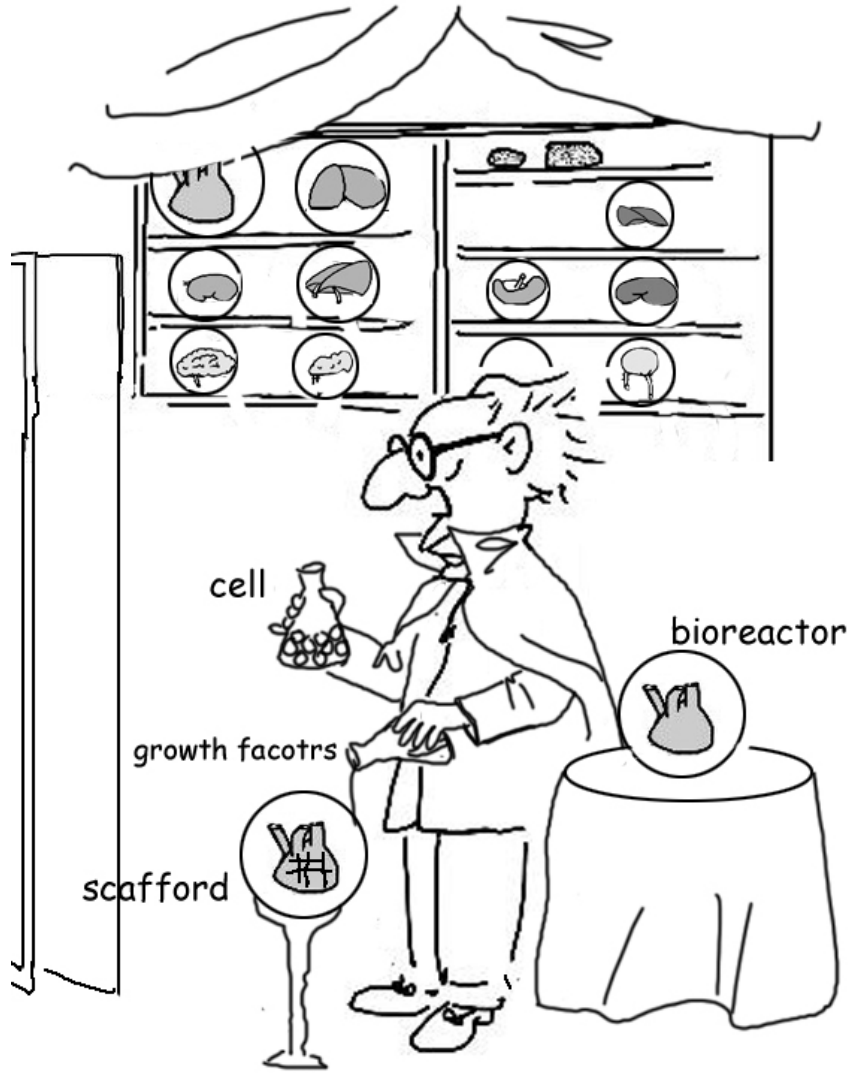
Tissue engineering TE

Background

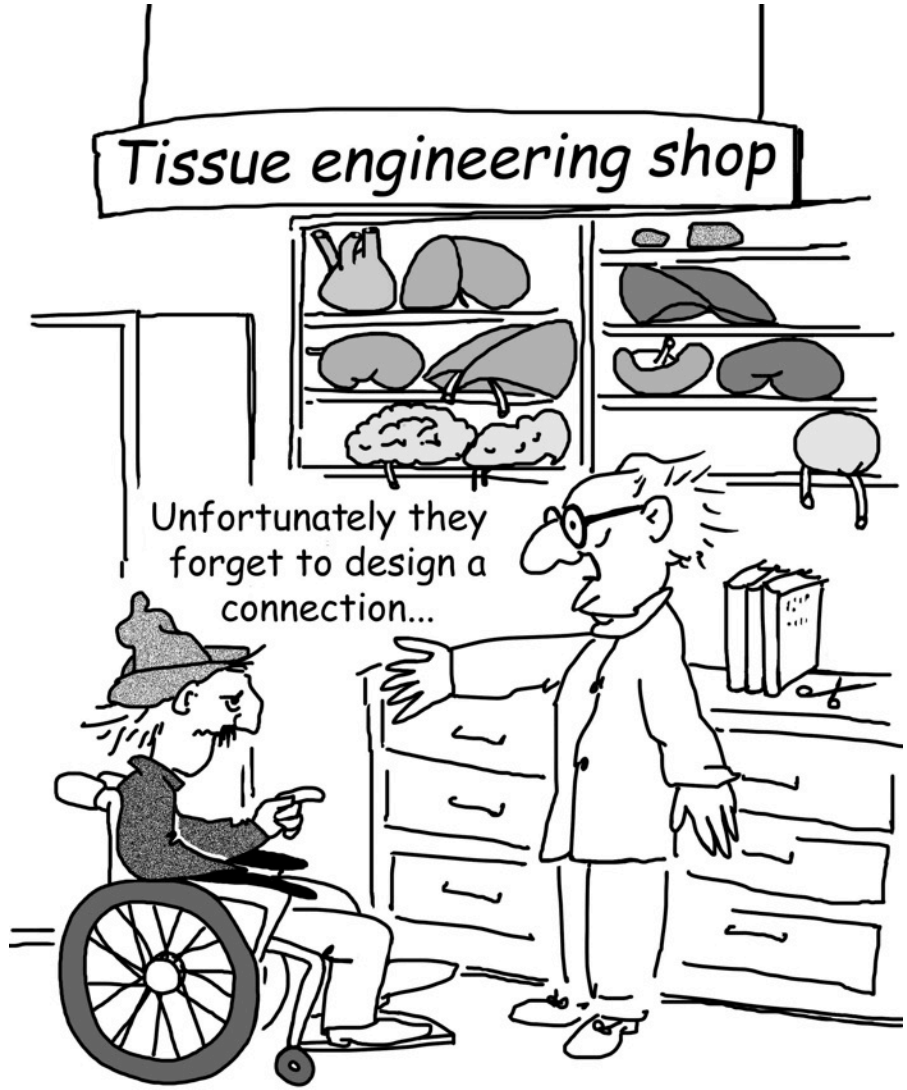
- TE aims to provide off-the-shelf organ substitutes
- The core technique is three-dimensional cell culture
- Cell, scaffold, growth factor and bioreactor comprise the four critical elements



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 a sufficient TE trachea substitute. Reepithelialization plays a key role and depends on a well vascularized wound surface



Host response to the tracheal substitute

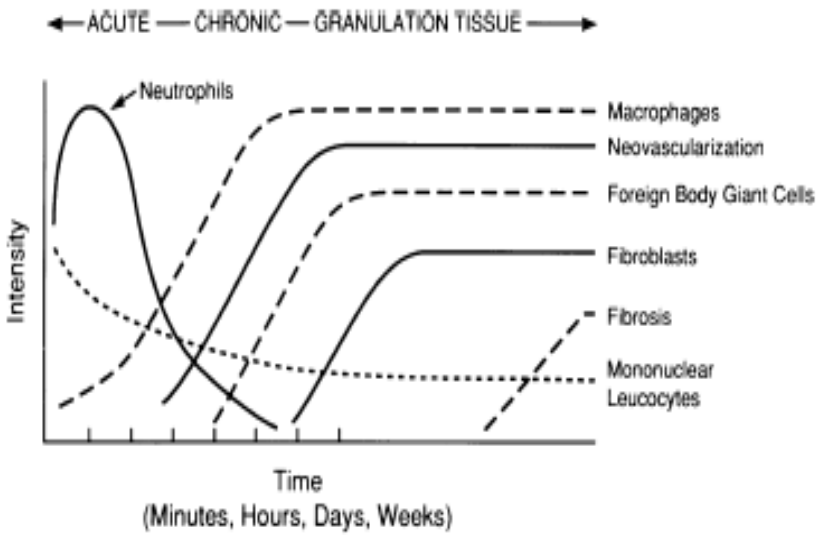


Fig. 1. The temporal variation in the acute inflammatory response, chronic inflammatory response and granulation tissue development, and foreign body reaction to implanted biomaterials. The intensity and time variables are dependent upon the extent of injury created in the implantation and the size,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

