Tracheal Reconstruction with Tissue Engineered Airway

Paolo Macchiarini, MD, PhD

Director, Department of General Thoracic and Regenerative Surgery and Intrathoracic Biotransplantation and BIOAIR (Laboratory of Biomolecular and Bioengineering Airway) University Hospital Careggi, Florence
Tracheal transplantation

Indications

• Benign diseases
  – Relapsing polychondritis
  – Tracheobronchopathia osteochondroplastica
  – Trauma patients

• Malignant diseases
  – Adenoid cystic
  – Squamous cell
Clinical Airway Allotransplantation

Trachea

Aorta

Subclavian Artery

Concern of Allotransplantation

Technically feasible

Requires Imunosuppression
Who wants airway transplant?

Laryngectomees 1990’s, UK

Without immunosuppression
Permanent stoma
Completely safe

80%
50%
75%
Need of a safer alternative

Regenerative Medicine:
Focuses on the restoration or regeneration of lost, damaged, or aging cells and tissues in the human body
Regenerative airway replacement

Scaffold

Cells

Bioreactor
Patients Past History

• Airway Tuberculosis in 2005 treated with medications, upper airway resection and stent implantation into left main bronchus, removed 6 months later because not tolerated

• Progressive functional and clinical decline

• Technically, only the removal of the entire lung along with a major airway would have had any chance of curing her

• Because of our very encouraging preclinical data, we offered her the tissue engineered replacement of her left main bronchus
Detergent Enzymatic Decellularization

- Trachea removed from human donor
- Connective & tissue removed from trachea
- Cellular element lysed by repeating (@ least twice) washing step in distilled water
- Cell membranes & intracellular components solubilization by 4% Na deoxycholate solution x 4h
- Nuclear content solubilization and DNA degradation by 1mL NaCl plus DNAse solution x 3hse

Washing step with distilled water
After decellularization

- Macroscopy
- Major Histocompatibility Antigens status
- Persistence basal membrane
- Molecular evaluation
- Biomechanical properties

Native trachea after 25 DEM cycles

Class I

Class II

Endoluminal

External surface

Normal human airway

Decellularized human airway

a: negative control
b: tracheal sample
c: positive control

Graph showing force (Newton) vs. length (cm) for different demineralization cycles.
Which cells should be used?

- **Tracheal Histology**

- **Type of autologous cells**
  - In vitro expansion and characterization of respiratory cells
  - In vitro expansion of autologous mesenchymal stem cells (MSCs)
  - MSCs differentiation using specific growth factors as recombinant human transforming growth factor-\(\beta\)3 (TGF-\(\beta\)3)

Epithelial morphology confirmation
- Red=cytokeratins 5 and 8 (epithelial)
- Blue=4'-6'-diamidino-2-phenylindole

Using specific serum-free medium, supplemented with specific growth factors differentiated cultures of epithelial cells at the 4th passage were obtained.

*J Thorac Cardiovasc Surg. 139(2):437-43, 2010*
How to reseed the decellularized scaffold

- Rotating bioreactor under sterile conditions
- Dynamic culture for 72 hours at 1 to 1.5 rpm resulted in an homogenous seeding of the matrix

Matrix staining:
- Red = Cytokeratins 5 & 8 (epithelial)
- Green = Collagen II (chondrocytes)
- Blue = DAPI (nuclei)
Outcome

- Surgery was on June 12, 2008
- Immediate post-operative implantation
- View 3 months post-operatively
- Normal lung function, got to full work by March 1st, 2009
- No clinical or immunological evidence of rejection, without immunosuppression

Graft brushing
- Red = cytokeratins 5& 8 (epithelial)
- Green = Collagen II (chondrocytes)
- Blue = DAPI (nuclei)

Anti HLA Antibodies
- FlowPRA - HLA I
- FlowPRA - HLA II

O2 MASS TRANSFER & REACTION

FGF

18 months post-transplant

Table: Lung function

pp O2 (%)

Biomaterials. 31(19):5131-6, 2010.
Angiogenic properties of DEM-scaffolds

- Strong immunoreactivity against anti b-FGF in the non-cartilaginous side of decellularized trachea

