Des cellules-souches dans le poumon : pourquoi faire ?

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Dept. of Genetic and Laboratory Medicine, University Hospitals
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- are the stem cells in the lung?
- what is their physiological function?
- can we use stem cells to treat lung disease?

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Evidence for Human Lung Stem Cells

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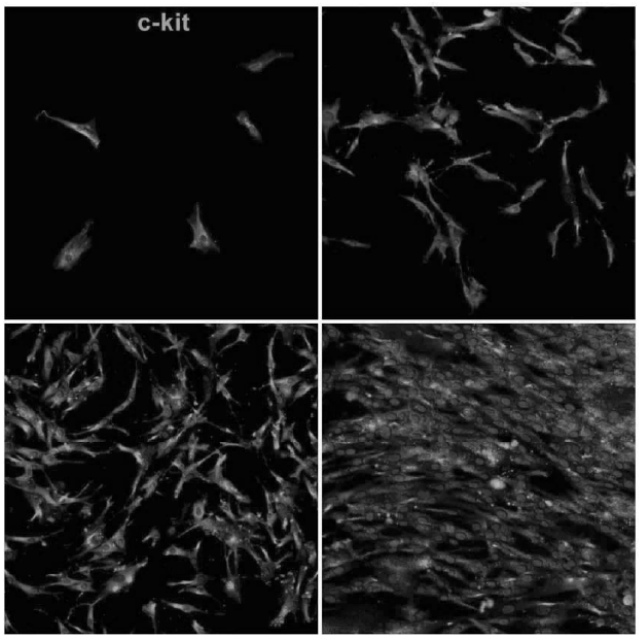
how to purify lung stem cells?

Flow cytometry cell sorting for purification of stem cells

- hematopoietic stem cells
  - CD34
- cardiac stem cells
  - CD117 (=c-kit)
- lung stem cells?
  - CD117 (=c-kit)



Supplementary Figure 1

BACKGROUND

Although progenitor cells have been described in distinct anatomical regions of the lung, description of resident stem cells has remained elusive.

METHODS

Surgical lung-tissue specimens were studied in situ to identify and characterize human lung stem cells. We defined their phenotype and functional properties in vitro and in vivo.

RESULTS

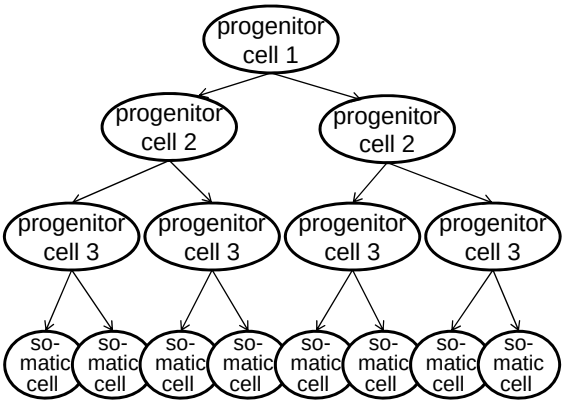
Human lungs contain undifferentiated human lung stem cells nested in niches in the distal airways. These cells are self-renewing, clonogenic, and multipotent in vitro. After injection into damaged mouse lung in vivo, human lung stem cells form human bronchioles, alveoli, and pulmonary vessels integrated structurally and functionally with the damaged organ. The formation of a chimeric lung was confirmed by detection of human transcripts for epithelial and vascular genes. In addition, the self-renewal and long-term proliferation of human lung stem cells was shown in serial-transplantation assays.