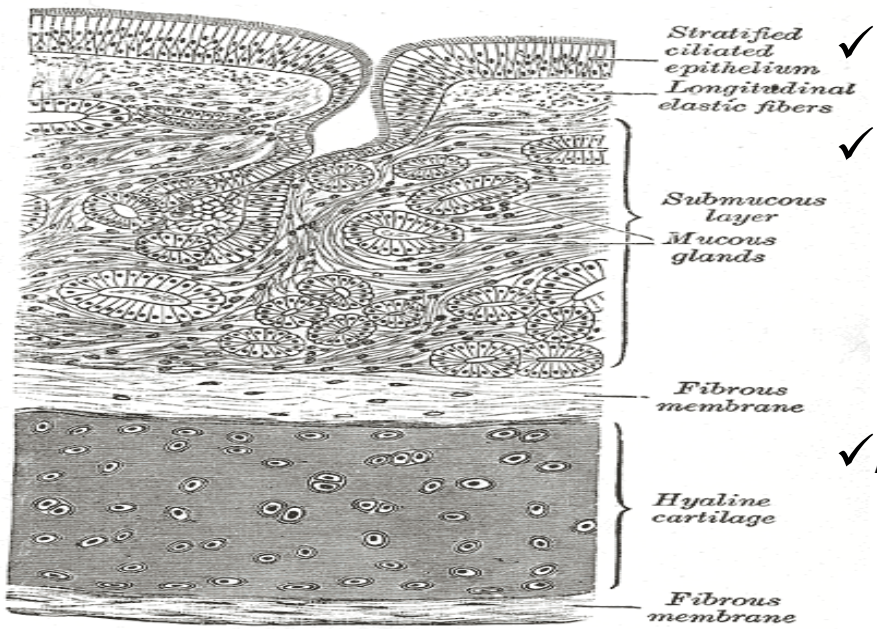
Stage I: Acute inflammation
Macrophage activation
Fibrin clot formation
Fibroblast, bacteria invasion

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

Stage III: Foreign body reaction
Granulation tissue and
fibrous encapsulation

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

Requirments for trachea prosthesis



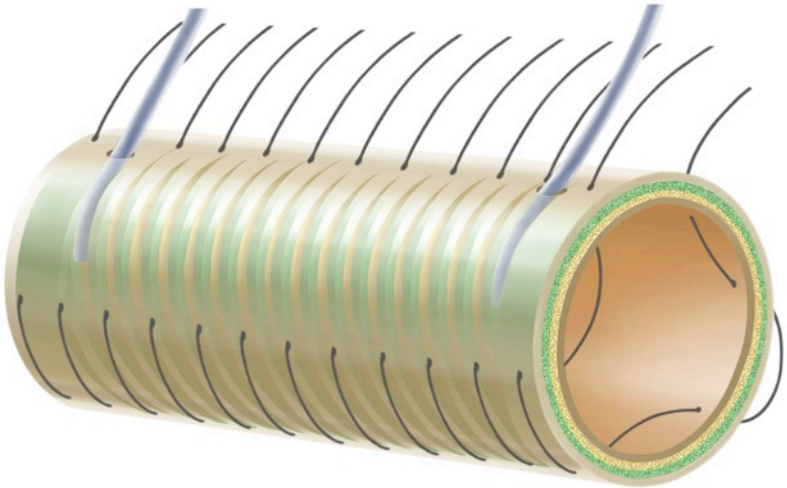
- ✓ Laterally rigid but longitudianlly flexible
- ✓ Intact surface of epithelium impervious to fibroblastic and bacterial invasion of the lumen
- ✓ Airtight

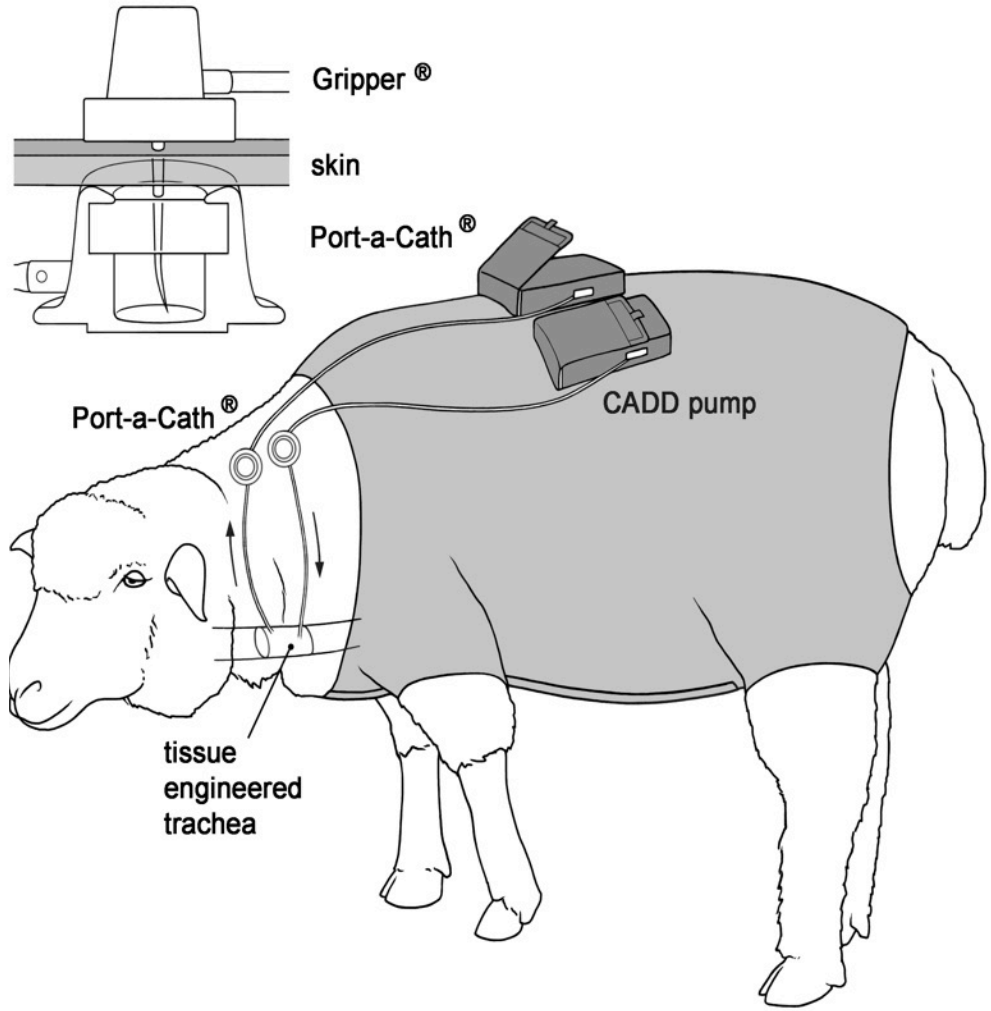
- ✓ Biocompatible
  - cause minimal foreign body reaction
  - incorportable by surrounding tissue
  - permit ingrowth of the repiratory epithelium along the lumen

