Stem cell-based approaches to prevent hyperoxic lung injury

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Lung Development, Injury and Repair

Antoine Laurent Lavoisier
Chimiste français (1743-1794)

“Considérations Générales sur la Nature des Acides” (1778)
“air” is responsible for combustion and the source of acidity
- Oxygen (Greek for acid-former)
- Azote (Greek for no life)
- Hydrogen (Greek for water-former)
"For as a candle burns much faster in dephlogisticated (oxygen-enriched) than in common air, so we might live out too fast, and the animal powers be too soon exhausted in this pure kind of air. A moralist, at least, may say, that the air which nature has provided for us is as good as we deserve"


"Many children, especially those prematurely born, die from inability to expand their lungs sufficiently when they take their first breath. I have no doubt that in many of those cases, lives could be saved by starting the respiration artificially by means of apparatus operating in the manner described above."

Alexander Graham Bell, 1889
Donald and Lord, 1953
Histological stages of lung development

Historical BPD originally described by Northway

The “New BPD”: An Arrest in Lung Development

Experimental Models mimicking BPD

- Prematurity
- Chorioamnionitis
- Oxygen
- Ventilation
- Dex

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Decreased Alveolarization in BPD: Potential Long Term Consequences

Alveolar Number (x 10^6)

12-year-old boy history of prematurity (26 wks-596g) and BPD who died of an acute asthma attack.

Age-matched control

Mechanisms of Lung Injury and Repair Remain Incompletely Understood

Alveolar Type 2 cell dedifferentiation

Bone Marrow-derived cell homing and engraftment

Thin section CT of a 20 year old non-smoking male born 1355g at 28 weeks gestation, dependent upon supplementary oxygen to 321 days postpartum. There is severe emphysema and almost complete replacement of the right upper lobe with a large bulla.
A stem cell is a cell that has the ability to divide (self-replicate) for indefinite periods—often throughout the life of the organism.

Under the right conditions, or given the right signals, stem cells can differentiate into many different cell types. That is, stem cells have the potential to develop into mature cells that have characteristic shapes and functions, such as heart cells, skin cells, or nerve cells.

**Bone Marrow-derived Hematopoietic Stem Cell:**
the best characterized Stem Cell

**Bone Marrow-derived Mesenchymal Stem Cell:**
a “high reparative potential” Stem Cell

**Stem Cell Potential**

- **Zygote or fertilized egg**
  - Totipotent

- **Embryo**
  - Pluripotent

- **Differentiated Cells**
  - Mesodermal
  - Endodermal
  - Neural
  - Epithelial
  - Hematopoietic

Friedenstein AJ. Int Rev Cytol 1976
Bone Marrow-derived Mesenchymal Stem Cell: a “high reparative potential” Stem Cell

[Image]

Experimental O₂-induced “BPD” in Newborn Rats

95% Oxygen

O₂

Birth (P0)

95% O₂ - induced P15

Alveolar stage (P4-14)

BMP imaging

Scanning Electron Microscopy

FITC Cells

Barium Angiogram

Control

95% O₂ - induced

Alveolar structures

Lung vascular structures

Decreased lung resident MSC in O₂-induced lung injury

[Graphs]

BM-MSC migrate towards O₂-injured lungs

[Bar Graph]

Decreased CFU-F number per 10^6

Normoxia

Hyperoxia

Tim van Haften, MSc
**In vitro** BMSC co-cultured with oxygen-injured lung adopt a lung alveolar epithelial cell phenotype

**In vitro** BMSC co-cultured with injured lung adopt morphological features of alveolar epithelial cells

**In vivo** BMSC co-cultured with oxygen-injured lung adopt a lung alveolar epithelial cell phenotype

**In vivo, intra-tracheal BMSC therapy is feasible and results in lung engraftment**

**Engrafted BMSCs express the alveolar epithelial cell marker SP-C**

**Fetal type II AEC**

**BMSC + culture media**

**BMSC + injured lung**

**Engrafted cells: 3.7% ± 2.9%**

**Conversion: 75.3% ± 24.5%**
Airway delivery of BM-derived MSC improves survival in \(O_2\)-induced BPD in newborn rats

![Graph showing % survival over time (Days) with different exposure conditions and markers.]

Airway delivery of BM-derived MSC improves exercise capacity in \(O_2\)-induced BPD in newborn rats

![Bar graph comparing distance (m) at different time points with different exposure conditions.]