CONCLUSIONS

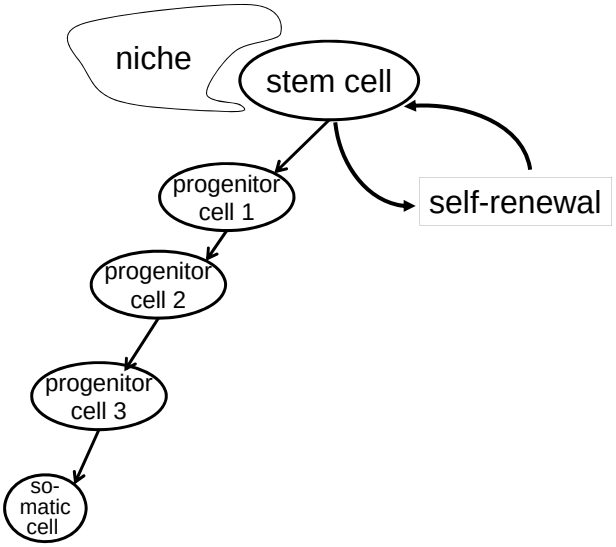
Human lungs contain identifiable stem cells. In animal models, these cells participate in tissue homeostasis and regeneration. They have the undemonstrated potential to promote tissue restoration in patients with lung disease. (Funded by the National Institutes of Health.)

What is a progenitor cell?

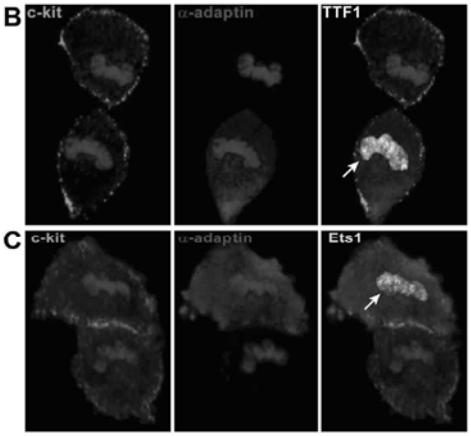
- a progenitor cell is a cell on the way to differentiate into a mature somatic cell
- progenitors are mostly dividing cells
- progenitors typically progress in differentiation after each division



What is a stem cell?



Assymetric division of lung stem cells



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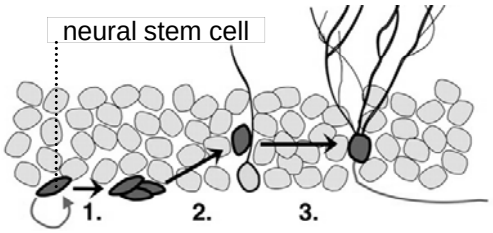
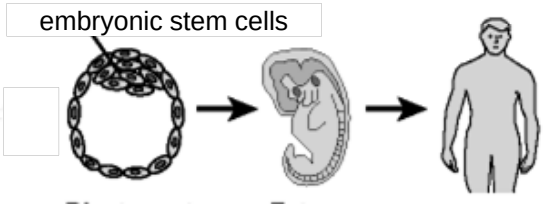
Multipotent vs. pluripotent stem cell



- multipotent stem cells
  - sources: fetus, umbilical cord, adult tissue

- pluripotent stem cells
  - sources: embryo, reprogramming



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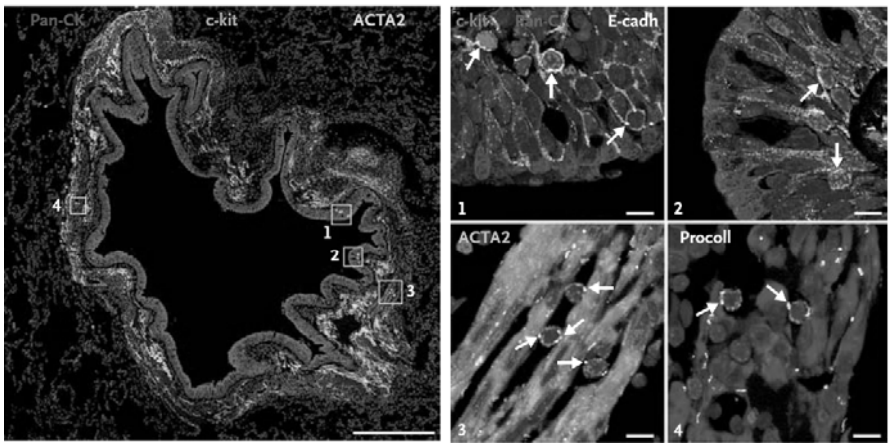
c-kit positive stem cells in human lung tissue



Take home message

- human lung tissue contains multipotent stem cells
  - ~1 stem cell / 24000 lung cells
  - predominantly found in bronchioli
  - associated with epithelial cells, smooth muscle cells, and fibroblasts
- human lung stem cells can be isolated and cultivated in vivo
  - immuno-isolation CD117 (c-kit)
  - symmetric and assymetric division

Which interest for physicians?