“In-vivo bioreactor” combine in-vitro reconstruction and in-vivo regeneration

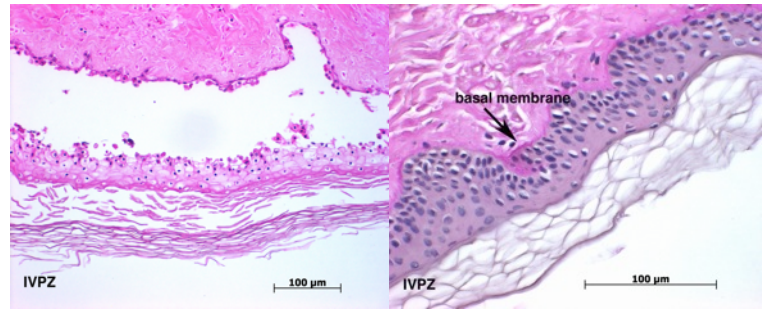
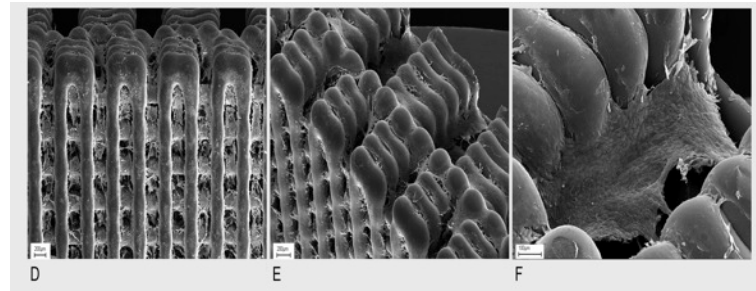
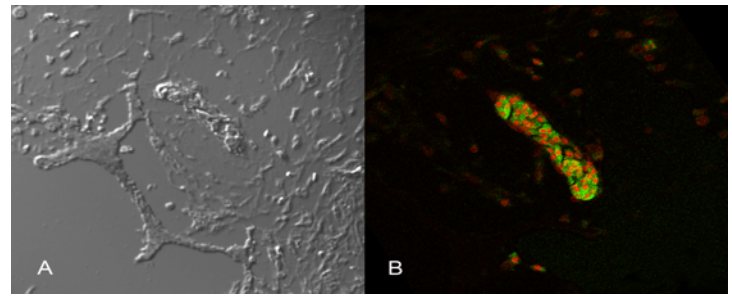
Concept

- Organ prosthesis connected to an extra-corporeal perfusion system





Advantages need to be proved by in-vitro pilot examinations

	Description	Examination result
Seed cells feeding	• Continuous medium flow mimicking blood stream support the survival of cells both inside (chondrocytes) and on the surface (epithelia) of the scaffold	
Refresh seed cells	• Prolong the cell seeding process to cover the whole regeneration period • Suitable to emergency operation	
Growth factors delivery system	• The expression levels can be readily adjusted by changing their medium concentrations	

Epithelial survival test

Study Design *microanalysis*

Split thickness skin graft harvest from pig

Wrapped around DegraPol scaffold

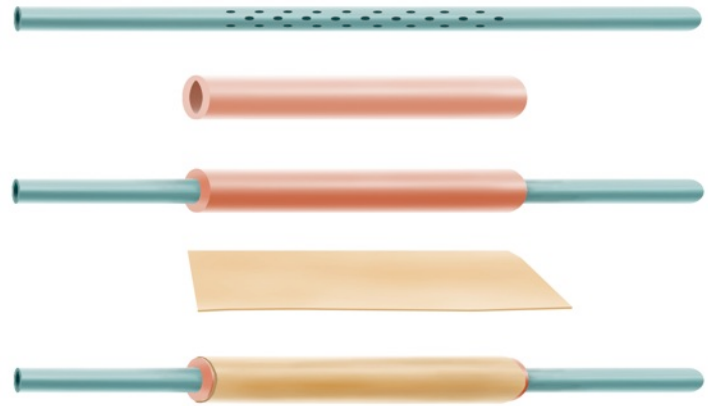
Connected to perfusion system

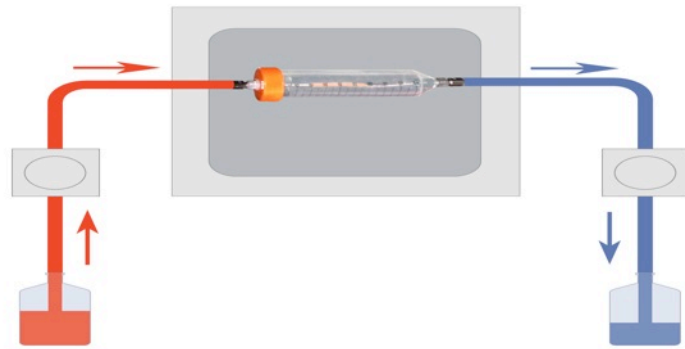
Continuously perfused for one week with

DMEM

Static culture as control

Four samples for each group



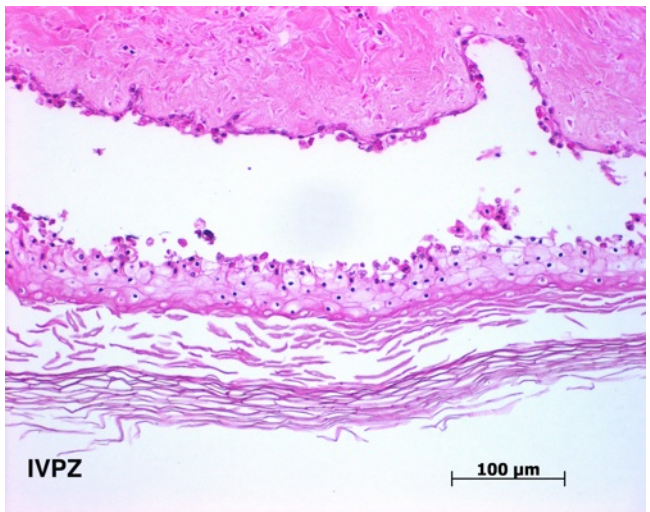


Assessments

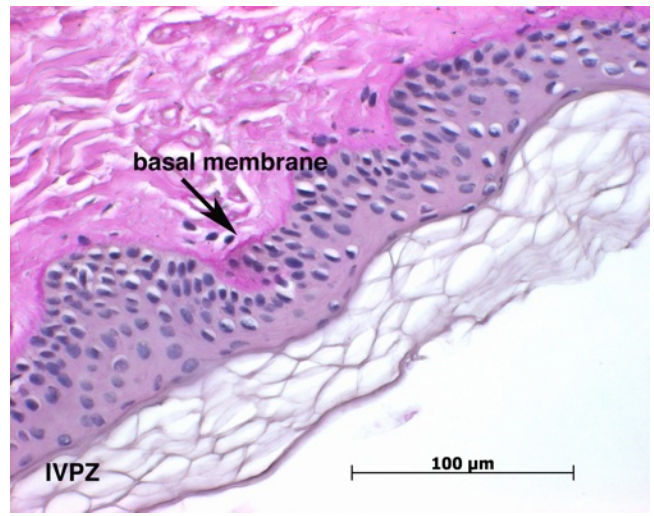
Histology

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



Static culture



Perfusion culture

Cell seeding project

Hypothesis

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

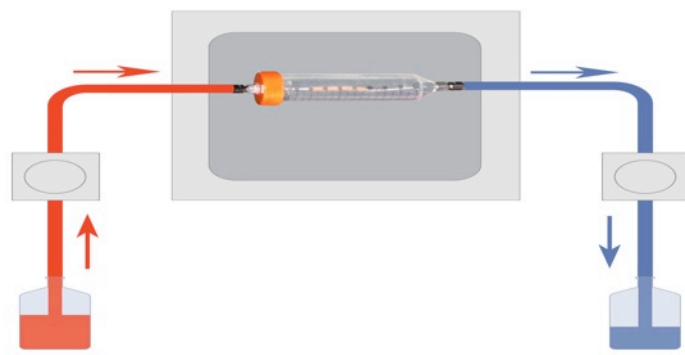
Study Design

Perfusion seeding group (four samples)

Harvest one flask chondrocyte every day for 5 days
Suspened in 1cc F-12 medium
Seeded to PEGT/PGT (1cm^3) 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 (1cm^3)
Immersed in F-12 medium after two hours
Static culture for 5 days



