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

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Running title: Airway-derived ECM bioinks

Supplementary File

Table S1: Materials used for 3D Bioprinting

Materials	Catalog #	Vendor
FlashForge Adventurer 3		FlashForge
Gelatin B	G7-500	Fisher Scientific
Gum Arabic	G9752-500G	Sigma
Pluronic F127	P2443-250G	Sigma
Ethanol (200 Proof)	BP28184	Fisher Scientific
Cellink-RGD	IKC210000301	Cellink
Calcium Chloride	C4901	Sigma

Table S2: List of Biorad Primers used for RT-qPCR.

Primer Name	Unique Assay ID
<i>YWHAZ</i>	qHsaCID0013897
<i>B2M</i>	qHsaCID0015347
<i>FOXJ1</i>	qHsaCID0016777
<i>MUC5B</i>	qHsaCID0011690
<i>MUC5AC</i>	qHsaCID0017663
<i>SCGB1A1</i>	qHsaCID0018013
<i>TP63</i>	qHsaCID0036332
<i>KI67</i>	qHsaCID0011882
<i>RSPH4A</i>	qHsaCID0017043
<i>KRT5</i>	qHsaCED0047798

Table S3: List of Primary and Secondary Antibodies used for IF Analysis.

Antibodies	Vendor
1°: CD163 Rabbit	Thermofisher
1°: iNOS, Rabbit	Thermofisher
1°: Myeloperoxidase, Rabbit	Thermofisher
2°: Goat Anti-Rabbit	Thermofisher

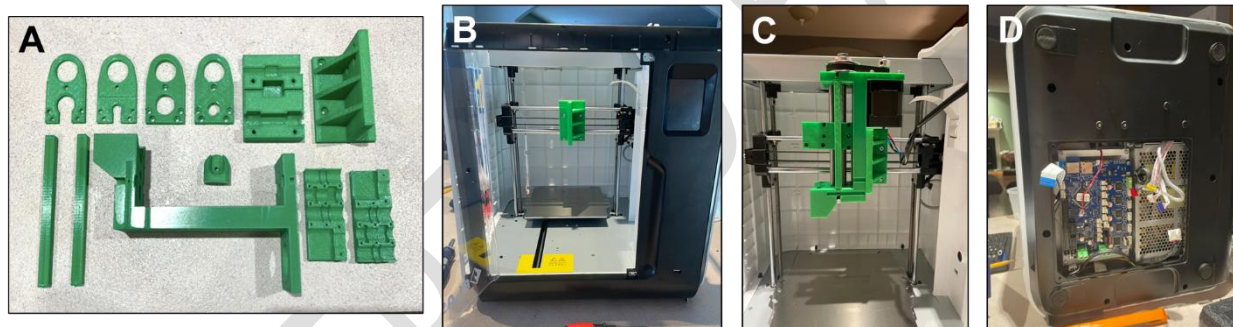


Figure S1: Fabrication of 3D bioprinter from FlashForge FDM 3D desktop printer. S1A: 3D printed PLA parts for fabricating bioprinter. **S1B:** Flashforge printer with original hot-end nozzle removed. **S1C:** Desktop printer with 3D printed Replistruder syringe parts assembled. **S1D:** Image of Duet wifi board replacement.