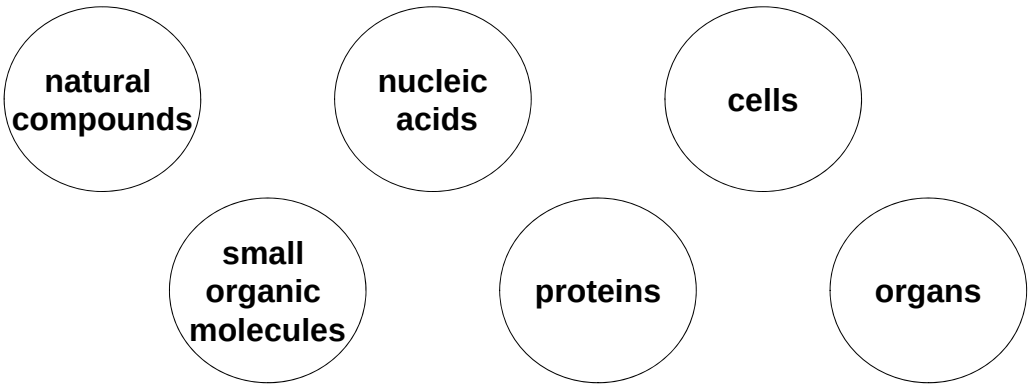
- better understand of lung physiology
- role of stem cells in
  - lung pathology
  - lung regeneration
  - lung aging
- sensitivity of lung stem cells to
  - irradiation
  - chemotherapy
- potential for cell therapy and tissue engineering?

Stem cells for therapy

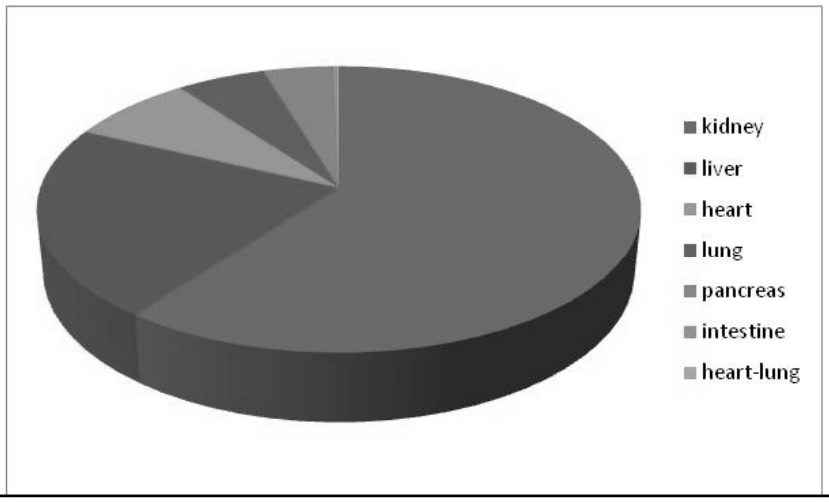
will stem cell therapy replace solid organ transplantation?

Medical treatments

from chemistry towards biology?



