seeded on 30 mg/mL AW-dECM + Cellink-RGD bioinks. Mean $\pm$ SD. $p < 0.05$ *; $p < 0.01$ **; $p < 0.001$ ***; $p < 0.0001$ ****, $n = 4$

technical replicates, N=2 biological replicates (two independent experiments). Data calculated using delta-delta Ct method. Statistical analysis performed on fold gene expression values ($2^{-\Delta\Delta Ct}$).

3.5. Acellular AW-dECM Cellink-RGD bioinks implanted subcutaneously in a rat model over one month demonstrate limited degradation and are biocompatible

Sprague-Dawley rats implanted with bioprinted Cellink-RGD and AW-dECM Cellink-RGD bioink samples over 30 days demonstrated excellent health with no signs of swelling or surgical site infection. Following tissue harvest on Days 3, 14 and 30, macroscopic evaluation of harvested implantation sites revealed no signs of necrosis, edema, infection or tissue rejection (**Figure S3A-S3G**). On Day 3, Cellink-RGD bioink samples were largely mobile within the skin, indicating a lack of encapsulation within the subcutaneous wall and muscle layer. AW-dECM Cellink-RGD bioinks were immobile on Day 3, and gross macroscopic evaluation of harvested implantation sites revealed the presence of a pocket-shaped hole within the subcutaneous wall where the bioinks were deposited (**Figure S3G**). By Days 14 and 30, both types of bioinks were encapsulated within the tissue as indicated by a lack of mobility within the skin. At the microscopic level, H & E staining of both bioink types at all time points revealed a lack of tissue integration (**Figure 5**), however, cellular infiltration was evident at the bioink-tissue interface (**Figure S4**). Measurement of this layer at Days 14 and 30 in animals implanted with both Cellink-RGD and AW-dECM Cellink-RGD bioinks revealed no significant differences in thickness between both samples in each animal and at both time points (**Figure S4B**).

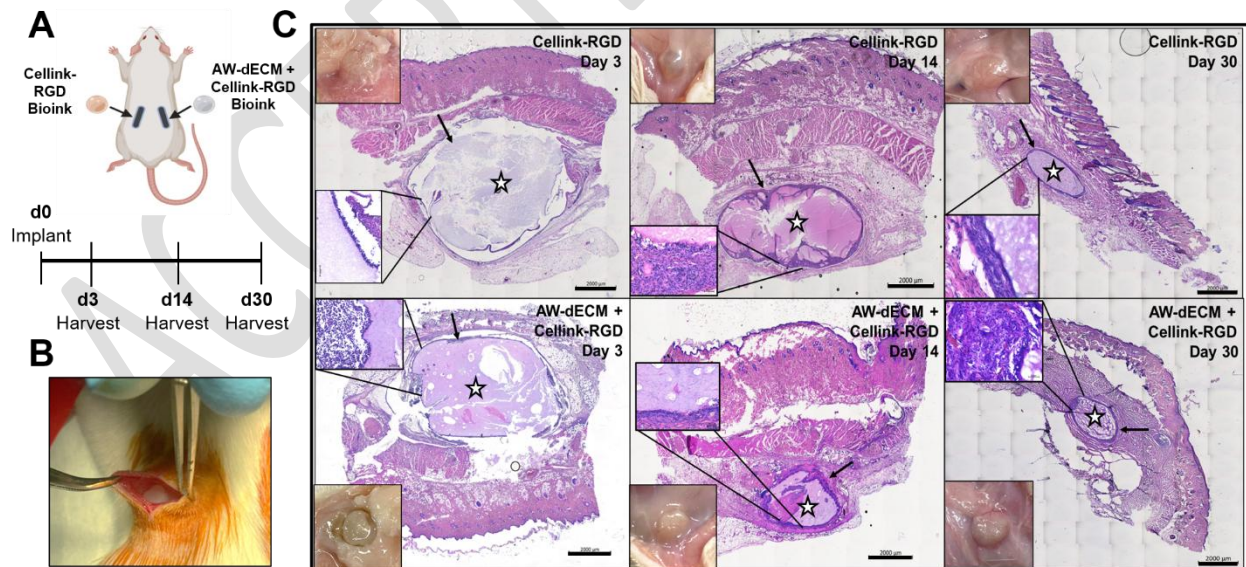


Figure 5: Representative H & E Staining of Acellular Cellink-RGD and AW-dECM +Cellink-RGD bioinks implanted subcutaneously in immune competent Sprague Dawley rats over 30 Days. 5A: Outline of experimental approach and timeline, n=8 rats/time point harvested. Images are representative paired observations from one animal per timpoint. **5B:**

