Tissue Model of the Epithelial Mesenchymal Trophic Unit

DEPARTMENT OF
Biomedical Engineering
UNIVERSITY OF WISCONSIN–MADISON

Dr. Kristyn Masters
Dr. Allan Brasier
February 24th, 2023
Carley Schwartz (Team Leader), Elijah Diederich (Co-Leader and BPAG), Nick Herbst (BWIG), Will Onuscheck (BSAC), Anuraag Shreekanth Belavadi (Communicator)

Problem Statement

- Chronic lung diseases can cause damage to epithelial tissues of the lungs
  - Pulmonary fibrosis, asthma, and COPD
  - Damage causes the sub-epithelial fibroblasts to increase production
- Currently no scaffolds that accurately model the lung extracellular matrix and its changes due to cell injury
  - Varying mechanical stiffness, porosity, incorporation of collagen and fibronectin, cell adhesive properties
- Dr. Brasier of the UW School of Medicine and Public Health requires such a scaffold that allows for lung epithelial cell culture in an ALI
  - Aim is to study cells in normal and fibrotic ECM conditions

Lung Extracellular Matrix Background

- Lung ECM has many components
  - Collagen, elastin, laminin, and fibronectin[1]
- Lung epithelial cells adhere to the ECM via integrins
  - Cell adhesive sequences like RGD [1]
- Fibroblasts and MMPs in the interstitial space remodel the ECM [1]
  - MMPs degrade ECM
  - Fibroblasts produce collagen
- Healthy stiffness ranges from 0.44 to 7.5 kPa due to heterogeneity
  - The ECM around the fibroblasts is ~1 kPa

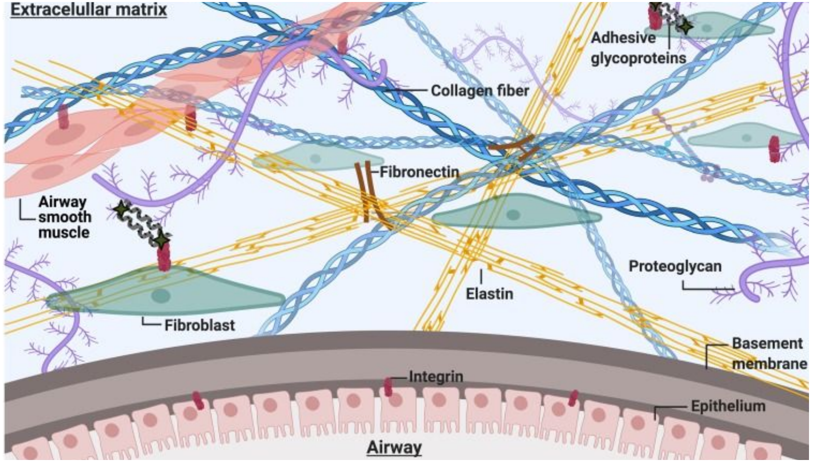

Figure 1: Lung ECM Diagram [2]

Cell Culture Scaffold Background

3D cell culture:

- More accurately mimic *in-vivo* ECM
- Allow cell-cell and cell-ECM interactions [3]

Air-liquid-interface (ALI):

- Basal surface (bottom): liquid culture medium
- Apical surface (epithelial cells): air [4]

Natural vs synthetic hydrogels:

- Natural
  - Gelatin, Alginate, Collagen
  - Biodegradable, Adhesive Properties
- Synthetic
  - Long lasting, replicable [5]

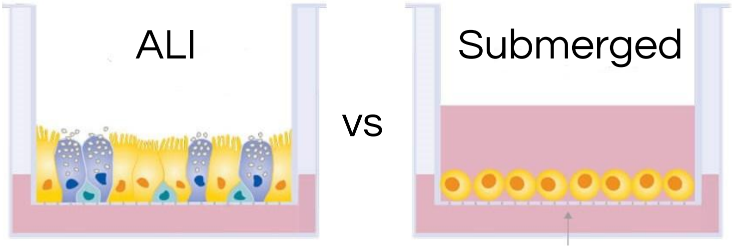

Figure 2: ALI vs submerged model [4]

Competing Designs

- 2D Models typically include layers of cells on top of polymer or glass dishes
  - Young's Modulus in 2-4 GPa range [6]
  - Negatively impacts gene expression
- 3D Models
  - Matrigel
    - Derived from Mouse Tumors
    - High variability in batches [7]
  - Human Lung ECM Hydrogels
  - Hyaluronic Acid (HA) Hydrogels
    - Incorporated in PEG hydrogels
    - Free Radical Toxicity

