A Fully Biological Gas Exchange Membrane for a Biomimetic Artificial Lung

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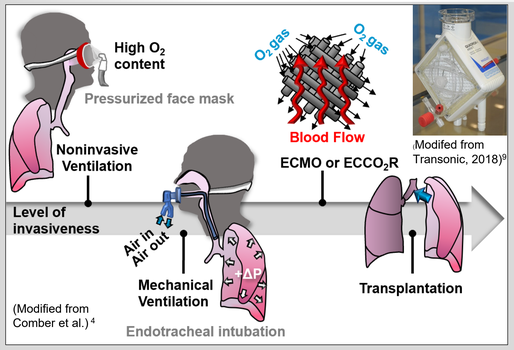
Clinical Motivation

- Chronic Obstructive Pulmonary Disease (COPD) - progressive lung disease associated with airflow obstruction and decreased gas exchange.
- Affects 16.4 million U.S. patients + kills 156,000/year[1]
- Causes >1 million hospitalizations annually.[2]
- < 2,700 lung transplants annually due organ shortages[3] and other therapies fail in terms of gas exchange efficacy and/or duration of use

Fig. 1: Alternative Therapies- Inadequate / Unavailable



Extracorporeal membrane oxygenation (ECMO)

ECMO diverts blood from the vasculature into an external oxygenator to supplement native organ function.

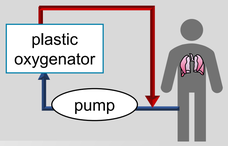
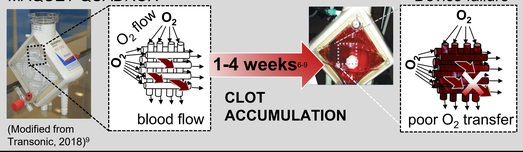


Fig. 2: Surface-induced clot on plastic oxygenators



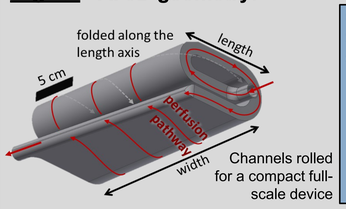
In contrast, *in vivo,* blood contacts endothelial cells which passively and actively inhibit coagulation.[11,12]

Device Design and Approach

Hypotheses:

- A biomimetic oxygenator with a fully biological gas exchange membrane that interfaces endothelial cells with blood will provide longer-lasting support than current devices.
- The membrane will likely require epithelial cells to minimize blood component efflux into the air space.

Fig. 3: **A. 3D geometry:**



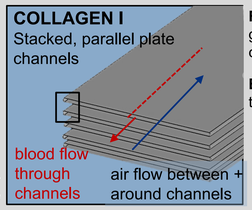
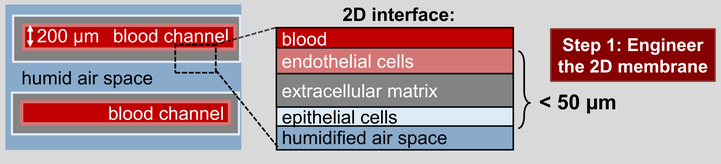