Imaging depicting placement of bioink into subcutaneous tissue pocket. **5C**: Representative H & E staining of implanted bioinks at different time points. White star symbol denotes subcutaneously implanted bioink. Scale bar= 1000 μ m. Inset H & E images of bioink edge = 50 μ m. Inset macroscopic images in upper left (**top panels**) and lower left (**bottom panels**) depict bioink following tissue harvest at **Day 3, Day 14** and **Day 30**.

Masson's Trichrome staining demonstrated the presence of a typical foreign body response evident by the formation of a fibrotic capsule surrounding the implanted bioink (**Figure 6**). Immunofluorescent staining of Day 3 bioink samples with a neutrophil-specific marker Myeloperoxidase confirmed the presence of acute inflammatory cells on the bioink surface and in the surrounding tissues following bioink implantation (**Figure S5**), that resolved by Day 14 (**Figure S6**). Additionally, immunofluorescent staining using CD163+ and iNOS+ antibodies suggested the presence of M1 and M2 macrophage cell populations in proximity to the bioink at Days 14 and 30 (**Figure 7**). Prior to mechanical evaluation of Day 30 implanted bioinks, fibrous tissue encapsulated around the structures was removed to allow for accurate assessment of potential degradation (biological replicates=4, technical replicates= 3). Comparison of the storage and loss moduli of Day 0 vs. Day 30 bioinks demonstrated no significant difference in values between both Cellink- RGD alone and AW-dECM + Cellink-RGD, suggesting minimal degradation occurred over the 30-day period following transplantation (**Figure 8A + 8B**). To further support this assessment, measurement of Day 30 bioinks compared to Day 0 revealed no change in diameter over time for both types of bioinks (**Figure 8C + 8D**).