United States, 2008:
> 27000 transplantations



Why do we need alternatives to

- lac
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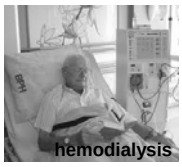
Table I-4. Unadjusted 1- and 5-Year Patient Survival by Organ

Organ Transplanted	1-Year Survival (Txp 2006-2007)	5-Year Survival (Txp 2002-2007)
Kidney		
Deceased donor	95.6%	81.9%
Living donor	98.5%	91.0%
Pancreas alone	97.8%	88.7%
Pancreas after kidney	97.0%	84.5%
Kidney-pancreas	95.7%	87.2%
Liver		
Deceased donor	88.4%	73.8%
Living donor	91.0%	79.0%
Intestine	89.3%	57.9%
Heart	88.3%	74.9%
Lung	83.3%	54.4%
Heart-lung	80.6%	44.9%
Kidney-liver	87.4%	71.4%
Kidney-heart	95.8%	77.6%
Liver-intestine	63.3%	58.0%

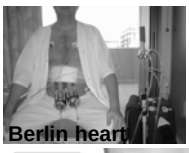
Source: *2009 OPTN/SRTR Annual Report*, Table 1.13.

What are the alternatives to organ transplantation?

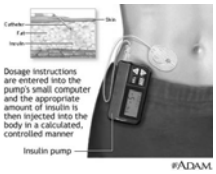
Mechanical organ equivalents



hemodialysis



Berlin heart



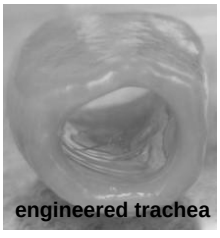
Biological organ equivalents

1. tissue engineering

2. cell replacement therapy

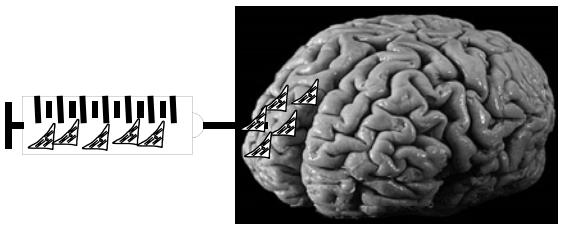
Biological organ equivalents

tissue engineering



engineered trachea

cell replacement therapy



Sources of cells for biological organ equivalents

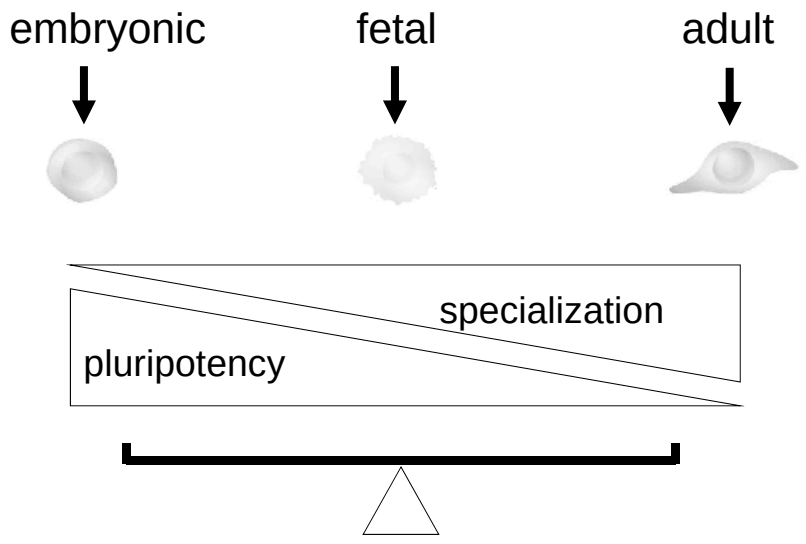
somatic cells

- e.g. islet transplantation, primary chondrocyte implantation
- advantages
  - little cell culture work
  - relatively little cell biology expertise required
- disadvantages
  - relatively poor survival of primary somatic cells
  - relatively poor integration into resident tissue
  - dedifferentiation

stem cells

- e.g. pluripotent stem cell therapy of spinal chord injury; satellite cells for therapy of heart failure
- advantages
  - improved survival of cells
  - relatively good integration into resident tissue
  - less problems of dedifferentiation
- disadvantages
  - much cell culture work
  - extensive cell biology expertise required

Properties of stem cells



Replacement of solid organ transplantation by stem cells?