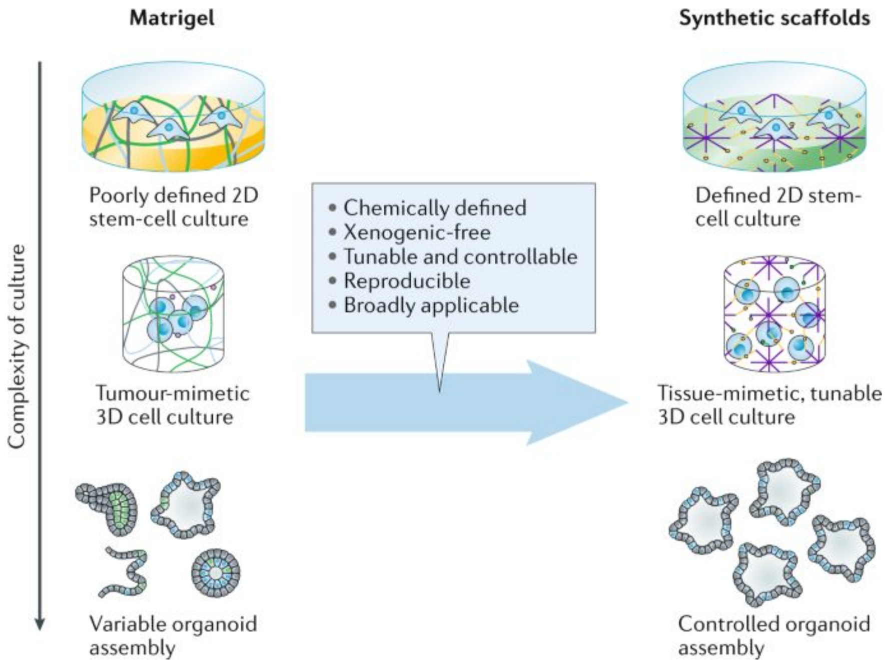

Figure 3: Competing Scaffold Design [8]

Design Specifications

- Preservation and facilitation of cell to cell communication
- Support of encapsulated fibroblasts and coated epithelia
- Epithelial polarization [4]
- Tunable mechanical properties [9]
    - Healthy 0.2–2 kPa
    - Fibrotic 3-35 kPa
- Cell adhesion to hydrogel
- Reconstructive allowance of hydrogel

Scaffold Design Matrix

Table 1: Design Matrix for Tissue Model Scaffold

		Design 1: Gelatin Methacrylate (GelMA)		Design 2: Polyethylene Glycol (PEG)		Design 3: Lung ECM	
Design Criteria	Weight	Score	Weighted Score	Score	Weighted Score	Score	Weighted Score
Mechanical Properties	20	4/5	16	4/5	16	2/5	8
Biochemical Properties	20	4/5	16	3/5	12	5/5	20
Ease of Fabrication	15	4/5	12	2/5	6	1/5	3
Ease of Use	15	2/5	6	1/5	3	1/5	3
Mechanical Tunability	10	4/5	8	4/5	8	1/5	2
Biochemical Tunability	10	3/5	6	4/5	8	1/5	2
Cost	10	5/5	10	3/5	6	1/5	2
Total:	**100**		74		59		40

Winner	Tie

- Matching mechanical properties
  - 2 kPa to 35 kPa [10]
  - Our focus: 2 kPa and 17 kPa
- Matching biochemical properties
  - Cell adhesion motifs
  - Reconstructive motifs
- Ability to fabricate
- Ability for client to use design
  - Reproduction
  - Client research
- Manipulating mechanical properties
  - Gradient of elasticity
- Manipulating biochemical properties

Preliminary Scaffold Designs

Polyethylene Glycol (PEG): 59

- Doesn't have any natural adhesive or degradation sequences[11]
  - RGD and MMP sequences can be added
- Mechanical properties are capable of being tuned within the native lung elastic modulus of 2 kPa to 16 kPa
- Fabrication process is difficult due to photoinitiator solubility issues
- Polyethylene glycol is inexpensive but the MMP and RGD are costly

Gelatin Methacrylate (GelMA): 74

- Inherently biochemically active, has natural RGD and MMP sequences [12]
- Mechanical properties can be altered by the degree of methacryloyl substitutions and created to mimic the elastic moduli of normal and fibrotic lung [13]
  - Can have batch to batch variation
- GelMA is significantly cheaper than PEG or Lung ECM

Lung ECM: 40 [14]

- Native lung ECM is constructed from decellularized lung and has the same biochemical properties
- Hydrogels constructed from lung ECM are not mechanically tunable
  - Won't maintain original mechanical properties
- Native lung tissue is expensive and difficult to obtain

Future Work

Testing

- Mechanical Stiffness
- Porosity/Permeability
- Translucency
- Cell Adhesiveness and Proliferation
- Degradability
- Reproducibility

Testing Upon Model's Success

- Client will:
  - Perform cell culture
  - Use scaffold for imaging
  - Incorporate fibroblasts into scaffold

Acknowledgements

Thank you to Dr. Kristyn Masters, Dr. John Puccinelli, and Dr. Allan Brasier & team!