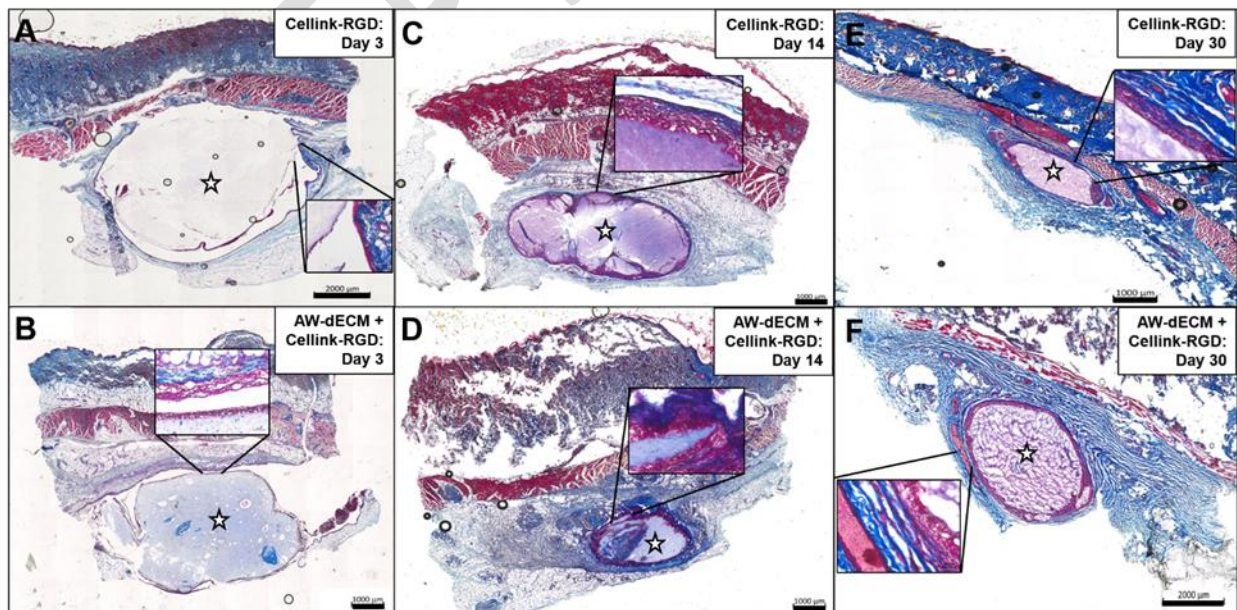


Figure 6: Representative Masson's Trichrome staining of acellular Cellink-RGD and AW-dECM +Cellink-RGD bioinks implanted subcutaneously in immune competent Sprague Dawley rats over 30 days. 6A + 6B: Cellink-RGD and AW-dECM + Cellink-RGD on Day 3. 6C + 6D: Cellink-RGD and AW-dECM + Cellink-RGD on Day 14. 6E + 6F: Cellink-RGD and AW-dECM + Cellink-RGD on Day 30. Star symbol denotes subcutaneously implanted bioink. Inset images, scale bar= 50 μ m.