- The big 5 of solid organ transplantation
  - kidney (60%)
  - liver (22%)
  - heart (8%)
  - lung (5%)
  - pancreas (4%)

Kidney International, Vol. 63 (2003), pp. 1195–1204

PERSPECTIVES IN BASIC SCIENCE

Tissue engineering the kidney[1]

MARC R. HAMMERMAN

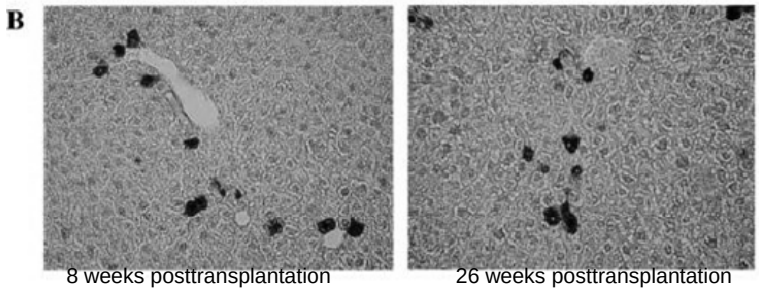
George M. O'Brien Kidney and Urological Disease Center, Renal Division, Departments of Medicine, and Cell Biology and Physiology, Washington University School of Medicine, St. Louis Missouri

- integration of new nephrons into the kidney
- growing new kidneys (in situ?)
- bioengineering of dialysis devices integrating kidney cells
- little research and development in the field. Most likely no significant progress within the next 25 years

Stem cells as alternatives to classical liver transplantation

- acute liver failure
  - transient hepatocyte transplantation to allow recovery through self renewal?
- chronic liver failure
  - transplantation of hepatocytes to repopulate the damaged liver
  - transplantation of engineered hepatic tissue
- hereditary metabolic disease
  - autologous transplantation of genetically corrected hepatocytes

Long-term engraftment of lentivector-transduced human hepatocytes in mouse liver



8 weeks posttransplantation 26 weeks posttransplantation

"Transduced hepatocytes represented 0.5% to 1% of the recipient liver and were distributed throughout the liver parenchyma"

Birraux et al. A step toward liver gene therapy: efficient correction of the genetic defect of hepatocytes isolated from a patient with Crigler-Najjar syndrome type 1 with lentiviral vectors. Transplantation. 2009 15;87:1006-12.

Stem cells as alternatives to classical heart transplantation

- cell replacement therapy
  - possible, but only a small fraction of cells gets integrated
  - satellite cells (skeletal muscle stem cells) have been used in clinical trials
- "cardiopatch"
  - minimal cardiac tissue engineering to be sutured to lesioned areas
  - technically simple, promising approach
- cardiac organ engineering
  - several initiatives
  - but unrealistic to get into clinics within 25 years

Stem cells as alternatives to pancreas transplantation

- cell replacement therapy rather than organ engineering
- major advances in differentiation of pluripotent stem cells into insulin-producing beta cells
- in mouse models: correction of diabetes with glucose-dependent insulin secretion
- diabetes = key target for pluripotent stem cell-based cell therapy

Pancreatic endoderm derived from human embryonic stem cells generates glucose-responsive insulin-secreting cells *in vivo*

Evert Kroon, Laura A Martinson, Kuniko Kadoya, Anne G Bang, Olivia G Kelly, Susan Eliazer, Holly Young, Mike Richardson, Nora G Smart, Justine Cunningham, Alan D Agulnick, Kevin A D'Amour, Melissa K Carpenter, Emmanuel E Baetge

Development of a cell therapy for diabetes would be greatly aided by a renewable supply of human β-cells. Here we show that pancreatic endoderm derived from human embryonic stem (hES) cells efficiently generates glucose-responsive endocrine cells after implantation into mice. Upon glucose stimulation of the implanted mice, human insulin and C-peptide are detected in sera at levels similar to those of mice transplanted with ~3,000 human islets. Moreover, the insulin-expressing cells generated after engraftment exhibit many properties of functional β-cells, including expression of critical β-cell transcription factors, appropriate processing of proinsulin and the presence of mature endocrine secretory granules. Finally, in a test of therapeutic potential, we demonstrate that implantation of hES cell–derived pancreatic endoderm protects against streptozotocin-induced hyperglycemia. Together, these data provide definitive evidence that hES cells are competent to generate glucose-responsive, insulin-secreting cells.

