ACCSCIENCE PUBLISHING

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RESEARCH ARTICLE

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

## Supplementary File

**Table S1. Materials used for three-dimensional bioprinting**

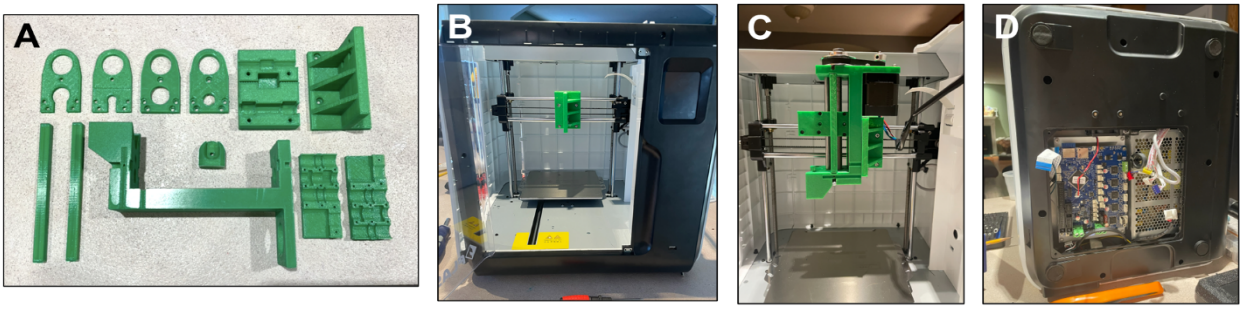
| Materials | Catalog # | Manufacturer (country) |
|---|---|---|
| FlashForge Adventurer 3 | – | FlashForge (USA) |
| Gelatin B | G7-500 | Fisher Scientific (USA) |
| Gum arabic | G9752-500G | Sigma (USA) |
| Pluronics F127 | P2443-250G | Sigma (USA) |
| Ethanol (200 Proof) | BP28184 | Fisher Scientific (USA) |
| Cellink-RGD | IKC210000301 | Cellink (Sweden) |
| Calcium Chloride | C4901 | Sigma (USA) |

**Table S2. List of Bio-Rad primers used for quantitative polymerase chain reaction**

| Primer name | Unique assay ID |
|---|---|
| YWHAZ | qHsaCID0013897 |
| B2M | qHsaCID0015347 |
| FOXJ1 | qHsaCID0016777 |
| MUC5B | qHsaCID0011690 |
| MUC5AC | qHsaCID0017663 |
| SCGB1A1 | qHsaCID0018013 |
| TP63 | qHsaCID0036332 |
| KI67 | qHsaCID0011882 |
| RSPH4A | qHsaCID0017043 |
| KRT5 | qHsaCED0047798 |

**Table S3. List of primary and secondary antibodies used for immunofluorescence analysis**

| Antibodies | Manufacturer |
|---|---|
| Primary: CD163 (Rabbit) | Thermo Fisher Scientific, United States |
| Primary: Inducible nitric oxide synthase (Rabbit) | Thermo Fisher Scientific, United States |
| Primary: Myeloperoxidase (Rabbit) | Thermo Fisher Scientific, United States |
| Secondary: Goat anti-rabbit | Thermo Fisher Scientific, United States |




**Supplementary Figure 1**: **Fabrication of 3D Bioprinter from FlashForge FDM 3D Printer. 1A**: 3D printed PLA parts for fabricating bioprinter. **1B**: Flashforge printer with original hot-end nozzle removed. **1C**: Desktop printer with 3D printed Replistruder syringe parts assembled. **1D**: Image of Duet wifi board.

**Figure S1.** Fabrication of a three-dimensional (3D) bioprinter from a FlashForge fused deposition modeling 3D desktop printer. (A) 3D-printed polylactic acid parts for fabricating the bioprinter. (B) FlashForge printer with original hot-end nozzle removed. (C) Desktop printer with assembled 3D-printed Replistruder syringe parts. (D) Image of Duet Wi-Fi board replacement.