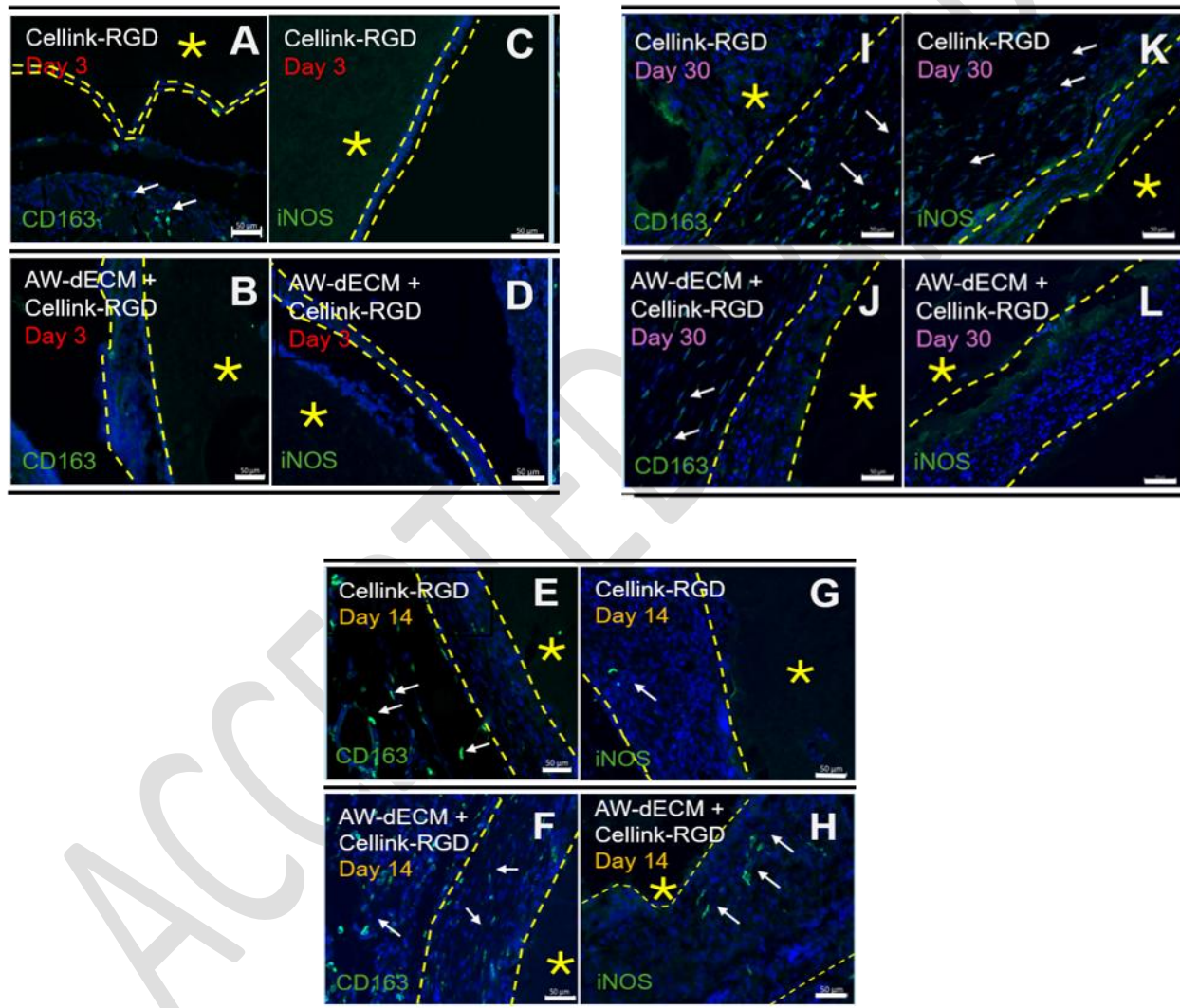


Figure 7: Representative immunofluorescent analysis of cell populations present at bioink-tissue interface at Days 3, 14 and 30. 7A + 7B: CD163⁺ staining of cells at bioink-tissue interface in Cellink-RGD and AW-dECM Cellink-RGD samples on Day 3. 7C + 7D: iNOS⁺ staining of cells at bioink-tissue interface in Cellink-RGD and AW-dECM Cellink-RGD samples on Day 3. 7E + 7F: CD163⁺ staining of cells at bioink-tissue interface in Cellink-RGD and AW-dECM Cellink-RGD samples on Day 14. 7G + 7H: iNOS⁺ staining of cells at bioink-tissue

interface in Cellink-RGD and AW-dECM Cellink-RGD samples on Day 14. **7I + 7J**: CD163+ staining of cells at bioink-tissue interface in Cellink-RGD and AW-dECM Cellink-RGD samples on Day 30. **7K + 7L**: iNOS+ staining of cells at bioink-tissue interface in Cellink-RGD and AW-dECM Cellink-RGD samples on Day 30. Yellow dotted lines demarcate bioink-tissue interface, yellow star denotes bioink sample. White arrows indicate cells with positive staining. Scale bars= 50 μ m.