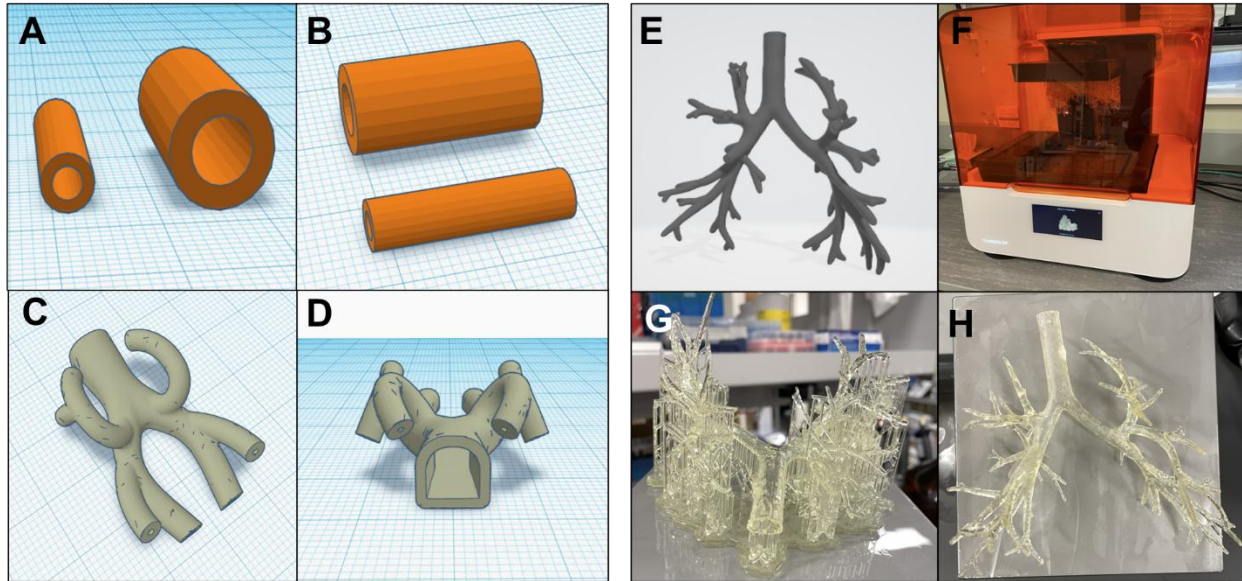


Figure S2: CAD files used for 3D bioprinting hollow cylinders and simplified or complex hollow airway structures. S2A+S2B: Hollow cylinders (5-10 mm diameters, 1 mm or 2 mm thickness) mimicking size of pediatric trachea. **S2C + 2D:** Simplified, branched hollow airway structure. **S2E:** Pediatric airway structure derived from CT-scan. **S2F:** Stereolithography (SLA) 3D printer used for testing printability of hollow pediatric airway structure derived from CT-scan. **S2G + S2H:** Hollow pediatric airway 3D printed using resin (with supports and without).

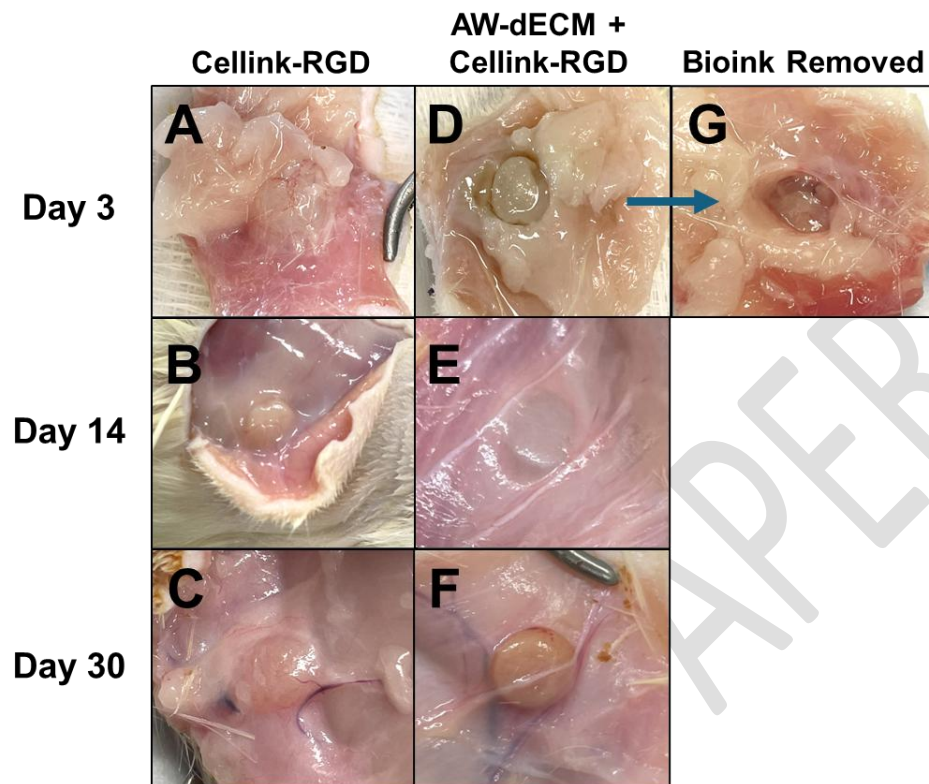


Figure S3: Representative macroscopic images of harvested bioink samples at days 3, 14 and 30. S3A, S3B, S3C: Cellink-RGD samples at three different time points. S3D, S3E, S3F: AW-dECM + Cellink-RGD samples at three different time points. S3G: Pocket formed in tissue by AW-dECM + Cellink-RGD bioink at Day 3.