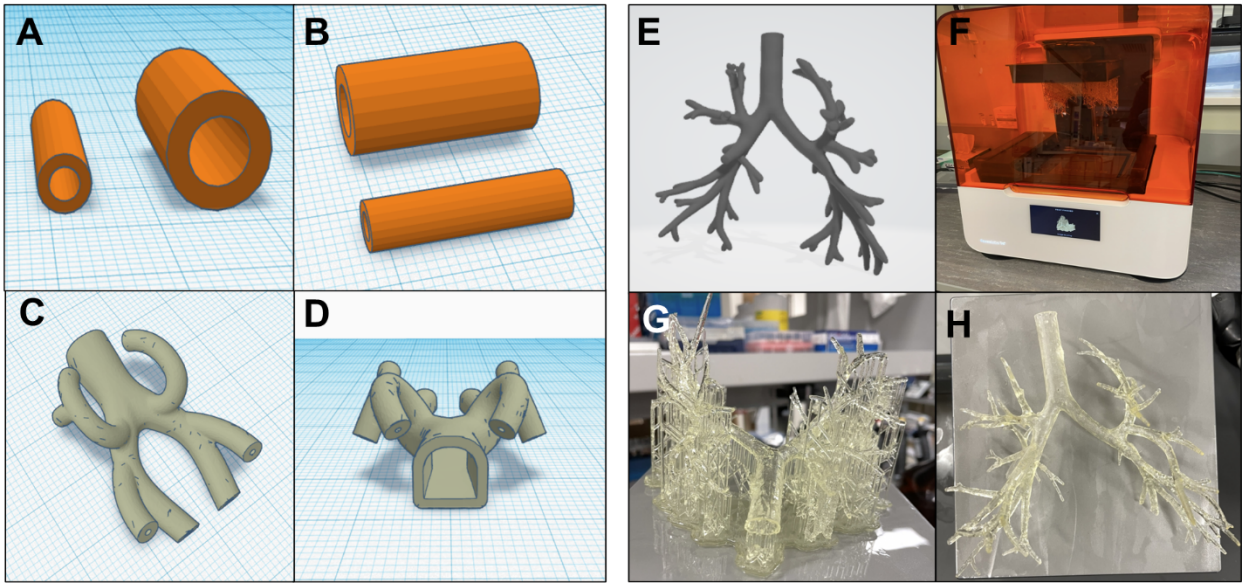



**Figure S2.** Computer-aided design files used for three-dimensional (3D) bioprinting hollow cylinders and simplified or complex hollow airway structures. (A, B) Hollow cylinders (5–10 mm diameters, 1 mm or 2 mm thickness) mimicking the size of the pediatric trachea. (C, D) Simplified, branched hollow airway structure. (E) Pediatric airway structure derived from the computed tomography scan. (F) Stereolithography (SLA) 3D printer used for testing the printability of the hollow pediatric airway structure derived from the computed tomography scan. (G, H) Hollow pediatric airway 3D-printed using resin (with supports and without).

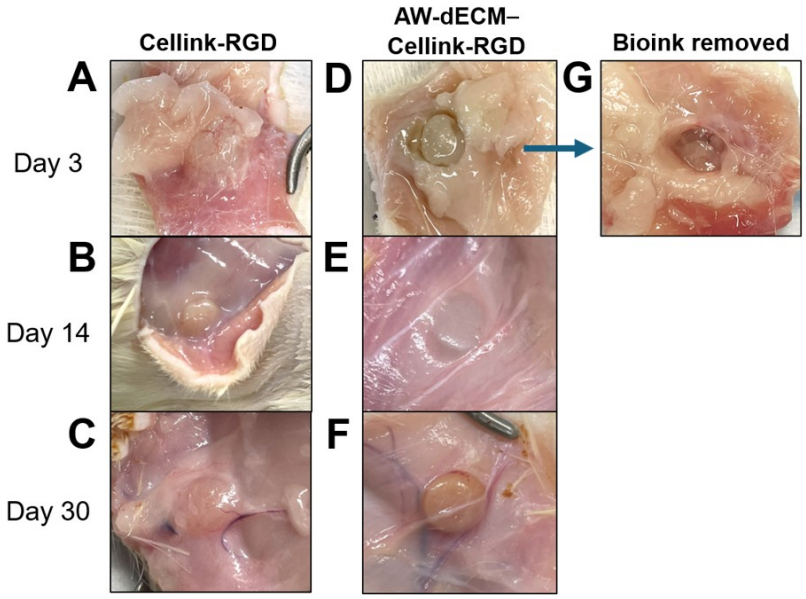



**Figure S3.** Representative macroscopic images of harvested bioink samples at Days 3, 14, and 30. (A–C) Cellink-RGD samples at three different time points. (D–F) Airway-derived decellularized extracellular matrix (AW-dECM)–Cellink-RGD samples at three different time points. (G) Pocket formed in tissue by AW-dECM–Cellink-RGD bioink at Day 3.