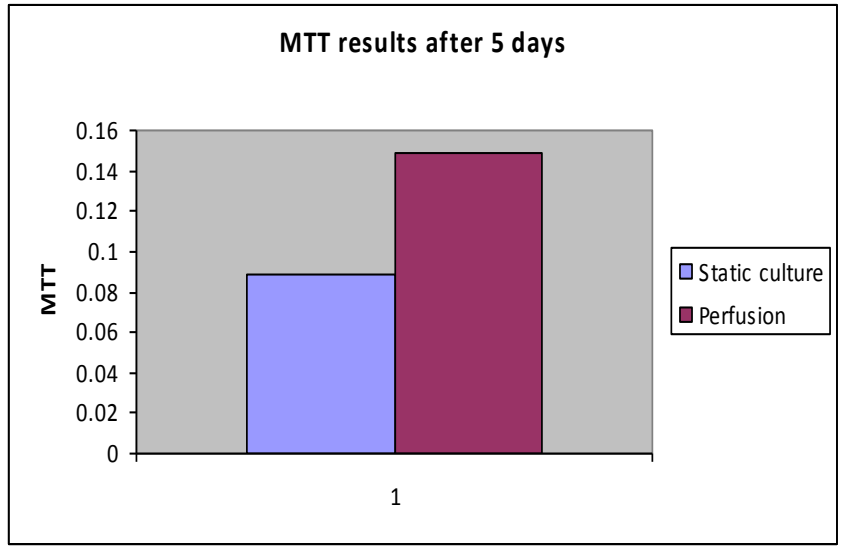
Assessments

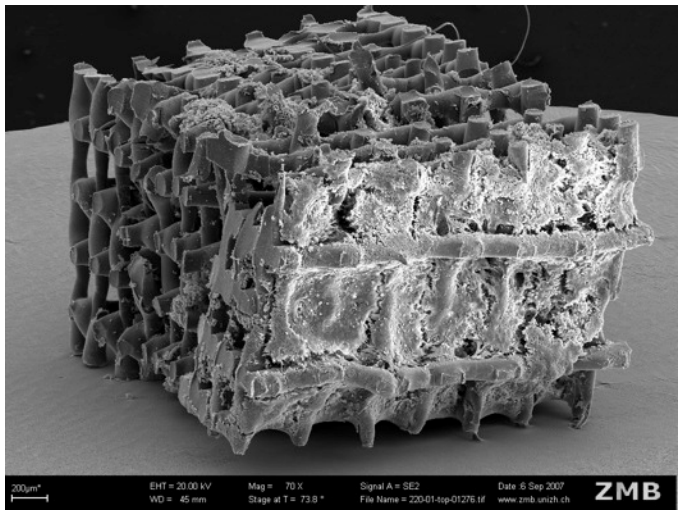
MTT, SEM

Cell seeding project

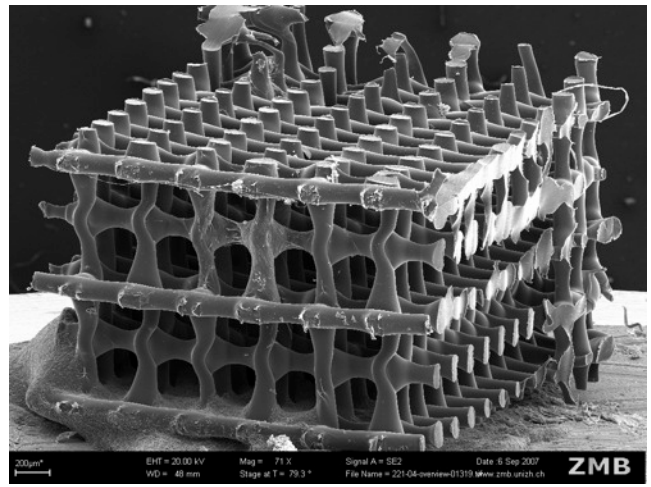
Progress report *in vivo part*

- Chondrocytes successfully seeded onto PEGT/PGT scaffold through perfusion
- MTT and SEM picture showed better three dimensional cell growth in the perfusion group





Perfusion Seeding



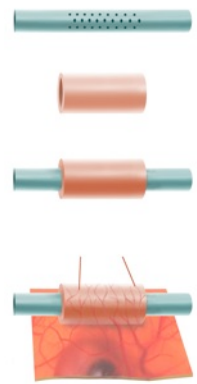
Static control

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.

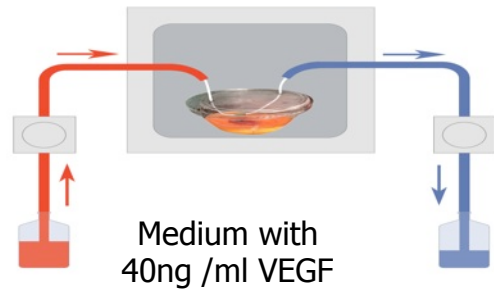
Study Design



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



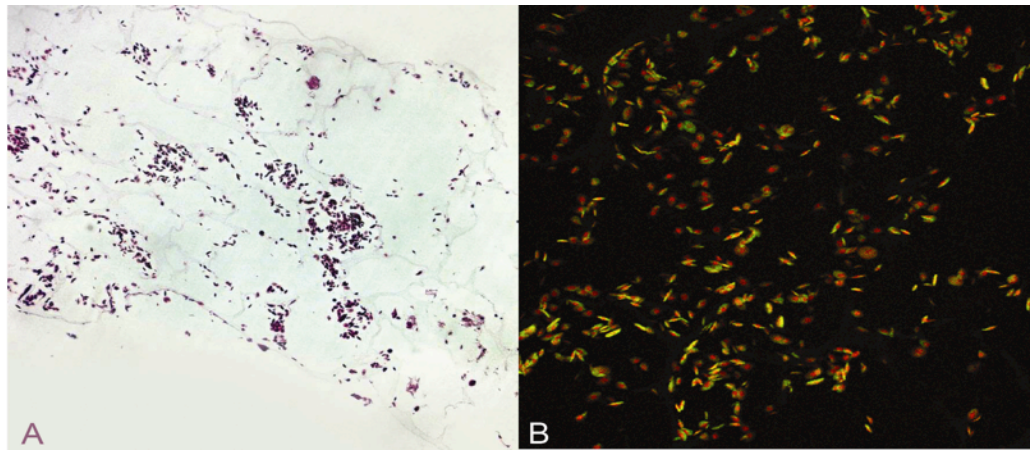
Assessments

Microinjection of bisbenyimide H33342 one hour before
sample harvest

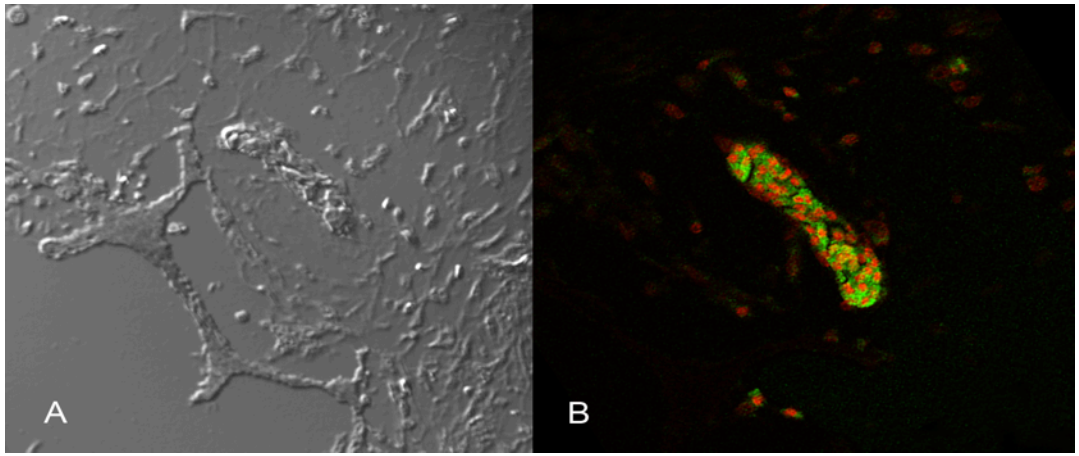
Histology and fluorecence image to test functional vessels