Kroon E, Martinson LA, Kadoya K, Bang AG, Kelly OG, Eliazer S, Young H, Richardson M, Smart NG, Cunningham J, Agulnick AD, D'Amour KA, Carpenter MK, Baetge EE. Pancreatic endoderm derived from human embryonic stem cells generates glucose-responsive insulin-secreting cells in vivo. Nat Biotechnol. 2008 Apr;26(4):443-52.

VIACYTE
FORMERLY NOVOCELL, INC.

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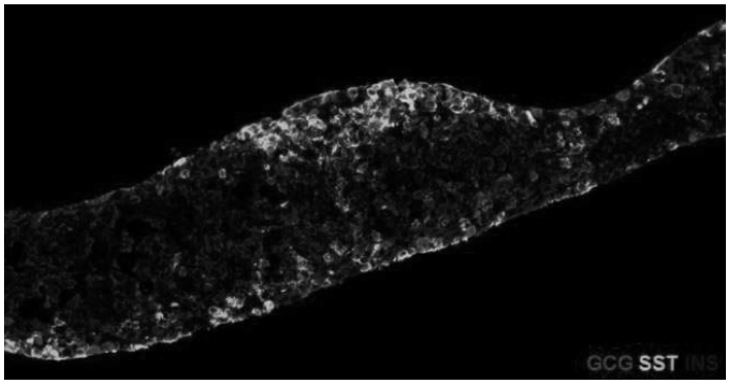
DIABETES TREATMENTS FROM STEM CELLS

ViaCyte, Inc. is a stem cell engineering company dedicated to creating and commercializing cell therapies to treat diabetes. The company's goal is insulin independence without immunosuppression, hypoglycemia, or other diabetes-related complications.



Device encapsulated stem cell-derived islet cells. In this image, insulin (INS, blue), glucagon (GCG, red) and somatostatin (SST, green) mark three of the five endocrine cells normally found in human islets.

LATEST NEWS

May 5, 2010
Novocell Becomes ViaCyte, Inc. as it Accelerates Pre-clinical Development of a Stem Cell Derived Treatment for Diabetes

May 5, 2010
ViaCyte, Inc., Receives Three Additional U.S. Patents, Supporting its Development of a Stem Cell Derived Treatment for Diabetes

October 28, 2009
Novocell is the Recipient of a Disease Team Award for $20 Million from the California Institute for Regenerative Medicine to Develop a Stem Cell Therapy for the Treatment of Diabetes

and what about the lung?

Stem cells as alternatives to classical lung transplantation

- cell replacement therapy

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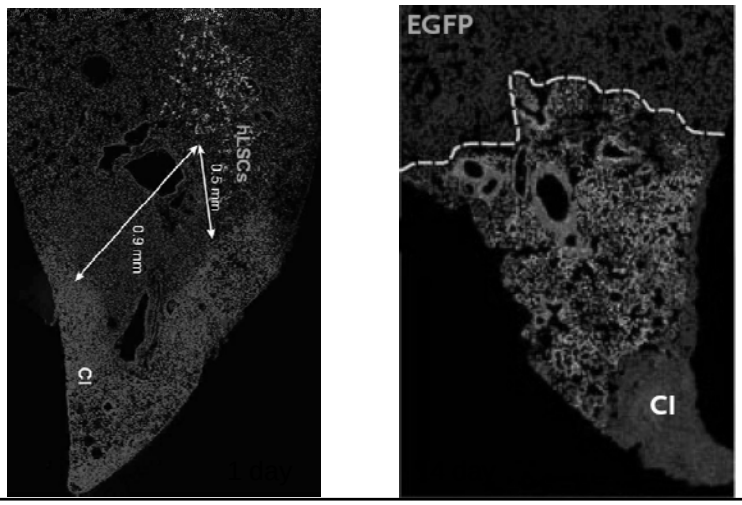
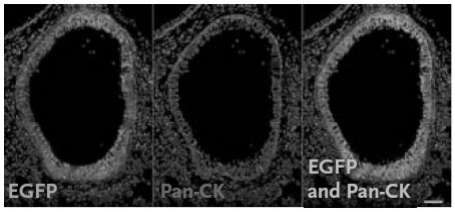
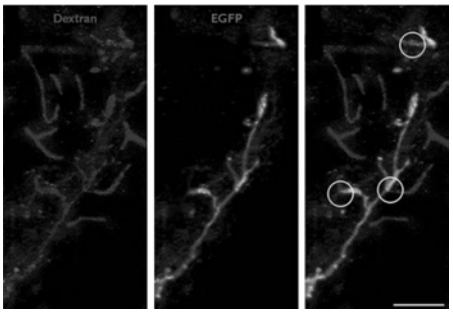
Migration of human lung stem cells into areas of regeneration after cryoinjury in the mouse lung