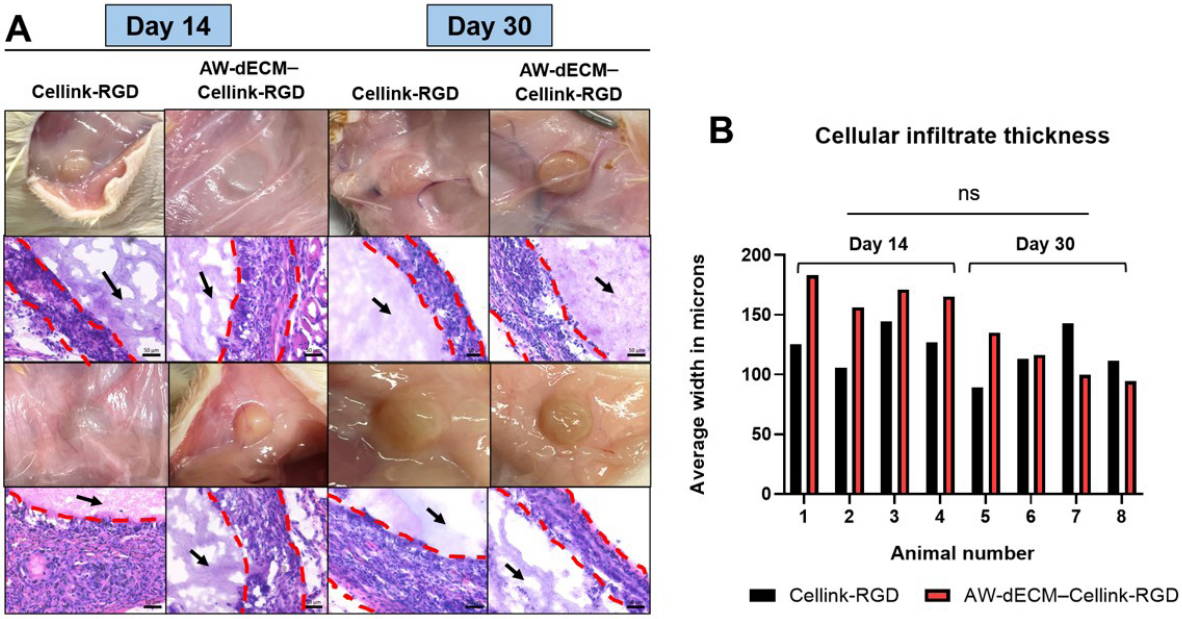



**Figure S4.** Representative hematoxylin and eosin (H&E) images of the bioink–tissue interface. (A) H&E images of the bioink–tissue interface at Day 14 and Day 30 revealing formation of a fibrotic capsule in Cellink-RGD and airway-derived decellularized extracellular matrix (AW-dECM)–Cellink-RGD bioink samples. Black arrows denote bioinks. The red dotted line delineates the cellular infiltrate at the bioink–tissue interface. (B) Measurement of cellular infiltration/fibrotic capsule thickness at the bioink–tissue interface in microns. $n = 4$ biological samples, 6 images/sample, 10 measurements/image. Data represented as mean ± standard deviation. Statistical significance measured using one-way analysis of variance and Tukey's multiple comparisons. Scale bars: 50 μm; magnification: 20×.