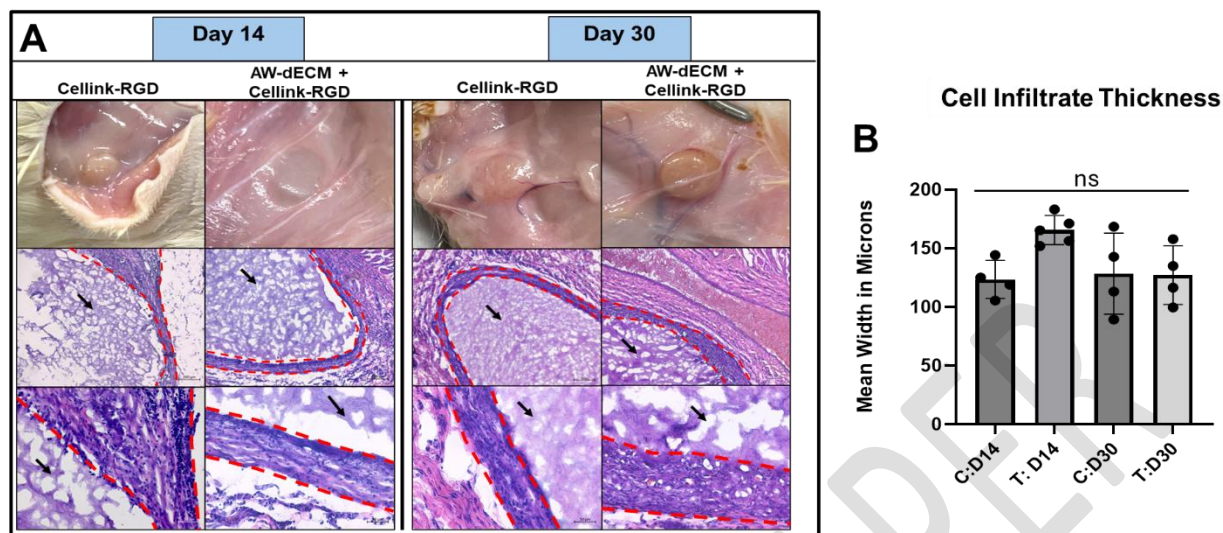


Figure S4: Representative H&E images of bioink-tissue interface. S4A: H&E images of bioink-tissue interface at Day 14 and Day 30 revealing formation of fibrotic capsule in Cellink-RGD and AW-dECM + Cellink-RGD bioink samples. Black arrow denotes bioink. Red dotted line delineates cellular infiltrate at bioink-tissue interface. **S4B:** Measurement of cellular infiltration/fibrotic capsule thickness at bioink-tissue interface in microns. n=4 biological samples, 6 images/sample, 10 measurements/image. Data represented as mean $\pm$ SD. Statistical significance measured using One-way Anova, Tukey's Multiple Comparisons.