Fig 3: A. Macro-scale 3D geometry with stacked collagen I channels

B. cross-sections showing the 2D interface.

B. Channel-stack cross section:



Biofabrication Methods and Characterization Results

Fig. 4: 2D Scaffold Fabrication and Acellular Characterization

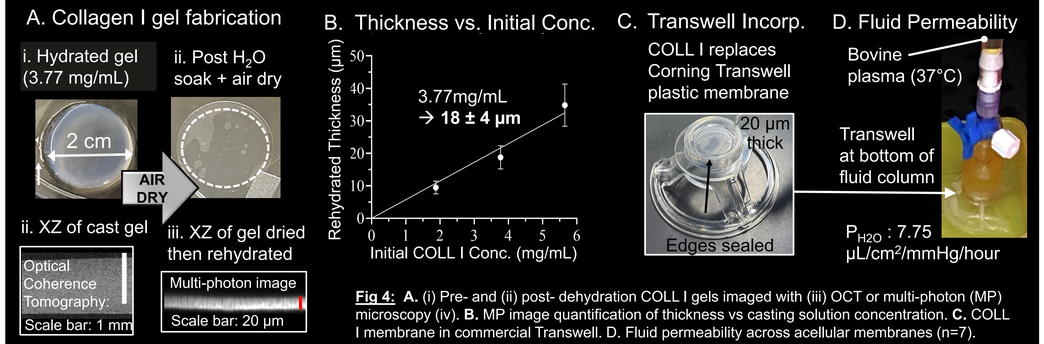


Fig 4: **A.** (i) Pre- and (ii) post- dehydration COLL I gels imaged with (iii) OCT or multi-photon (MP) microscopy (iv). **B.** MP image quantification of thickness vs casting solution concentration. **C.** COLL I membrane in commercial Transwell. D. Fluid permeability across acellular membranes (n=7).

Fig. 5: Cellularization & Live-Dead of Air Liquid Culture

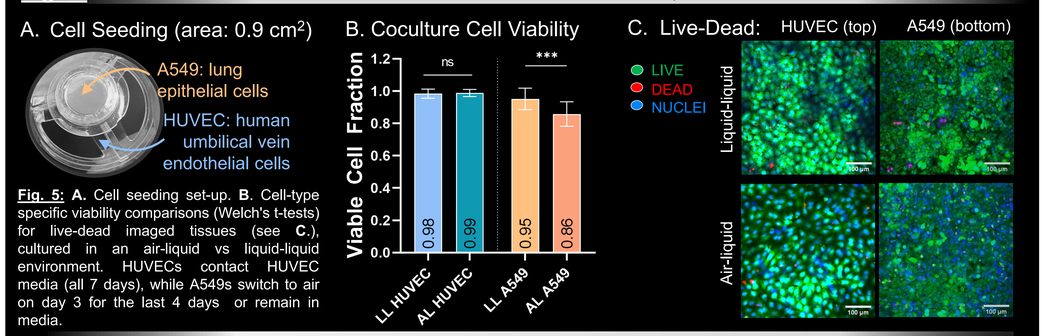


Fig. 5: **A.** Cell seeding set-up. **B.** Cell-type specific viability comparisons (Welch's t-tests) for live-dead imaged tissues (see **C.**), cultured in an air-liquid vs liquid-liquid environment. HUVECs contact HUVEC media (all 7 days), while A549s switch to air on day 3 for the last 4 days or remain in media.

Fig. 6: Permeability to Blood Protein

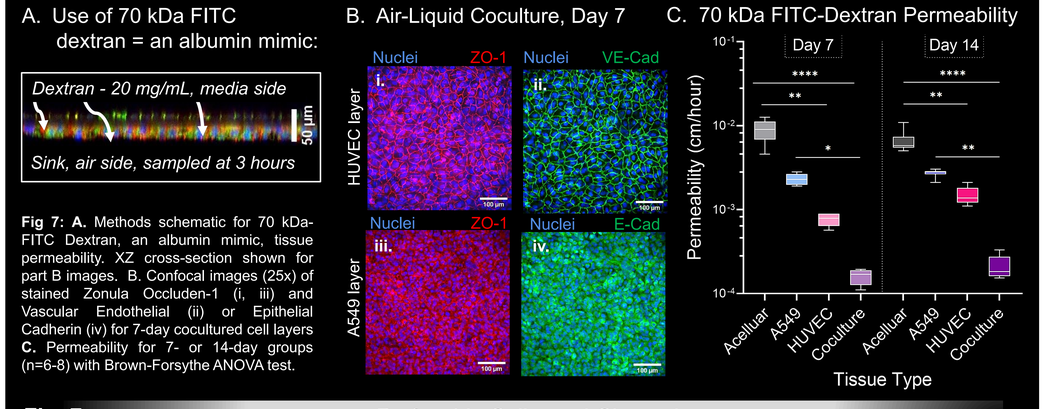


Fig 7: **A.** Methods schematic for 70 kDa-FITC Dextran, an albumin mimic, tissue permeability. XZ cross-section shown for part B images. B. Confocal images (25x) of stained Zonula Occluden-1 (i, iii) and Vascular Endothelial (ii) or Epithelial Cadherin (iv) for 7-day cocultured cell layers **C.** Permeability for 7- or 14-day groups (n=6-8) with Brown-Forsythe ANOVA test.