Integration of human lung stem cells into bronchioli and vessels in areas of lung regeneration





Stem cells as alternatives to classical lung transplantation

- cell replacement therapy
  - cellular source? adult stem cells vs. pluripotent stem cells
  - autotransplantation vs. immunosuppression
- given the complexicity of lung architecture whole organ engineering extremely challenging
- cell-depleted lung as a natural growth matrix for generating new rat lung

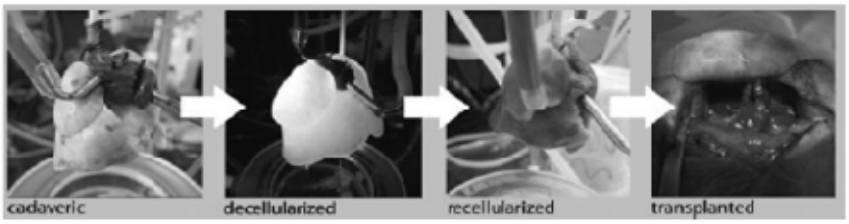
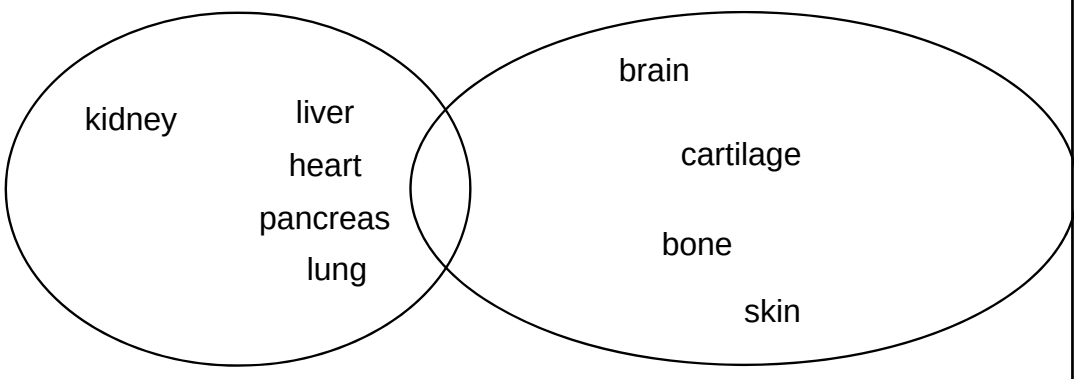


Figure 1: Bioartificial lung engineering on the basis of perfusion-decellularized matrix. Deceased donor lungs are cannulated via the pulmonary artery, vein and trachea. Cells and cellular debris are removed through perfusion with detergents and phosphate buffered saline via the pulmonary vasculature. Resulting acellular native lung ECM scaffolds are then mounted in a bioreactor and seeded with epithelial and endothelial cells via the trachea and pulmonary artery, respectively. After *in vitro* testing, the left lungs are removed, cannulated and transplanted in an orthotopic position after left pneumonectomy in rats. Grafts are perfused and ventilated via the recipient's vasculature and tracheobronchial tree.



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