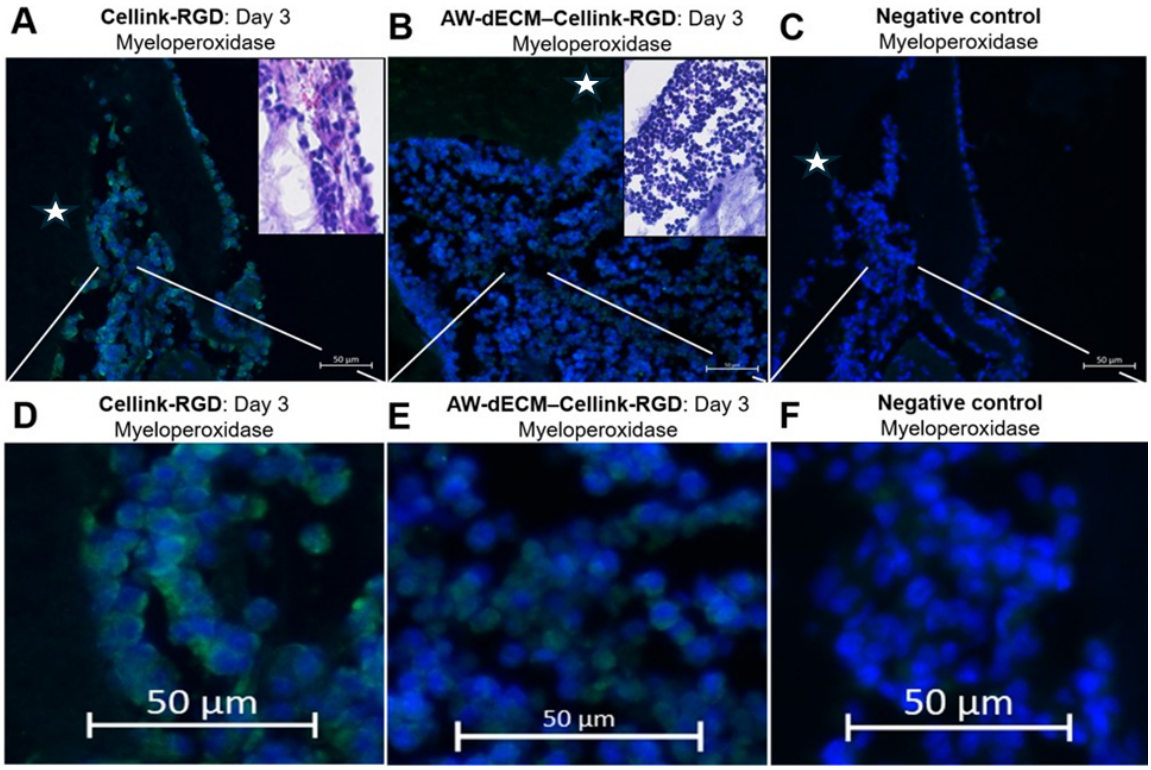



**Figure S5.** Immunofluorescence analysis of immune cells at the bioink–tissue interface on Day 3. (A) Representative myeloperoxidase staining of cells surrounding airway-derived decellularized extracellular matrix (AW-dECM)–Cellink-RGD bioink samples at Day 3. (B) Representative myeloperoxidase staining of cells surrounding AW-dECM–Cellink-RGD bioink sample at Day 3. (C) Negative control. (D–F) Corresponding magnified immunofluorescent images of A–C. White stars indicate bioink samples in proximity to immune cells. Scale bar: 50 µm; magnification: 20×.

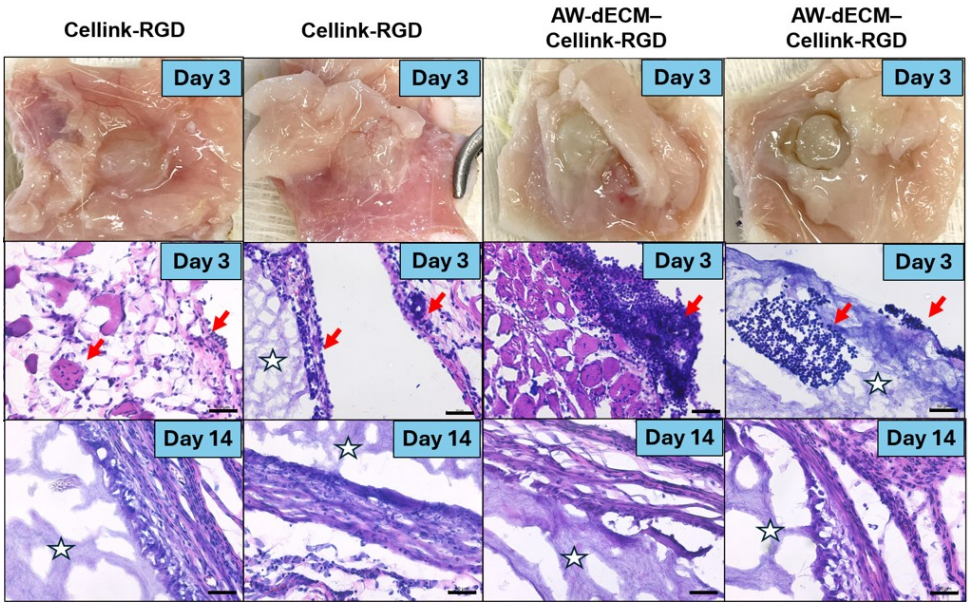



**Figure S6.** Representative hematoxylin and eosin images showing resolution of acute inflammatory response by Day 14 of bioink implantation. The white stars indicate the locations of the bioink within the tissue. Red arrows indicate inflammatory cells at the bioink surface and in the surrounding tissue. Scale bar: 50 µm; magnification: 20×.
Abbreviation: AW-dECM: Airway-derived decellularized extracellular matrix.