Fig. 7: Perfusable Collagen I Channels

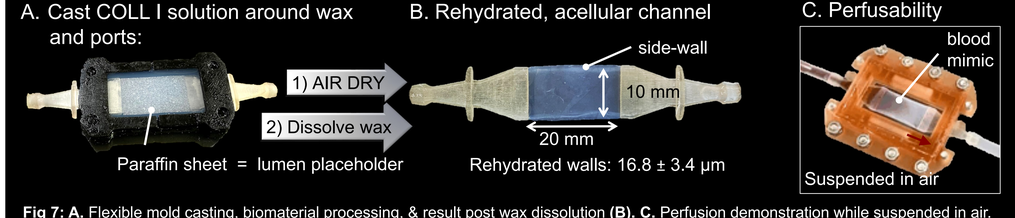


Fig 7: **A.** Flexible mold casting, biomaterial processing, & result post wax dissolution **(B). C.** Perfusion demonstration while suspended in air.

Results and Conclusions

- 2D scaffold was fabricated + measured to be 18.8 ± 4 µm.
- Acellular COLL I membranes minimize water movement from plasma to the air-side to 7.75 µL/cm²/mmHg/hour
- Cocultured tissues (42.1 ± 7 µm) are nutrient supported in air-liquid culture with cell fractions >85% viable.
- Cellularization significantly decreased permeability of an albumin mimic (70 kDa-FITC Dextran) and cocultures were the least permeable (ex: 14-day coculture: 2.11 E-4 cm/hr).
- Dextran permeability did not change between 7 & 14 days.
- The 2D interface was formed as a COLL I channel, analogous to those in the full-scale device design.
- Channels are cellularizable and perfusable while suspended in air.

Future Work

- Channels will be cellularized and perfused.
- Channels will then be used to quantify gas exchange and compatibility with blood.
- Results will inform whether the fully biological interface has potential for use as the membrane of a biomimetic oxygenator.
- We will then switch to primary cell types and scale up surface area with a multi-channel device.

Acknowledgments

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References

1. American Lung Association. COPD Mortality (2018). Available at https://www.lung.org/research/trends-in-lung-disease/copd-trends-brief/copd-mortality
2. NBHLB, N. I. of H. Morbidity and Mortality: 2012 Chart Book on Cardiovascular, Lung, and Blood Diseases. (National Institute of Health, 2012).
3. National Data - OPTN. Available at: https://optn.transplant.hrsa.gov/data/view-data-reports/national-data/#. (Accessed: 4th March 2020)
4. Comber, E. M., Palchesko, R. N., NG, W. H., Ren, X. & Cook, K. E. De novo lung biofabrication: clinical need, construction methods, and design strategy. Translational Research (2019). doi:10.1016/j.trsl.2019.04.008
5. Haneya, A. et al. Extracorporeal Circulatory Systems as a Bridge to Lung Transplantation at Remote Transplant Centers. Ann. Thorac. Surg. 91, 250–255 (2011).
6. Camboni, D. et al. First Experience With a Paracorporeal Artificial Lung In Humans. ASAIO J. 55, 304–306 (2009).
7. Fischer, S. et al. Bridge to lung transplantation with the novel pumpless interventional lung assist device NovaLung. J. Thorac. Cardiovasc. Surg. 131, 719–723 (2006).
8. Strueber, M. et al. Bridge to Thoracic Organ Transplantation in Patients with Pulmonary Arterial Hypertension Using a Pumpless Lung Assist Device. Am. J. Transplant. 9, 853–857 (2009).
9. National Heart Lung and Blood Institute. How the Lungs Work. NIH (2018). Available at: https://www.nhlbi.nih.gov/health-topics/how-lungs-work. (Accessed: 13th August 2018)
10. Gehr, P., Bachofen, M. & Weibel, E. R. The normal human lung: ultrastructure and morphometric estimation of diffusion capacity. Respir. Physiol. 32, 121–140 (1978).
11. Van Hinsbergh, V. W. M. Endothelium - Role in regulation of coagulation and inflammation. Seminars in Immunopathology (2012). doi:10.1007/s00281-011-0285-5
12. Yau, J. W., Teoh, H. & Verma, S. Endothelial Cell Control of Thrombosis. N. Center for Health Statistics, N. National Hospital Ambulatory Medical Care Survey: 2014 Emergency cell control of thrombosis. BMC Cardiovasc. Disord. (2015). doi:10.1186/s12872-015-0124-z