Bioengineering Lungs in Decellularized Mouse Lungs Using Human iPS Cells in a Bioreactor System

Akansha Prasad | Angela Panoskaltsis-Mortari | University of Minnesota – Twin Cities

Problem / Question

Will lungs bioengineered using human iPS cells be functional to use as organs for patients who are in need of organ transplants? Will human iPS cells engraft on decellularized lungs?

Hypothesis

- I believe that using human iPS cells to bioengineer lungs using decellularized lungs will yield functional and transplant-capable lungs.
- Prior research has shown some success in this field and the bioreactor matrix provides a suitable environment for engraftment.

Project Overview

- Due to a shortage of donor lungs, new sources of transplantable lung tissue need to be developed for patients with end-stage lung disease. A decellularized lung matrix bioreactor system was used to assess if human iPS cells could differentiate into lung tissue. The lungs, with attached heart and trachea, from 2-3 month old female BALB/c mice were decellularized through a series of succeeding solutions of distilled water, triton, deoxycholate, sodium chloride, and DNase. The acellular lung matrices were then cannulated and suspended in Essential 8 Flex Medium (control) or Bronchial Epithelial Cell Growth Medium (BEGM). Human iPS (induced pluripotent stem) cells were infused into the airways through the cannula (i.e. the trachea), and the bioreactor system was placed in an incubator and attached to a ventilator to simulate respiration. The bioreactors were left in the incubator for 7 days, after which the lung matrices were analyzed histologically using scanning electron microscopy and histochemical staining.

Variables / Research

Controlled variables	Independent variable	Dependent variable

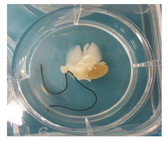
• Mouse strain
• iPS cell culture
• Decellularization process
• Cannulating process
• Media additives (fungizone, primocin)
	• Media
	  • Bronchial Epithelial Cell Growth Medium (BEGM)
	  • Essential 8 Flex Medium (E8)
		• Cell engraftment
		  • Measured using histology analysis using histochemical staining and scanning electron microscopy

Materials

Materials (detailed list)	Quantity (be specific)
Wells	30
BEGM and E8 Media	1 bottle each
19 Gauge Syringe	10
25 Gauge Syringe	10
Petri Dish	5
Ventilator	1
T12.5 Flask	2

Procedure

Step 1

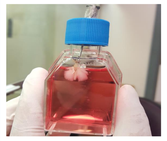


Mice lung were decellularized using a series of washes

Step 2

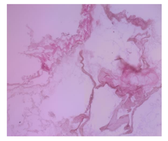
iPS cells were cultured for use in bioreactor system

Step 3



Bioreactor system was set up and allowed to ventilate

Step 4



Histological staining and scanning electron microscopy

Data / Observations

- Bioreactor systems are highly prone to contamination
- First bioreactor pair were contaminated after 3 days of ventilation
- Second bioreactor pair showed no signs of contamination after 7 days of ventilation
  - Lack of contamination may be because of media additives
- The lobe of the lung used for histological testing in the E8 media did not inflate before freezing in blocks
  - This will affect the histology imaging visualization
- The lobe of the lung used for histological testing in the E8 media did not inflate before freezing in blocks
- BEGM lung inflated nicely

Results

Cell Type	Media Type	Cell Engraftment
iPS	BEGM	Initial imaging did not show the presence of cells
iPS	E8	Initial imaging did not show the presence of cells

- Cells were not seen clearly engrafted onto the lung matrix using an electron microscope on both the BEGM and E8 media samples
- Certain photographs show signs of possible cell presence that must be further analyzed for viable data on the E8 media sample
- Blocks must be further sectioned to get data on deeper lung tissue to search for evidence of cell engraftment

Conclusion

- After histochemical staining and histology using scanning electron microscopy, no cells were clearly visible on either lung sample
- There may be cells on deeper tissue of the lung requiring further testing
- Future work on this project includes more histology on deeper sections and the set up of more bioreactors to continue working on cell engraftment

Works Cited

- Price, Andrew P. et al. “Development of a Decellularized Lung Bioreactor System for Bioengineering the Lung: The Matrix Reloaded.” *Tissue Engineering. Part A* 16.8 (2010): 2581–2591. *PMC*. Web. 4 Oct. 2015.
- Ott, Howard et al. “Engineering pulmonary vasculature in decellularized rat and human lungs.” Nat Biotechnol. 2015 Oct;33(10):1097-102. doi: 10.1038/nbt.3354. Epub 2015 Sep 14.

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