Tissue engineering TE

Background

- TE aims to provide off-the-shelf organ substitutes
- The core technique is three-dimensional cell culture
- Cell, scaffold, growth factor and bioreactor comprise the four critical elements
Revascularization and reepithelialization remain key obstacles in TE trachea

**Background**

- Delayed revascularization process in large size TE substitutes limits their clinic application.

- Tubular cartilage tissue default as a sufficient TE trachea substitute. Reepithelialization plays a key role and depends on a well vascularized wound surface.
Host response to the tracheal substitute

Stage I: Acute inflammation
- Macrophage activation
- Fibrin clot formation
- Fibroblast, bacteria invasion

Stage II: Chronic inflammation
- Overgrowth of granulation tissue
- Bacterial mucous plug formation
- Lumen obstruction

Stage III: Foreign body reaction
- Granulation tissue and fibrous encapsulation

Fig. 1. The temporal variation in the acute inflammatory response, chronic inflammatory response and granulation tissue development, and foreign body reaction to implanted biomaterials. The intensity and time variables are dependent upon the extent of injury created in the implantation and the size, shape and topography, and chemical and physical properties of the biomaterial (used with permission from Ref. [247]).

Development of tracheal prostheses made of porous titanium: a study on sheep
Schultz P, et al., 2007 Apr
Requirments for trachea prosthesis

- Biocompatible
  - cause minimal foreign body reaction
  - incorportable by surrounding tissue
  - permit ingrowth of the repiratory epithelium along the lumen

- Laterally rigid but longitudanlly flexible
- Intact surface of epithelium impervious to fibroblastic and bacterial invasion of the lumen
- Airtight
In-vivo bioreactor” combine in-vitro reconstruction and in-vivo regeneration

**Concept**

- Organ prosthesis connected to an extra-corporeal perfusion system
Advantages need to be proved by in-vitro pilot examinations

### Description

- **Continuous medium flow** mimicking blood stream support the survival of cells both inside (chondrocytes) and on the surface (epithelia) of the scaffold
- **Prolong the cell seeding process** to cover the whole regeneration period
- **Suitable to emergency operation**
- **The expression levels can be readily adjusted** by changing their medium concentrations

### Examination result
Epithelial survival test

Study Design microanalysis
Split thickness skin graft harvest from pig
Wrapped around DegraPol scaffold
Connected to perfusion system
Continuously perfused for one week with DMEM
Static culture as control
Four samples for each group

Assessments
Histology
Epithelial survival test

• Histology showed skin graft survive with an **intact basement membrane** after one week under perfusion

• Histology results showed **epidermis and dermis tissue separation** in static culture group
Cell seeding project

Hypothesis

Continuously seeding cells through “in-vivo bioreactor” to combine the cell seeding and cell culture systems.

Study Design

**Perfusion seeding group (four samples)**
Harvest one flask chondrocyte every day for 5 days
Suspened in 1cc F-12 medium
Seeded to PEGT/PGT (1cm³) through perfusion system
Pause the perfusion for 2 hours to facilitate cell adhesion
Cultured under perfusion at speed of 2ml/hour

**Static control (four samples)**
Harvest 5 flask chondrocytes
Directly seeded onto PEGT/PGT (1cm³)
Immersed in F-12 medium after two hours
Static culture for 5 days

Assessments

MTT, SEM
Cell seeding project

Progress report \textit{in vivo part}

- Chondrocytes successfully seeded onto PEGT/PGT scaffold through perfusion

- MTT and SEM picture showed better three dimensional cell growth in the perfusion group
Angiogenesis project

Hypothesis

Functional concentration of growth factors can be maintained inside tissue engineered prosthesis through continuous perfusion of in-vivo bioreactor to accelerate angiogenesis.

Study Design

- Tubular Degrapol scaffold put on the surface of ex ovo chorioallantoic chick embryo (CAM) as angiogenesis test model
- Perfusion seeding group (four samples)
  - Intra-scaffold continuous perfusion with DMEM containing 40ng/ml VEGF for 5 days
- Static control (four samples)
  - Degrapol scaffold immersed in DMEM with high concentration (4ug/ml) VEGF for one hour

Assessments

- Microinjection of bisbenyimide H33342 one hour before sample harvest
- Histology and fluorescence image to test functional vessels
Angiogenesis project

- Erythrocytes migrated all over the scaffold in perfusion group due to increase vessel permeability

- Normal functional vessel were only detected in two samples from perfusion group
Conclusions

“*In-vivo Bioreactor*”, defined as the integration of in intra-scaffold medium flow supported by an extra-corporeal portable pump system for in situ TE regeneration, can deliver, and further maintain, the survival of seed cells while facilitating ideal effect exertion of the growth factor.
Artificial oxygen carrier (OxygentTM) project