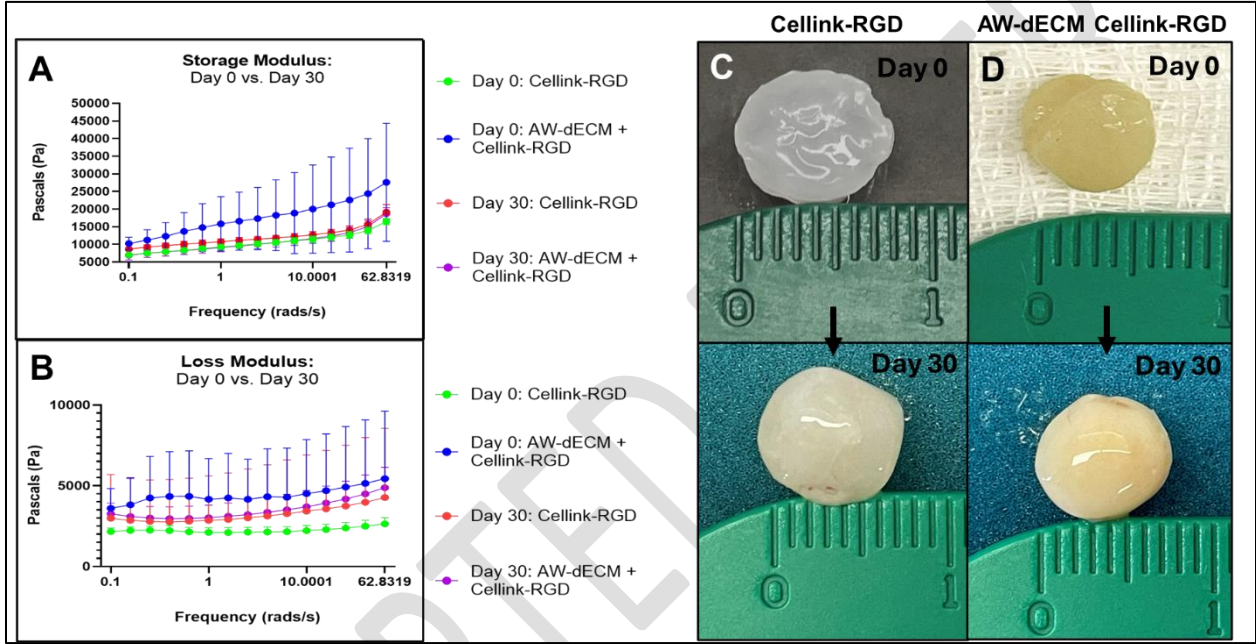


Figure 8: Mechanical assessment and structural evaluation of Cellink-RGD and AW-dECM Cellink-RGD bioinks following subcutaneous implantation in rats over 30 Days. 8A: Storage Modulus in Pascals (Frequency Sweep) of Cellink-RGD vs. AW-dECM Cellink-RGD bioinks at Day 0 vs. Day 30 of SQ implantation. **8B:** Loss Modulus in Pascals (Frequency Sweep) of Cellink-RGD vs. AW-dECM Cellink-RGD bioinks at Day 0 vs. Day 30 of SQ implantation. Data represented as mean $\pm$ SD, n=6 bioinks/time point (Day 0), n=4 bioinks/time point (Day 30). **8C + 8D:** Measurement of Cellink-RGD and AW-dECM Cellink-RGD bioinks at Day 0 vs. Day 30.

4. Discussion

Despite significant advances in airway engineering, biofabrication of physiologically relevant proximal airway constructs remains challenging because current approaches often cannot simultaneously recapitulate tissue-specific biochemical composition, mechanical properties, sufficient printability, and anatomically relevant hollow architectures. Here, we developed and characterized a human airway-derived decellularized extracellular matrix (AW-dECM)-based polymer-blended bioink that combines tissue-specific extracellular matrix components with

polymer-enhanced printability and structural stability. This biofabrication strategy enabled the successful fabrication of both simplified hollow airway structures and anatomically relevant airway-like constructs generated from CT scans while exhibiting bulk mechanical properties within the range measured for native airway tissue. Primary human airway epithelial cells seeded on the bioinks and cultured at an air-liquid interface for 28 days remained viable, adhered and spread across the constructs, and exhibited gene expression patterns consistent with differentiation toward mucociliary and secretory phenotypes. *In vivo* studies using immunocompetent rats demonstrated that acellular bioinks were tolerated without evidence of infection or tissue necrosis over 30 days, although a typical foreign body response developed following implantation. Collectively, these findings demonstrate the feasibility of incorporating tissue-specific ECM into polymer-blended bioinks for proximal airway biofabrication.

The present findings also provide insight into several limitations that remain in the field of proximal airway engineering. While past studies have demonstrated successful bioprinting of airway-like constructs, many have relied on purified ECM proteins or synthetic biomaterials that prioritize printability over biological complexity^{38,39}. Conversely, tissue-specific dECM materials often provide a more physiologically relevant microenvironment but lack the mechanical properties required for fabrication of stable hollow structures. This limitation is due, in part, to the decellularization and bioink formation process, during which pepsin digestion disrupts collagen telopeptides and reduces the mechanical stability of the resulting biomaterial⁴⁰. This often renders the material too soft for extrusion-based bioprinting, limiting its ability to maintain shape following deposition into a support bath or culture dish. The addition of natural or synthetic polymers substantially enhances the mechanical properties of dECM bioinks, improving printability and shape fidelity. In the present study, commercially available alginate conjugated to RGD was selected as the polymer component because it is naturally derived, inexpensive, and demonstrates enhanced biocompatibility, cell adhesion, and printability compared to alginate alone and other synthetic bioinks such as PEG and PLGA⁴¹.