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

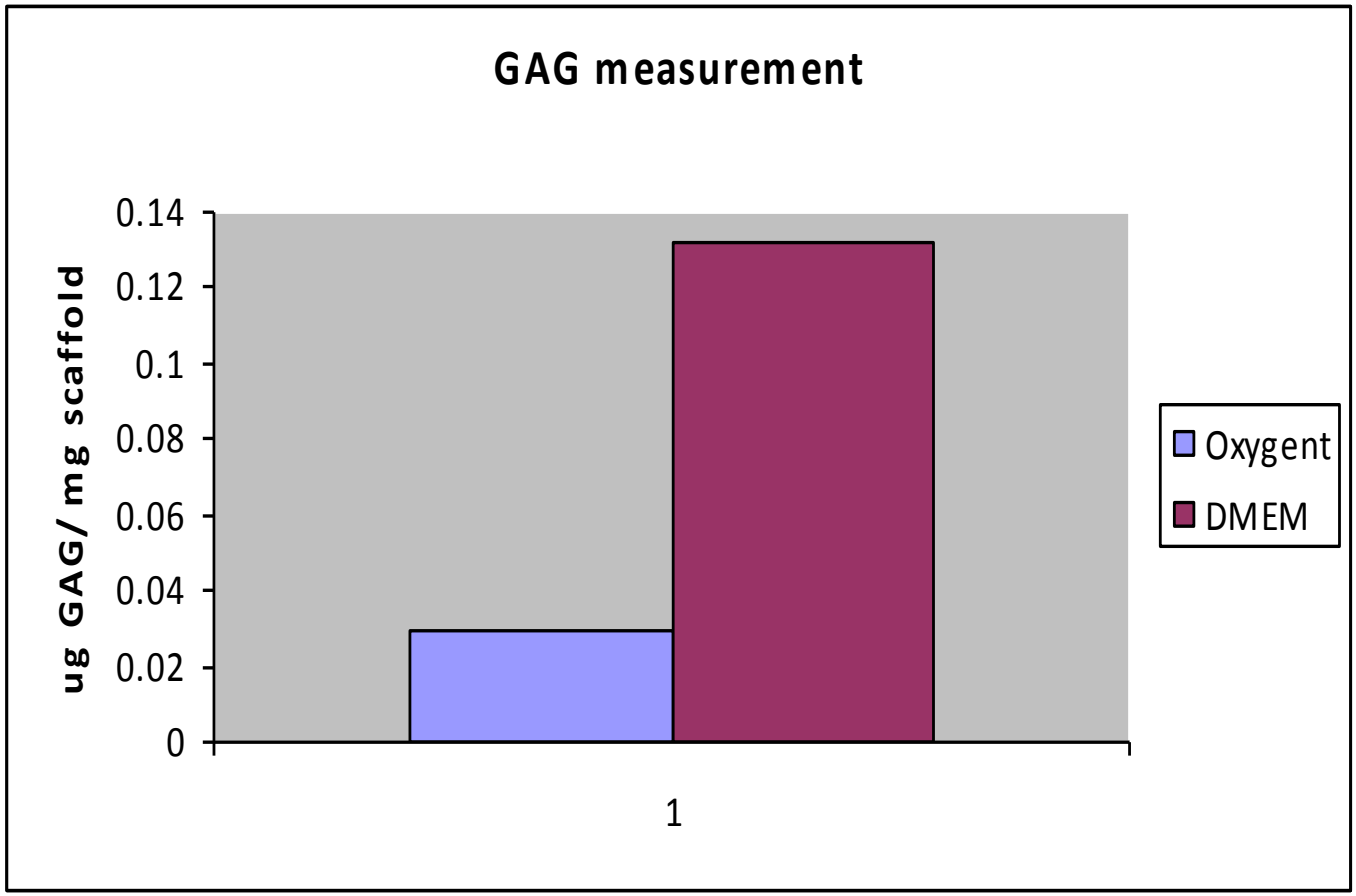
while facilitating ideal effect exertion of the growth factor

Artificial oxygen carrier (OxygentTM) project

Item	Study design
Effect on cartilage tissue	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
Effect on angiogenesis	• 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
Effect on epithelial cells	• PtO2 measurement, Microdialysis

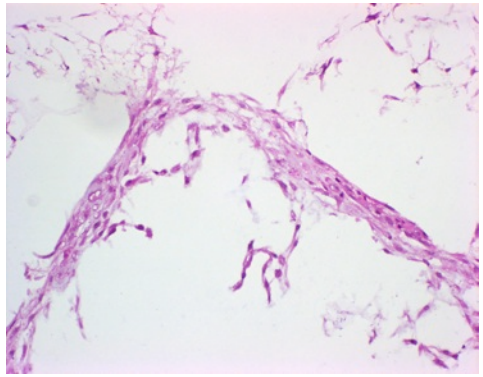
OxygentTM project

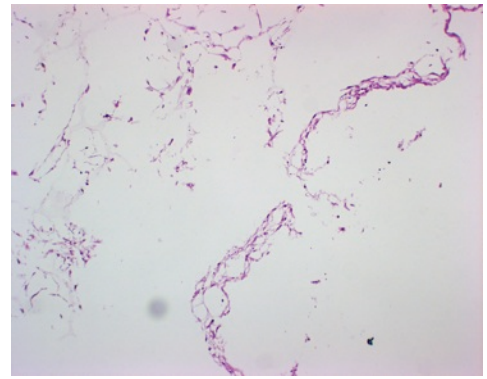
Lower GAG expression in OxygentTM group

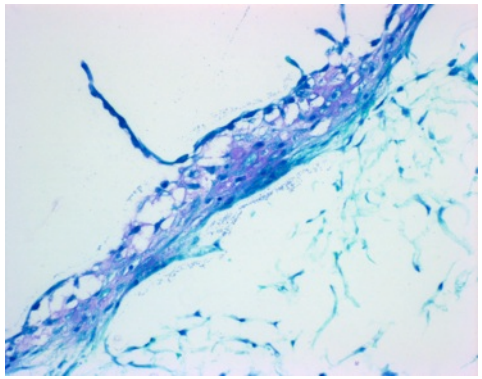


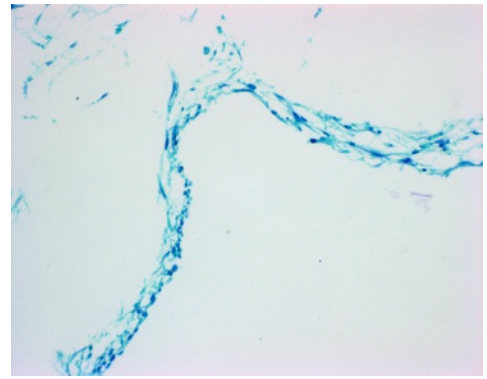
OxygentTM project

Poor acid mucopolysaccharides formation in OxygentTM group







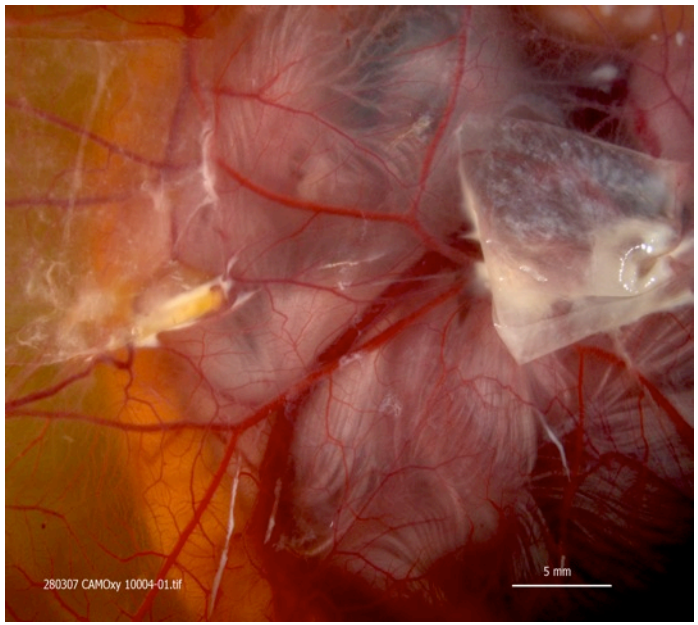


DMEM

OxygentTM

OxygentTM 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

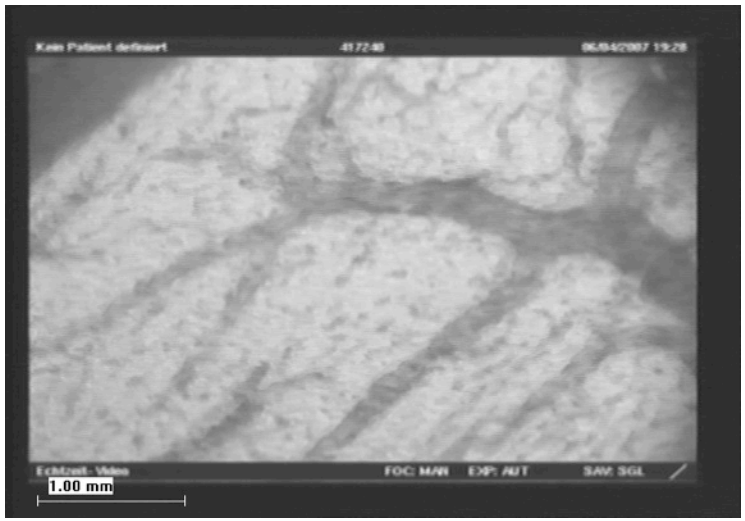
Orthogonal Polarization Spectral (OPS) imaging system