- In vitro cell migration assay
  - A significant increase in HUVEC migration was observed either for non-cartilaginous and cartilaginous tracheal samples

- In vivo angiogenic assay Chick embryo chorioallantoic membrane assay
  - Significant correlation between pro-angiogenic effect of bioengineered tracheal matrices and time
  - Acellular tracheal matrices were adherent to the CAM, totally enveloped by CAM and newly formed blood vessels arranged under the implants

Endothelial cells

HUVEC

Negative Control
(Non-cartilaginous sample)
(Cartilaginous sample)
Positive Control

Non-Cartilaginous trachea

Cartilaginous trachea

Number of blood vessels

Time (day)

*p<0.05 from negative control, p<0.05 from non-cartilaginous tracheal samples

*p<0.05 from day 2; p<0.05 from day 3
Towards Bionics Regenerative Medicine
Cellular & Molecular Advances

- The human body reacts to injury with an amazing site-specificity to achieve a regenerative response.
- Wound healing is closely linked to inflammatory responses (IL-6, IL-1 & TNF).
- If controllable, stem cell activation following injury has the therapeutic potential for modulating regeneration in acute or chronic wounds.
Human FmSCs express the receptor for erythropoietin (EPO).

EPO influences FmSC growth in the absence (inhibitory) or presence (stimulatory) of IL-6/TNF-alfa.

EPO switches from its inhibitory function to a supportive role for boosting skin regeneration.

Detection of the EPO receptor mRNA-expression in human skin fibroblastic mesenchymal stem-cell-like cells.

Expression of EpoR, beta-cR, and growth hormone receptor in parallel in mouse tissues and stem cells.

Day0 Day1 Day3 Day5 Day7

Cell number
**In-situ regeneration**

- Topically applied EPO considerably enhanced wound healing and improved chronic wounds conditions.
- Human’s FmSCs cytophenotype did not change with or without the presence of trauma cytokines.
- *In vivo* studies of EPO administration in ischemic or acute injuries found a trauma dependent stimulation of wound healing independent of the tissue organ studied.
Experimental rationales

- Bionic regeneration

- Bypassing stem in vitro cell cultures

- Purely intraoperative or bedside procedure

- Intraoperative stem cell activation with and without templates to achieve restitutio ad integrum

- Commitment factors (differentiation)

- Boosting factors (acceleration)

- Permission factors (local trauma)

- Recruitment factors (increase/use of available stem cells)

Normal trachea

Bionic trachea (7 days)

PCR Human Tracheas and Esophagus

GAPDH Human

271 bp

1  2  3  4  5  6  7  8

200 bp

100 bp

EPOR human

231 bp (with Intron 311 bp)

8  1  2  3  4  5  6  7

ßCR human

213 bp

Skin (Positive control)

Dermis (Positive control)

Negativ control

Native

Bionic (3 weeks)
Adult bionic airway transplantation

- *In-situ* regeneration
- Fate of human cell differentiation

Viable chondrocytes

Bone Marrow (60 mL), G-CSF (200 IU), Erythropoietin (10 000 IU), TGF-β1 (50 µg)

- Recruit (locally & systemically) progenitor endothelial cells
- Accelerate and boost regeneration
- Differentiate adult mesenchymal SCs into condrocytes

2 months post-transplant - Epithelium with ciliated cells
Pedriatic bionic airway transplantation

Tracheo-aortic fistula in a 10 yrs old boy

Bionic boosting and recruitment

Aortic patch repair & total windpipe replacement (7 cm)

Bone Marrow (60 mL)

G-CSF (200 IU)

Erythropoietin (10,000 IU)

TGF-β1 (50 µg)

Recruit (locally & systemically) progenitor endothelial cells

Accellerate and boost regeneration

Differentiate adult mesenchymal SCs into condrocytes

Head TGF-1 stem cells activation

Islets of native mucosa

Bioabsorbable stent
Conclusions

- Transplantation of tissue engineered human trachea is feasible without immunosuppression, in adults and children;
- Decellularization preserves proangiogenic proteins that *in vivo* induce organ-angiogenesis;
- Bionic tissue engineering uses the own body as bioreactor, avoids cell cultures, would be available for everyone & much more cost-effective.