By combining airway-specific dECM with a mechanically supportive polymer blend, the present approach addresses the limited mechanical stability of dECM while retaining tissue-specific biochemical cues important for airway engineering. Our previous characterization of the AW-dECM used in this study demonstrated that it is highly enriched in collagen I, VI, laminin, fibronectin, and other cartilaginous collagens, all of which are important extracellular matrix proteins involved in cell attachment, growth, and differentiation²⁹. Consistent with these findings, other studies have demonstrated that tissue-specific bioinks more closely recapitulate the native tissue microenvironment because of their inherent biochemical composition, making them attractive for clinically relevant tissue engineering applications and drug screening platforms⁴²⁻⁴⁶. Likewise, our previous work also demonstrated that AW-dECM is biocompatible and supports mucociliary and secretory differentiation of primary human bronchial epithelial cells²⁹, providing the rationale for incorporating AW-dECM into the polymer-blended bioinks evaluated in the present study. Although tissue-specific dECM bioinks have been investigated for several organ systems, relatively few studies have evaluated human airway-derived dECM in combination with

printable polymer blends for fabrication of hollow airway constructs. Consequently, the present work extends previous studies by incorporating regionally isolated human airway-derived ECM into a mechanically reinforced bioink while evaluating its printability, epithelial cell compatibility, and *in vivo* host response.

Compared to AW-dECM alone, AW-dECM blended with Cellink-RGD exhibited enhanced mechanical properties that substantially improved structural fidelity, allowing development of stable hollow airway constructs. The storage moduli (~8-10 kPa) of the polymer blended bioinks more closely reflect average bulk mechanical values reported for native proximal airway tissue (~10-20 kPa) compared to AW-dECM alone (~100 Pa), enabling the successful bioprinting of hollow airway-like structures with improved shape fidelity⁴⁷. In addition to elastic modulus, shear-thinning behavior is an important determinant of extrusion bioprinting performance because it permits bioinks to flow under shear while rapidly recovering viscosity following deposition⁴⁸. This characteristic contributed to the ability of the AW-dECM Cellink-RGD formulation to generate stable hollow geometries using the FRESH approach. While the current study focused primarily on bulk mechanical characterization, native proximal airway tissue is mechanically heterogeneous⁴⁹. Therefore, future studies should evaluate regional mechanical heterogeneity throughout printed constructs and its influence on airway cell behavior. Incorporation of chondrocytes and stromal cell populations into 3D bioprinted airway constructs may also better mimic the mechanical properties found within different regions of the upper airways.

Based on our *in vitro* findings, gene markers associated with mucociliary, and secretory differentiation were elevated in airway epithelial cells seeded on AW-dECM Cellink-RGD bioinks, albeit at lower levels compared to AW-dECM alone. Despite this, epithelial cells remained viable and underwent differentiation toward both ciliated and secretory phenotypes, suggesting that incorporation of Cellink-RGD did not eliminate the instructive effects of airway-derived ECM but instead may alter the efficiency of epithelial differentiation. This may be due to differences in cell-matrix interactions associated with the polymer blend, including reduced matrix remodeling resulting from the lower degradability of alginate, altered ligand presentation, or other changes in the biochemical and mechanical environment⁵⁰. Future studies will investigate the effects of Cellink-RGD alone to determine its full contributions to airway epithelial differentiation.

Overall, similar tradeoffs between printability and biological activity have been reported for numerous polymer-blended dECM bioinks⁵¹. Because these mechanisms were not directly evaluated in the present study, additional work will be required to determine their relative contributions. Furthermore, protein-level characterization and morphological analyses using multiple epithelial cell donors will also be necessary to confirm successful epithelial differentiation and function. Nevertheless, the present findings demonstrate that polymer-blended AW-dECM bioinks support preliminary airway epithelial cell viability and growth while providing the mechanical properties required for extrusion bioprinting, highlighting their potential for future airway biofabrication applications.