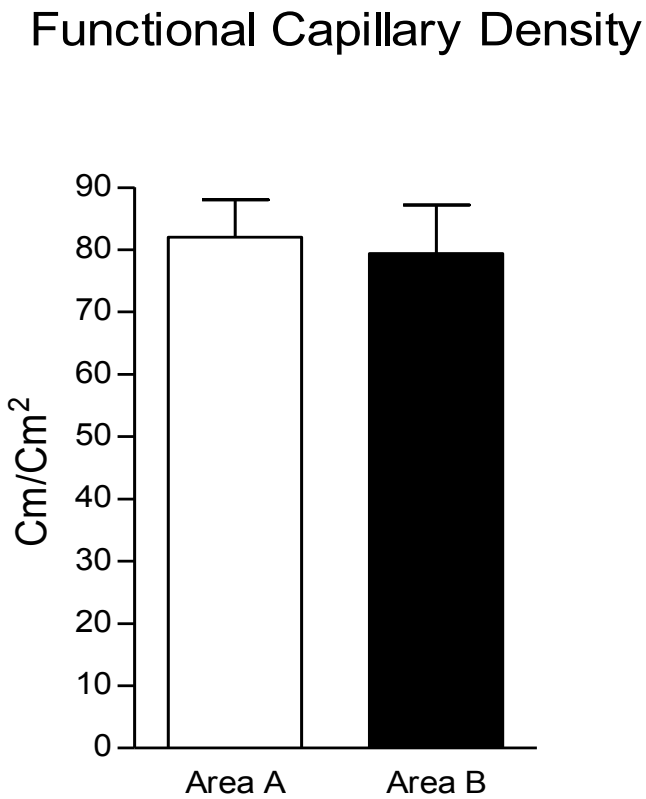
Capillary diameter

Capillary red blood cell velocity

Functional capillary density

Oxygent project





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

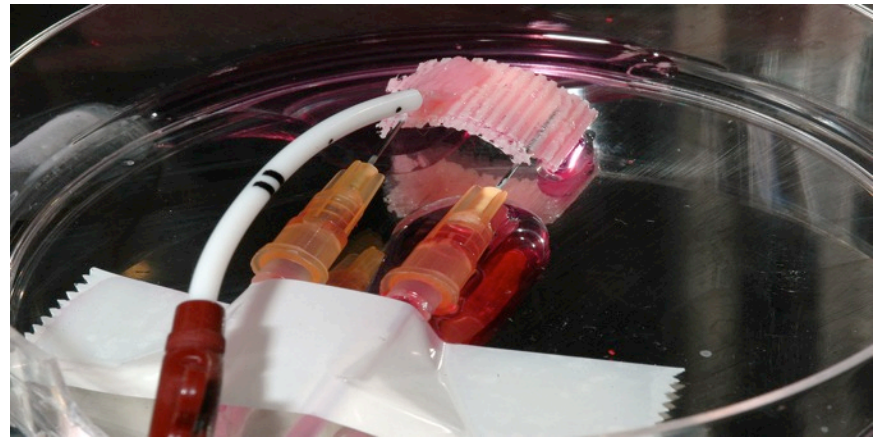
  measure capillary density around and inside the scaffold
- The grouping of concentration and combination of

  GFs following mathmatic optimization principle

  e.g. genetic arithmetic, orhtoganal design

OxygentTM project *Epithelial survival*

Study Design *PtO2 measurement*



Polarographic microprobe measures

tissue partial oxgen tension (PtO2)

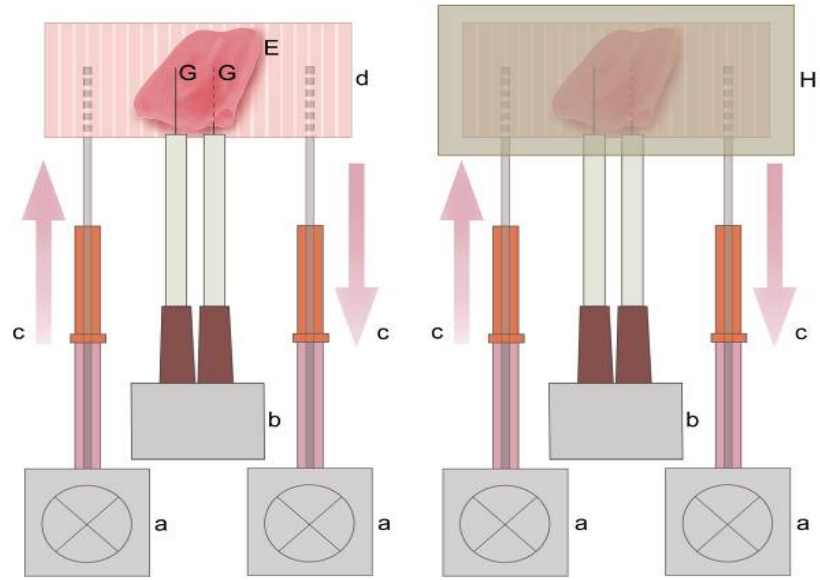
Two probes at different thickness of TE trecheal epithelium