Airway delivery of BM-derived MSC prevents alveolar injury in \(O_2\)-induced BPD in newborn rats

![Image showing mean linear intercept (\(\mu m\)) with different exposure conditions.]

Airway delivery of BM-derived MSC attenuates \(O_2\)-induced lung vascular injury

![Diagram illustrating decreased pulmonary hypertension and improved vascular morphometry with different exposure conditions.]

CFSE (green) labeled BM-MSC

Improved Vascular Morphometry

Decreased Pulmonary Hypertension

Normoxia

Hyperoxia

Hyperoxia + BM-MSC

Hyperoxia + PASMC

RV/LV+S

PAAT (sec.)

\(\ast\) P<0.05 vs Normoxia

\(\ast\) P<0.001 vs Hyperoxia (Fisher's PLSD) n = 10/group
Interim Summary (1/3):
Adult BM-MSC prevent $O_2$-induced lung injury

Bone Marrow-derived cell homing and engraftment

- Migrate preferentially towards injured lung
- can be administered intratracheally similar to routinely used surfactant
- engraft into the injured lung
- adopt a lung phenotype
- improve survival
- prevent $O_2$-induced lung injury

Harnessing the Therapeutic Potential of Human Umbilical Cord Blood derived cells

- Clinically relevant
- Discarded source of potent stem cells
- Strong reparative potential
- Devoid of ethical dilemma

Juliana Rey, MD, PhD
HUCB-derived cells attenuate O$_2$-induced Lung Injury in Newborn Nude Rats

Normoxic Control  Hypoxia (BPD model)  Hypoxia+UCB

Hyperoxia+UCB

*P<0.02 vs Hyperoxia

Normoxic Control  Hyperoxia (BPD model)  Hyperoxia+UCB

Hyperoxia (BPD model)

Normoxic Control  Extrapulmonary Bleomycin + HUCB

Hyperoxia+UCB

*P<0.05

HUCB-cells improve Exercise Capacity in Bleomycin (BLM)-induced PF in Mice

Minutes

0 5 10 15 20 25 30 35 40 45

Before 7 days after treatment 14 days after treatment 21 days after treatment

Control n=7  BLM + HUCB cells n=7  BLM n=6

*P<0.05

HUCB-cells improve Lung Function in BLM-induced PF in Mice

Compliance

Elastance

Compliance cmH$_2$O/ml

Elastance cmH$_2$O/ml

Control BLM BLM+ HUCB

Control BLM BLM+ HUCB

*P<0.05

Idiopathic Pulmonary Fibrosis (PF)

• Progressive dyspnea and abnormal lung function
• Characterized by epithelial injury, fibroblast proliferation
• ECM growth, collagen deposition
• No proven prevention or treatment
• Life expectancy 3-5 years


HUCB-cells improve Lung Function in BLM-induced PF in Mice

Compliance cmH$_2$O/ml

Elastance cmH$_2$O/ml

Control BLM BLM+ HUCB

Control BLM BLM+ HUCB

*P<0.05

10
**Interim Summary (2/3):**

HUCB-derived cells prevent experimental lung injury

**Image:**
Bone Marrow-derived cell homing and engraftment

**Graphs:**
- **HUCB-cells decrease BLM Lung Fibrosis**
- **Engrafted BMSCs express the alveolar epithelial cell marker SP-C**

**Bars:**
- Hydroxyproline (g/mg)
- Total collagen (mg/mg)

**Table:**

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<th>Control</th>
<th>BLM</th>
<th>BLM + HUCB</th>
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<td>Hydroxyproline (g/mg)</td>
<td>0.00</td>
<td>0.60</td>
<td>0.40</td>
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<tr>
<td>Total collagen (mg/mg)</td>
<td>0.00</td>
<td>0.60</td>
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**Engrafted cells:** 3.7% ± 2.9%
Conversion: 75.3% ± 24.5%

**Roisin Byrne, MSc**
BMSC-derived CdM decreases O2-induced apoptosis of rat AEC2

- Room air
- Hyperoxia 80%

% of Apoptotic AEC2 in hyperoxia

- DMEM
- BM-MSC Conditioned media

*P<0.05

BMSC-derived CdM improves AEC2 wound healing in vitro

- Before scratch
- Scratch
- 3h after scratch
- 6h after scratch

% Wound Closure

- DMEM
- BMSC-CdM

Unpaired T-Test p<0.05

BMSC-derived CdM enhances lung endothelial cell cord formation in vitro

- RLMVEC + DMEM
- RLMVEC + BMSC-CdM

- Norm
- 85% O2

Total Cord Length

Intersections

RLMVEC: Rat Lung Microvascular Endothelial Cells

Unpaired T-Test, p<0.05

In vivo effect of BMSC-derived CdM in acute LPS-induced lung injury

- 8-10 week old male C57 mice

- 4 hours

- no treatment
- Intra-Tracheal DMEM
- Intra-Tracheal BMSC CdM

Bronchoalveolar Lavage (BAL)