References

[1] E. S. White, “Lung extracellular matrix and fibroblast function,” *Annals of the American Thoracic Society*, Mar-2015. [Online]. Available: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4430981/

[2] P. C. Wijsman, L. H. van Smoorenburg, D. M. de Bruin, J. T. Annema, H. A. M. Kerstjens, O. M. Mets, M. van den Berge, P. I. Bonta, and J. K. Burgess, “Imaging the pulmonary extracellular matrix,” Current Opinion in Physiology, vol. 22, p. 100444, Aug. 2021.

[3] H. Chamkouri and M. Chamkouri, “A Review of Hydrogels, Their Properties and Applications in Medicine,” *Biomedgrid*, 03-Feb-2021. [Online]. Available: https://biomedgrid.com/fulltext/volume11/a-review-of-hydrogels-their-properties-and-applications-in-medicine.001682.php#Types of Hydrogels.

[4] “Air-Liquid Interface Culture for Respiratory Research,” *STEMCELL Technologies*. [Online]. Available: https://www.stemcell.com/air-liquid-interface-culture-respiratory-research-lp.html.

[5] “2D vs 3D - Creative Biolabs,” *Cell Culture: 2D Vs 3D - Creative Biolabs*. [Online]. Available: https://www.creative-biolabs.com/adc/2d-vs-3d.htm.

[6] D. T. Butcher, T. Alliston, and V. M. Weaver, “A tense situation: Forcing tumour progression,” Nature reviews. Cancer, 01-Feb-2009. [Online]. Available: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2649117/. [Accessed: 22-Feb-2023].

[7] S. Kim, S. Min, Y. S. Choi, S.-H. Jo, J. H. Jung, K. Han, J. Kim, S. An, Y. W. Ji, Y.-G. Kim, and S.-W. Cho, “Tissue extracellular matrix hydrogels as alternatives to Matrigel for culturing gastrointestinal organoids,” Nature News, 30-Mar-2022. [Online]. Available: https://www.nature.com/articles/s41467-022-29279-4. [Accessed: 23-Feb-2023].

[8] E. A. Aisenbrey and W. L. Murphy, “Synthetic alternatives to Matrigel,” Nat Rev Mater, vol. 5, no. 7, Art. no. 7, Jul. 2020, doi: 10.1038/s41578-020-0199-8.

[9] G. Burgstaller, B. Oehrle, M. Gerckens, E. S. White, H. B. Schiller, and O. Eickelberg, “The instructive extracellular matrix of the lung: Basic composition and alterations in chronic lung disease,” European Respiratory Journal, vol. 50, no. 1, p. 1601805, 2017.

[10] E. Hui, L. Moretti, T. H. Barker, and S. R. Caliari, “The combined influence of viscoelastic and adhesive cues on fibroblast spreading and focal adhesion organization,” Cellular and molecular bioengineering, 02-Jun-2021. [Online]. Available: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8548477/. [Accessed: 10-Feb-2023].

[11] Yom-Tov, O., Seliktar, D., & Bianco-Peled, H. (2016). PEG-Thiol based hydrogels with controllable properties. European Polymer Journal, 74, 1-12. https://www.sciencedirect.com/science/article/pii/S0014305715300434#:~:text=Poly(ethylene%20glycol)%2DThiol,drug%20delivery%20and%20regenerative%20medicine

[12] K. Yue, G. Trujillo-de Santiago, M. M. Alvarez, A. Tamayol, N. Annabi, and A. Khademhosseini, “Synthesis, properties, and biomedical applications of gelatin methacryloyl (gelma) hydrogels,” Biomaterials, vol. 73, pp. 254–271, 2015.

[13] Y. Piao, H. You, T. Xu, H.-P. Bei, I.Z. Piwko, Y.Y. Kwan, X. Zhao, “Biomedical applications of gelatin methacryloyl hydrogels,” Engineered Regeneration, 2 (2021), pp. 47-56.

[14] R. H. de Hilster, P. K. Sharma, M. R. Jonker, E. S. White, E. A. Gercama, M. Roobeek, W. Timens, M. C. Harmsen, M. N. Hylkema, and J. K. Burgess, “Human lung extracellular matrix hydrogels resemble the stiffness and viscoelasticity of native lung tissue,” American Journal of Physiology-Lung Cellular and Molecular Physiology, vol. 318, no. 4, Apr. 2020.