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

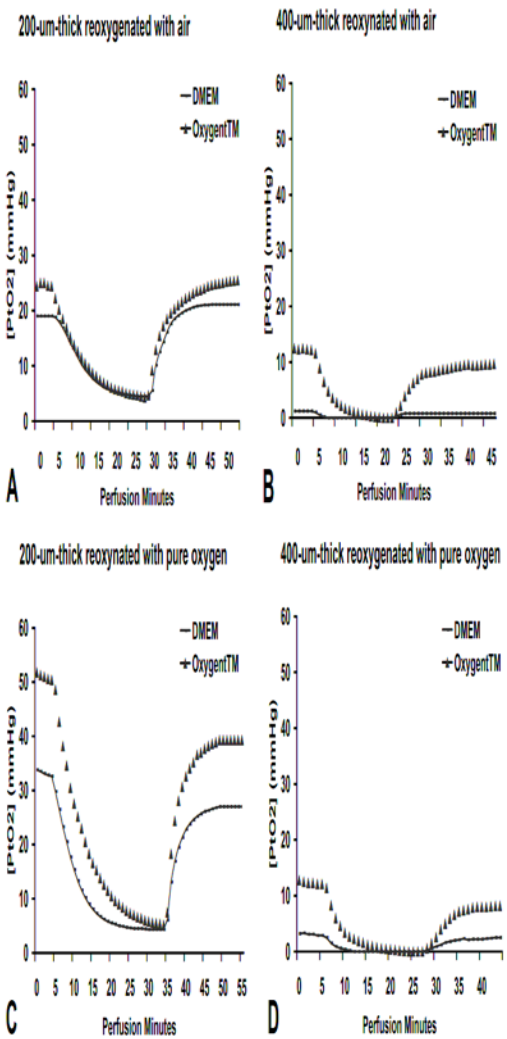


Fig. 02

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

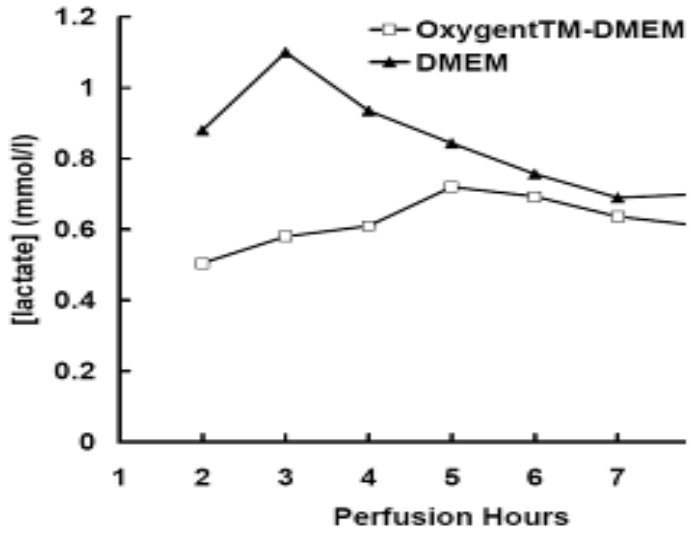
   Reoxygenation with pure oxygen

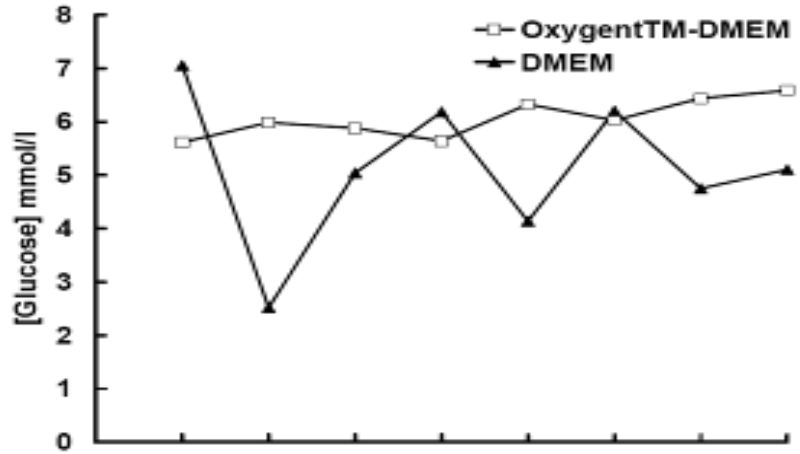
   73.79% more in the 200-μm-thick

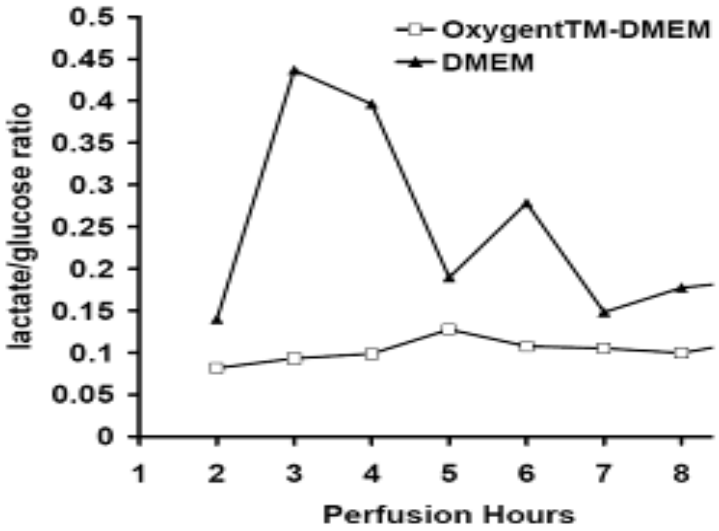
   607.22% more in the 400-μm-thick

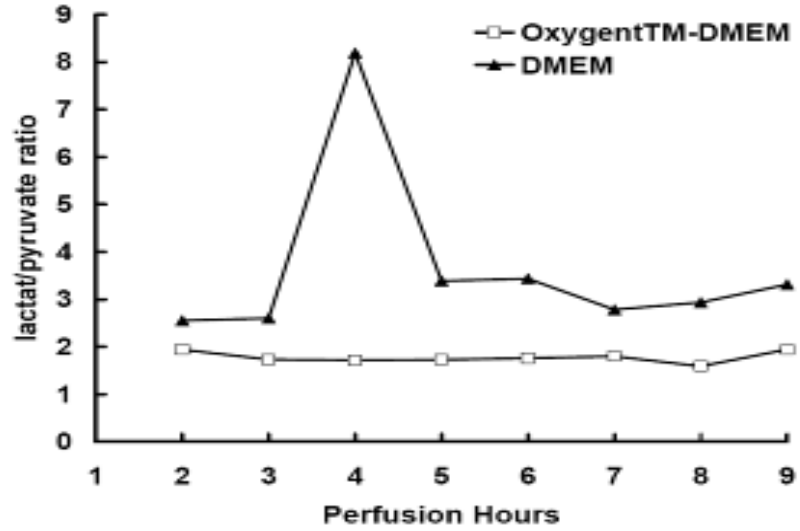
3. OxygentTM supplement can support around 200-um-thick epithelium

Tissue metabolite concentrations measured by microdialysis









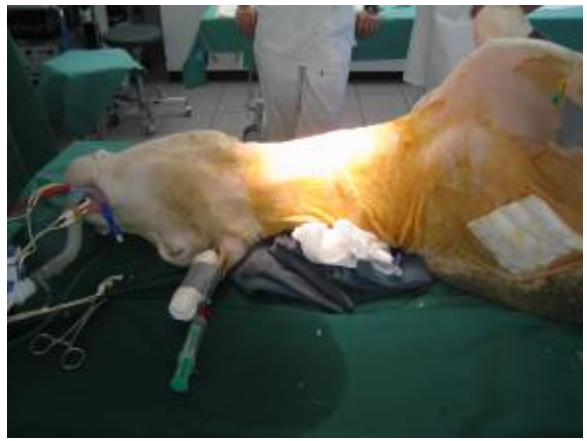
Conclusions

OxygentTM 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

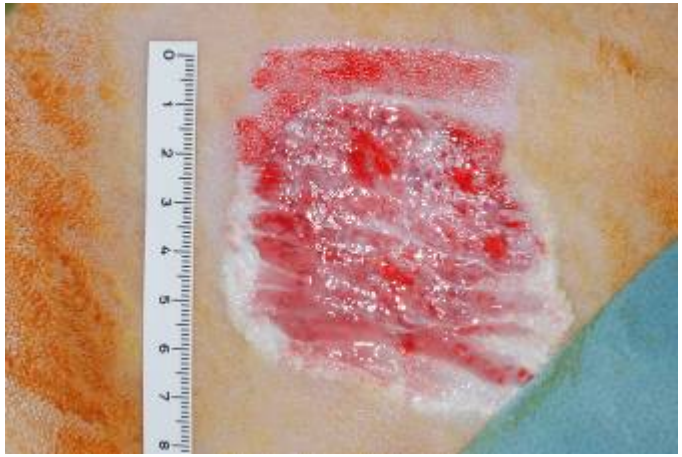
Clinical evaluation for three months

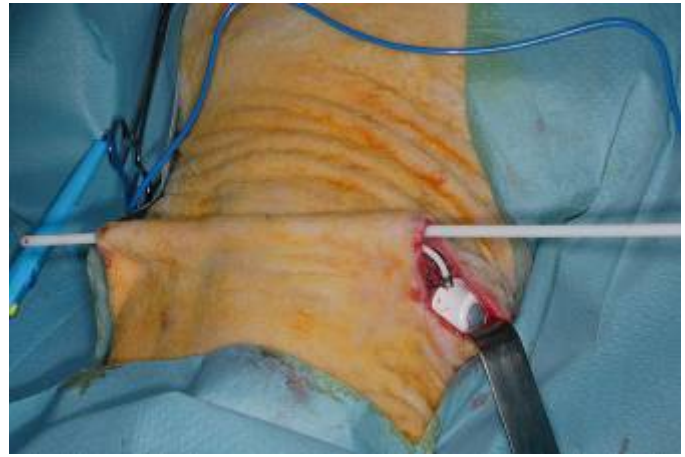
General situation checked everyday
Bronchoscopy every month