12 - 48 hours

Harvest

Lung Histology
**BMSC-derived CdM reduces BAL total cell and neutrophil count in LPS-induced lung injury**

**Interim Summary (3/3):**
MSCs may act via a paracrine mechanism

- Bone Marrow-derived cell homing and engraftment
- Bone Marrow-derived cell secretion of repair modulating factors

Today in the Lab: HUCB derived cells prevent lung injury
**Perspectives**

- To improve our understanding of lung development-injury-repair
- To improve our understanding of stem cell biology (homing, engraftment, plasticity…) and identify of the best “reparative” cell
- To carefully assess the short- and long-term safety (tumor formation…?) and efficacy (structure and function) of stem cell-based therapies
- No single magic bullet for a multi-factorial disease
- Incremental improvement and combined use of available and new therapeutic strategies

The Team

- Paul Wozak
- Arul Vadivel
- Rajesh Anthuvan
- Christina Luong
- Beverly Morgan
- Farah Eaton
- Juliana Rey
- Rossin Byrne
- Lavinia Coltan
- Gaia Weissmann

Collaborators:
- Jacques Galipeau, Montreal
- Mary, Yoder, U Indianapolis
- Jonathan Davis, Boston
- Fabio Mosca, Milano
- Lomana Larijani, Milano
- John Akeham, U of Alberta

Before Thomas Edison, it was evident that we would always produce light with a candle
Preparation of BMSC Conditioned Media CdM

- BMSCs freshly isolated from adult Sprague Dawley rats or C57 mice.
- Supernatant replenished with serum for in vitro studies and concentrated and de-salted for in vivo studies.

BMSC-CdM Secretome: Fold Change vs. Lung Fibroblast CdM

- Growth and Angiogenic Factors
- Immunomodulatory Factors

Stem Cells 80% Confluent → Serum-Startve for 24 hours → Media Collection
Experimental design

1. Oxygen-Induced Injury of fetal rat Type II Alveolar Epithelial Cells
   
   ![Cell Image]

   85% O2
   36 hours
   Apoptosis & Proliferation

2. Wound Healing Assay with fetal rat Type II Alveolar Epithelial Cells

   ![Cell Image]

3. Rat lung microvascular endothelial cell cord formation assay

   ![Cell Image] MATRIGEL

**Patrick Bouvier Kennedy** (August 7, 1963 - August 9, 1963) was the younger son of United States President John F. Kennedy and First Lady Jacqueline Bouvier Kennedy. He was born five and a half weeks prematurely by caesarean section at the Otis Air Force Base Hospital, with a birth weight of 2,112 g…

... Kennedy was aware that Drs. Paul Swyer and Maria Delivoria had successfully ventilated an infant with RDS in Toronto nearly two years earlier, who became one of the few survivors of neonatal positive pressure ventilation until that time. A decision was made to transfer the baby there for care, but political pressures intervened. At the last minute, the child was transferred to Boston Children's Hospital, where he was placed in a hyperbaric oxygen chamber, and died two days later of **hyaline membrane disease**.

His obituary in *The New York Times* pointed out that, at that time, all that could be done "for a victim of hyaline membrane disease is to monitor the infant's blood chemistry and to try to keep it near normal levels. Thus, the battle for the Kennedy baby was lost only because medical science has not yet advanced far enough to accomplish as quickly as necessary what the body can do by itself in its own time".

More than any other single event, the death of this infant served to ignite public and medical awareness to the need for neonatal intensive care and soon led to the establishment of NICUs around the country.
Monday 21st JULY 1969

The still unnamed white substance, Dr. Clements said, is a surface active agent normally secreted by mammalian lungs to coat the inside walls of the alveoli and thus prevent lung collapse...

Hyaline Membrane Disease of Premature Infants is due to Surfactant Deficiency

The drug is a credit to microbiologists, physiologists and pathologists who were the first to define the substance, which occurs naturally in the body but is deficient in premature babies. Testing with laboratory animals yielded encouraging results. Trials with humans established synthesized surfactant's efficacy, and it was approved for use in 1990!