**Item**

**Effect on cartilage tissue**

**Effect on angiogenesis**

**Effect on epithelial cells**

**Study design**

- Quantitatively measure GAG concentration
- Reconstruction of TE cartilage for one month
- Continue culture with vs without Oxygent for one month
- GAG measure
- Five samples in each group

- porcine acellular dermis matrix put on the surfaces of 8 CAM models for 7 days
- Add medium with vs. without Oxygen twice per day
- Orthogonal polarization spectral (OPS) imaging
- measure functional capillary density

- PtO2 measurement, Microdialysis
OxygentTM project

Lower GAG expression in OxygentTM group

GAG measurement

[Bar graph showing lower GAG expression in OxygentTM group compared to DMEM]
Oxygent™ project

Poor acid mucopolysaccharides formation in Oxygent™ group

DMEM

Oxygent™
Oxygent™ project

Angiogenesis

Study Design

Porcine a cellular dermis put on the surfaces of 8 CAM models for 7 days
Add medium with vs. without Oxygen twice per day
Orthogonal Polarization Spectral (OPS) imaging system

- Capillary diameter
- Capillary red blood cell velocity
- Functional capillary density
Oxygent project

Functional Capillary Density

- Area A: 80 Cm/Cm²
- Area B: 90 Cm/Cm²
Further plan for angiogenesis project

Aim
Find out the best spatial and temporal combination of three growth factors: VEGF; bFGF; PDGF

Study design

- CAM as an angiogenesis model
- Acellular porcine matrix as scaffold
- Three kinds of growth factors: VEGF; bFGF; PDGF
- Orthogonal polarization spectral (OPS) imaging system to measure capillary density around and inside the scaffold
- The grouping of concentration and combination of GFs following mathematical optimization principle, e.g., genetic arithmetic, orthogonal design
OxygentTM project *Epithelial survival*

**Study Design PtO2 measurement**

*Polarographic microprobe* measures
tissue partial oxygen tension (PtO2)

Two probes at different thickness of TE tracheal epithelium

*200-um-thick and 400-um-thick*

Continuously perfused with

*DMEM v.s. DMEM + 5% OxygentTM*

Perfusate reoxygenated with *air* and *pure* oxygen
PtO2 measurement results

Fig. 02
Summary of tpO2 measurement

1. The epithelia PtO2 level is much higher under continuous perfusion culture than that of static culture,

   32 v.s 4.3 mmHg in the DMEM group pre-charge with 100% oxygen

   50 v.s 5.2 in the Oxygent group pre-charge with 100% oxygen

2. Increased oxygen content under the OxygentTM DMEM perfusion

   Reoxygenation with air

   10.34% more at 200-µm-thick

   3427.44% more at 400-µm-thick

   Reoxygenation with pure oxygen

   73.79% more in the 200-µm-thick

   607.22% more in the 400-µm-thick

3. OxygentTM supplement can support around 200-um-thick epithelium
Tissue metabolite concentrations measured by microdialysis
Conclusions

Oxygent™ supplement

*increases* epithelial PtO2

*improves* epithelial metabolism

*does not impair* angiogenesis

*compromises* cartilage tissue formation
Dolley’s Anatomy
-- sheep experiment of TE trachea

Qiang Tan
Clinic of Thoracic Surgery
University Hospital, Zurich
Animal study design

**Operations**

Anterior trachea defect 4cm long, 2cm wide repair with neovel TE trachea

PEGT/PBT patch + Chondrocyte + skin fleet

**Two Groups**

Control group: TE trachea

In-vivo bioreactor group: TE trachea supported with in-vivo bioreactor

+ 20cc / hour DMEM
+ 10% autologous serum
+ autologous chondrocytes

**Assessments**

**Clinical evaluation for three months**

General situation checked everyday

Bronchoscopy every month

**Histological**

Ulex Europaeus agglutinin (UEA) & Peanut Agglutinin (PNA) for trachea epithelial

endothelin-1 for vessel
Operation

Split-thickness skin graft

Port-A-Catch implantation

Trachea defect
Operation

Repair with split-thickness skin graft

Scaffold implantation with catheter fixation
Result

One hour after operation
Resume from the anaesthesia
No dyspnea
No stridor

Three hours after operation
Normal food intake
No dyspnea
No stridor
Fluent perfusion
Blood inside the sucking tube
Results

Next morning (20 hours after operation)

normal movement
the sucking tube was block
subcutaneous emphysema
the sheep clinically fine with normal sound

One week later
fever 39.3
stop perfusion
Histology result after one month

- Lumen
- Normal tracheal epithelia
- Skin
- Scaffold
- New cartilage tissue form
- Seeded cells
“In-vivo bioreactor” combine with implantable biosensors might make monitoring and control of the tissue engineered organ regeneration process feasible.
Shanghai Chest Hospital & University Hospital Zurich

Thanks for your attention!