Our *in vivo* results of subcutaneously implanted bioinks were tolerated without evidence of necrosis or infection in immunocompetent Sprague Dawley rats over 30 days. After 30 days,

the bioinks maintained their original size and bulk mechanical properties as indicated by macroscopic and rheological evaluation of harvested bioinks at Day 0 and Day 30. However, a typical foreign body response (FBR) to both Cellink-RGD alone and AW-dECM Cellink-RGD bioinks was evident by Days 14 and 30. Histological staining revealed the development of a well-formed fibrous capsule in both conditions by the end of one month. Development of a fibrous capsule following implantation is a common outcome for implanted biomaterials and does not necessarily indicate material failure⁵². Rather, the extent of this response can be influenced by several factors including biomaterial composition, degradation rate, surface characteristics and mechanical properties⁵³. Overall, these findings are consistent with previous reports describing host responses to implanted hydrogel-based biomaterials^{23, 54-56}. Additionally, limited data currently exists related to the *in vivo* host response to alginate-RGD biomaterials, as most studies primarily focus on its *in vitro* performance⁵⁷⁻⁵⁹. Therefore, the present study extends characterization of this airway bioprinting approach by providing information regarding the *in vivo* host response to commercially available alginate-RGD biomaterials following implantation into immunocompetent animals.

To reduce the severity of the FBR, pre-clinical studies have suggested incorporating anti-inflammatory bioactive peptides or cytokines, such as IL-4, into biomaterials to promote macrophage polarization toward M2 phenotypes⁶⁰⁻⁶². In the present study, assessment of the cell types within the fibrous capsule at Day 30 suggested the presence of both M2 and M1 macrophages, as indicated by CD163+ and iNOS+ staining. This is consistent with previous studies demonstrating the important role of macrophages in regulating the FBR to implanted hydrogels⁶³. The mixed M1/M2 macrophage phenotype observed in this study may reflect distinct immunogenic signals from the Cellink-RGD bioink and tissue-specific dECM components, with alginate potentially contributing to the FBR⁶⁴ while AW-dECM may provide immunomodulatory cues that influence macrophage polarization^{65, 66}. However, as the thickness of the cell infiltrate/fibrotic capsule did not differ between Cellink-RGD and AW-dECM + Cellink-RGD implants, the contribution of AW-dECM to this response may be negligible. Currently, there is no standard for predicting the immunogenicity of tissue-specific dECM; therefore, studies evaluating residual MHC and antigen glycan content, cryptic particle abundance, and ECM structural alterations following processing will help elucidate its full contribution to the FBR response⁶⁷. These findings will also help guide future modifications to bioink composition aimed at promoting a more favorable host immune response.

As such, future studies could evaluate the incorporation of anti-inflammatory components into AW-dECM Cellink-RGD bioinks to determine whether host response and integration can be improved. In addition to biochemical modification of the bioink, modulation of its mechanical properties may provide another strategy for influencing the host immune response following implantation. One study demonstrated that stiffer hydrogels (~10 kPa) promoted a more pro-inflammatory macrophage phenotype compared with softer hydrogels (<2 kPa)⁶⁸. Therefore, future studies could investigate modulation of bioink stiffness, for example through reduced

crosslinking time, to determine whether a slightly softer substrate promotes a more favorable host immune response while maintaining sufficient structural integrity for airway biofabrication.

Another study also investigated the utility of alginate-blended (without RGD) *bulk lung* bioinks for 3D bioprinting lung-specific tissue. Researchers found that the material exhibited excellent printability and biocompatibility of embedded and seeded primary human epithelial cells, however the samples were subcutaneously implanted in a mouse model of *transplant immunosuppression*⁶⁹. By comparison, the present study evaluated Cellink-RGD and AW-dECM Cellink-RGD bioinks in immunocompetent hosts, providing complementary information regarding the host response when the innate immune system remains intact. Although these materials have demonstrated favorable *in vitro* cytocompatibility, the present findings indicate that they remain susceptible to a typical foreign body response following implantation. These observations highlight the importance of evaluating both *in vitro* performance and *in vivo* host responses when developing biomaterials intended for regenerative applications.