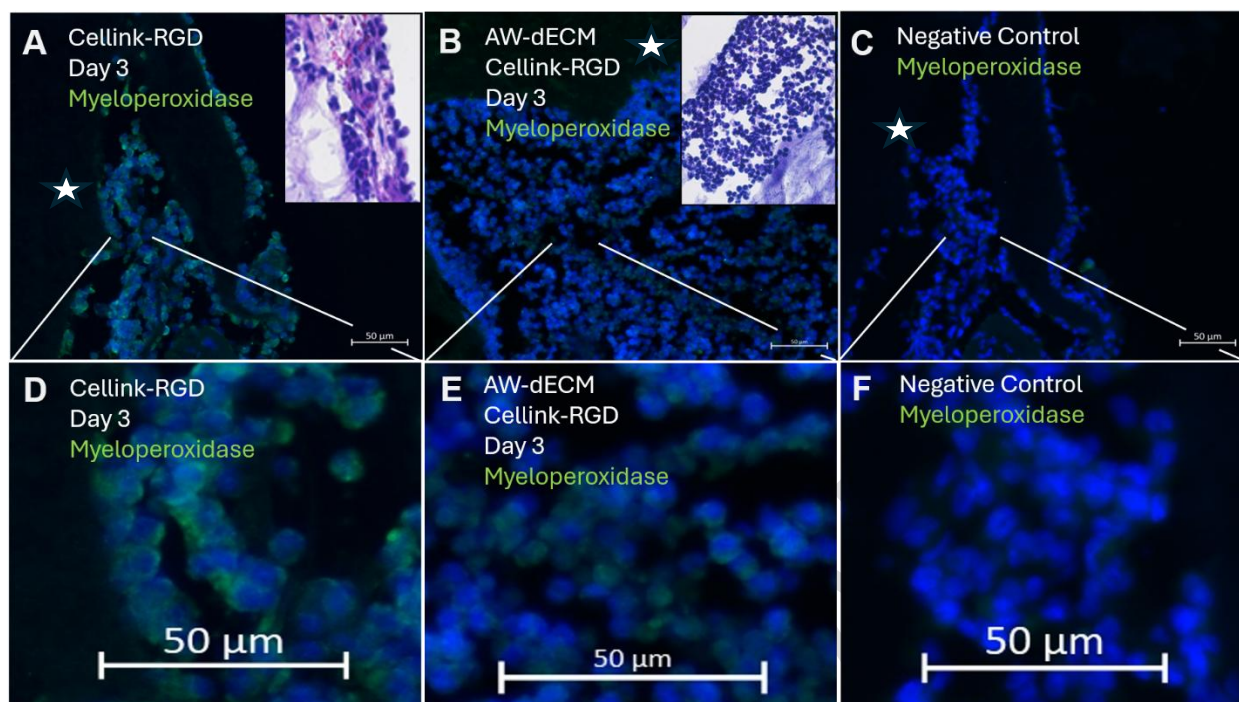


Figure S5: Immunofluorescence analysis of immune cells at Bioink-tissue interface on Day 3. **S5A:** Representative myeloperoxidase staining of cells surrounding AW-dECM +Cellink-RGD bioink samples at Day 3. **S5B:** Representative myeloperoxidase staining of cells surrounding AW-dECM Cellink-RGD bioink sample at Day 3. **S5C:** Negative control. **S5D-S5F:** Corresponding magnified IF images of **S5A-S5C**. White arrows indicate location of bioink samples in proximity to immune cells. Scales bars= 50 μ m.

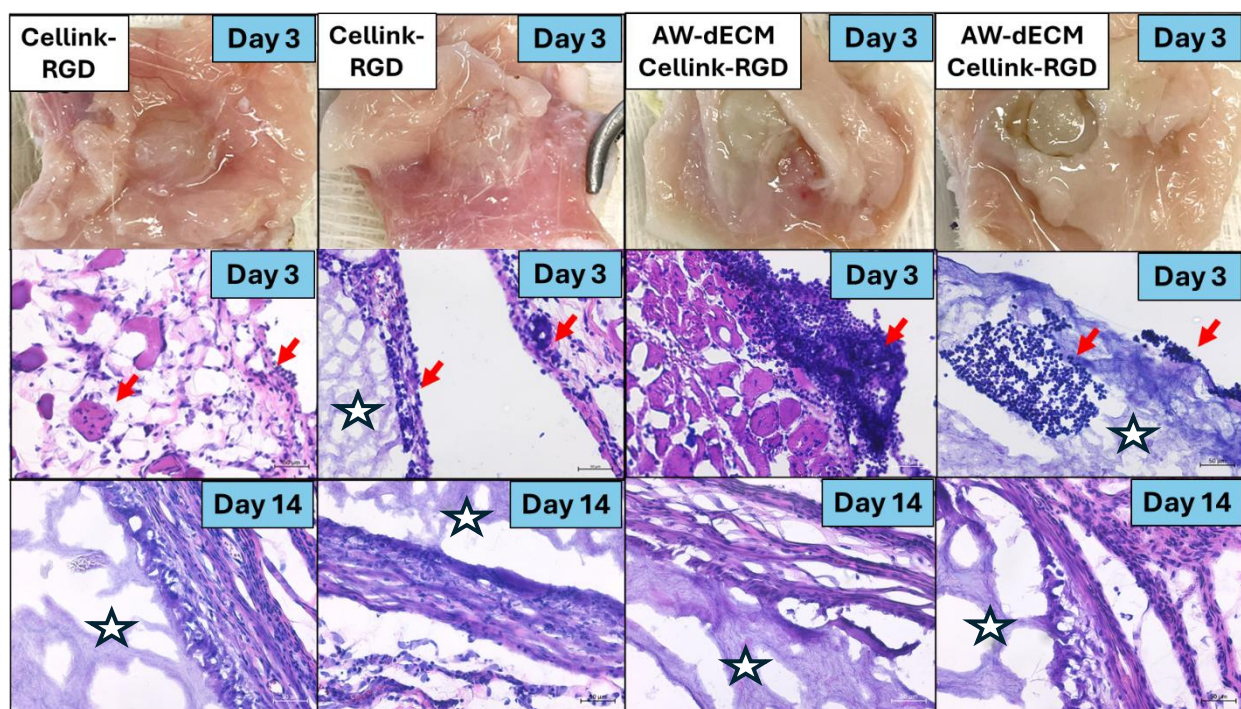


Figure S6: Representative H & E images showing resolution of acute inflammatory response by Day 14 of bioink implantation. White star indicates location of bioink within tissue. Red arrows indicate inflammatory cells at bioink surface and in surrounding tissue. Scale bars= 50 μm.