Another aspect that will require further investigation is the degradation rate of the bioink. Controlled degradation represents an important design consideration because airway replacements must initially withstand repetitive respiratory forces while eventually permitting replacement by host-derived tissue⁷⁰. Consequently, the degradation rate must provide sufficient structural support during the early stages of implantation while allowing cellular infiltration and matrix remodeling over time. For load-bearing tubular tissues such as the airway, premature degradation could compromise lumen patency and mechanical integrity, whereas excessively slow degradation may delay host integration and adaptive remodeling. Therefore, an intermediate degradation profile that provides initial structural support while permitting gradual host-mediated remodeling and vascularization is most desirable⁷¹. In the present study, AW-dECM + Cellink-RGD bioinks remained structurally intact following 30 days of subcutaneous implantation, suggesting that substantial degradation had not yet occurred. However, longer-term implantation studies (6 months to 1 year) will be necessary to determine whether the degradation profile is appropriate for future airway applications. If the bioinks demonstrate prolonged persistence *in vivo*, one potential strategy would be to incorporate MMP-sensitive peptides to promote controlled matrix degradation. In one study, incorporation of MMP2-sensitive peptides into alginate hydrogels laden with human mesenchymal stem cells increased material degradation and improved tissue integration following subcutaneous implantation in xenograft mouse models⁷².

Limitations of this study include the use of xenogeneic materials to assess host immune response and overall biocompatibility, as well as evaluation of acellular constructs rather than cell-laden implants. Future studies using decellularized rat airway tissue combined with Cellink-RGD will determine whether the host response and tissue integration are improved in immunocompetent animals. While the subcutaneous implantation model provides valuable information regarding biocompatibility and host response, it cannot fully replicate the biomechanical loading, airflow, mucosal environment, or microbial exposure encountered within the native airway. Consequently, interpretation of these findings should be limited to assessment of material compatibility rather than prediction of orthotopic airway performance. Therefore, the host response and structural

integrity of bioprinted airways following orthotopic implantation will need to be evaluated, as the airway represents a substantially different mechanical and immunological environment. To address this, future studies will focus on the development of a small animal airway defect model to iteratively evaluate these responses and further assess the translational potential of AW-dECM Cellink-RGD bioinks. Finally, quantitative validation of print fidelity and reproducibility, including comparisons between CAD designs and printed constructs (wall thickness measurements) and assessment of inter-print variability, will be incorporated in future studies as well.

5. Conclusion

In conclusion, this study demonstrates the feasibility of a human airway-like biofabrication approach for producing mechanically stable hollow airway constructs using polymer-blended AW-dECM bioinks. Through characterization of rheological behavior, mechanical properties, printability, preliminary epithelial cell compatibility, and *in vivo* host response, these findings demonstrate that tissue-specific AW-dECM can be successfully integrated with commercially available printable polymers to fabricate hollow airway constructs possessing structural and mechanical characteristics relevant to proximal airway tissue. Although further optimization of immune compatibility, matrix remodeling, and orthotopic implantation will be required prior to clinical translation, this work provides a foundation for the continued development of physiologically relevant *ex vivo* airway models and future tissue-engineered airway replacements.

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Conflict of interest

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Ethics approval and consent to participate

All animal work was approved by the Institutional Animal Care and Use Committee at the University of Connecticut Health Center (Protocol #: AP-210081-1126). Deidentified human lungs were obtained from the University of Vermont (UVM) autopsy services. Ethical clearance was not required for use of the materials. Per UVM policy, research involving deceased people is not considered human subject research and does not require IRB review. Deidentified lung tissues for human bronchial epithelial cell (HBEC) isolation was obtained from donor lungs from the University of Iowa Cells and Tissues core in the Precision Medicine Center for Cystic Fibrosis with approval of the University of Iowa Institutional Review Board (ID #: 199507432). Primary human bronchial epithelial cells were provided through the Cells, Tissues and Models core funded by NIH-NIDDK P30 DK054759-22 (to ALR).

Consent for publication

Not applicable.

Availability of data

Data are available from the corresponding authors upon reasonable request.

Further disclosure

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