Hitological

Ulex Europaeus agglutinin (UEA) & Peanut Agglutinin (PNA) for trachea epithelial
endothelin-1 for vessel

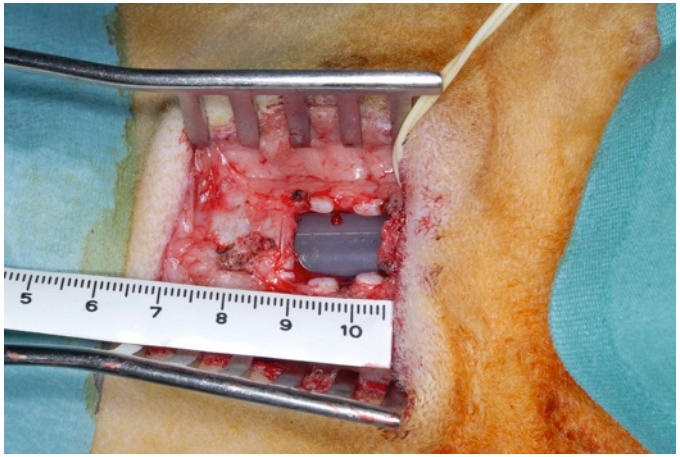
Operation

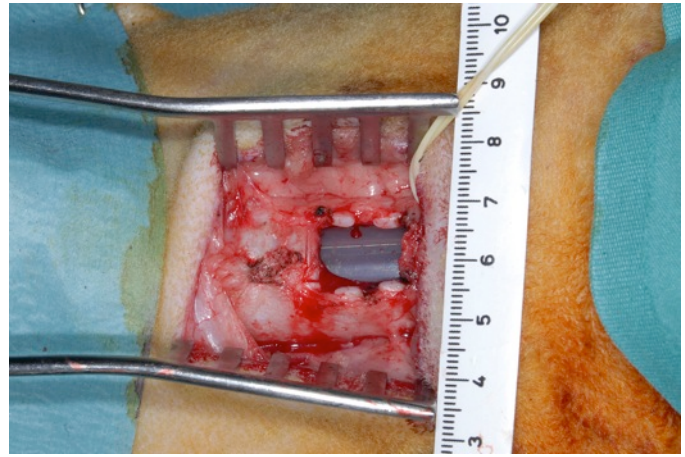




Split-thickness skin graft

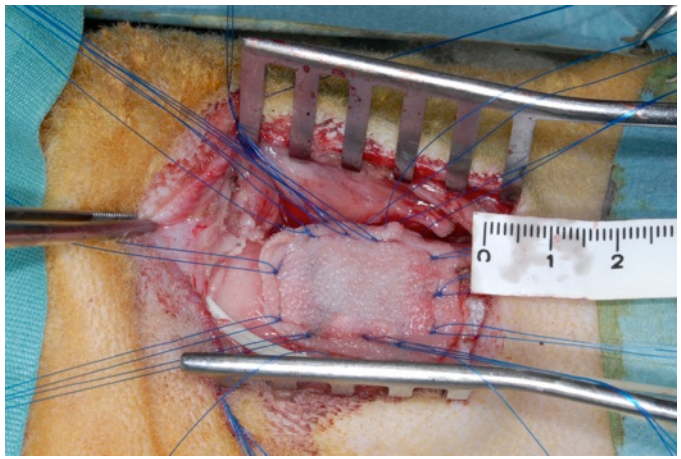
Port-A-Catch implantation

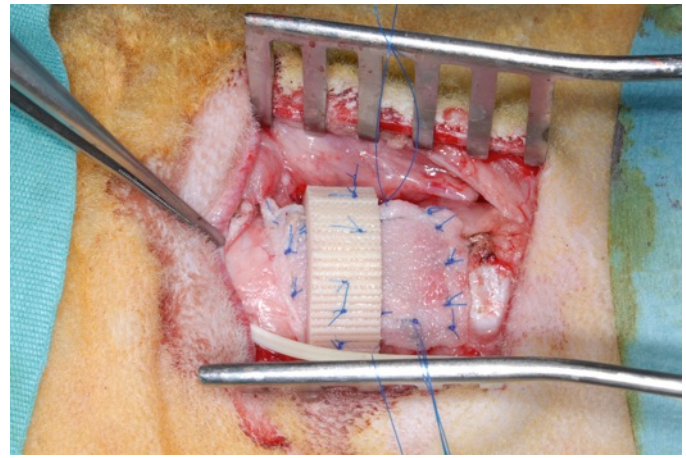




Trachea defect

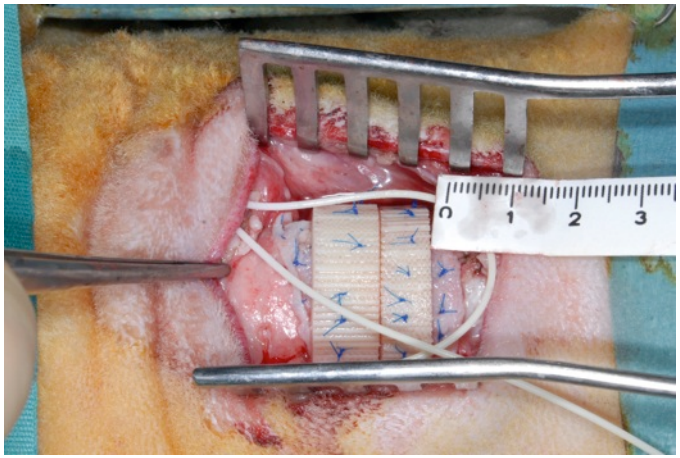
Operation

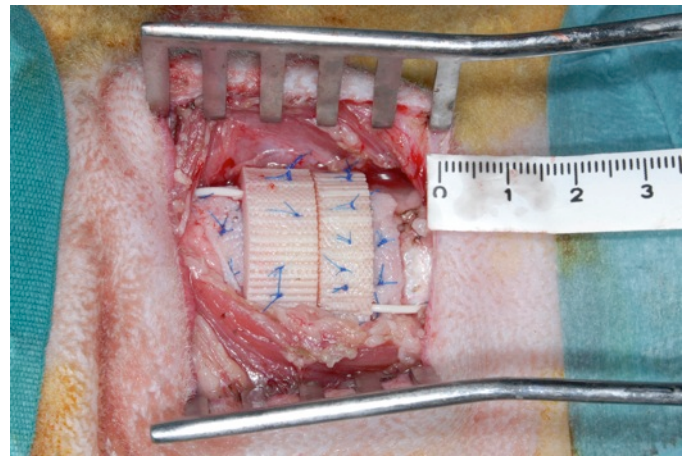




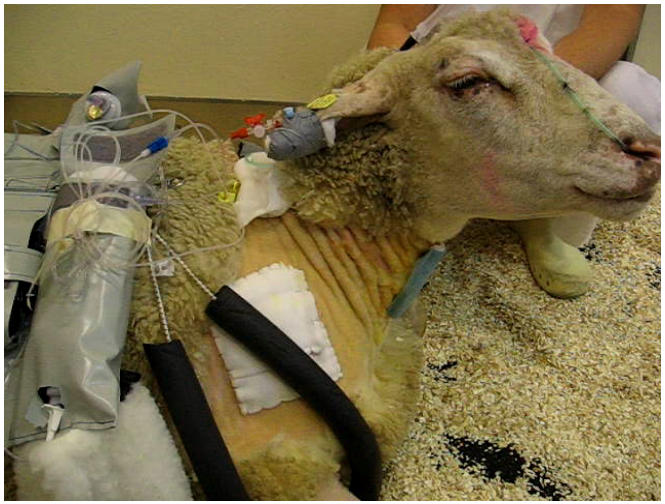
Repair with split-thickness skin graft

Scaffold implantation with catheter fixation



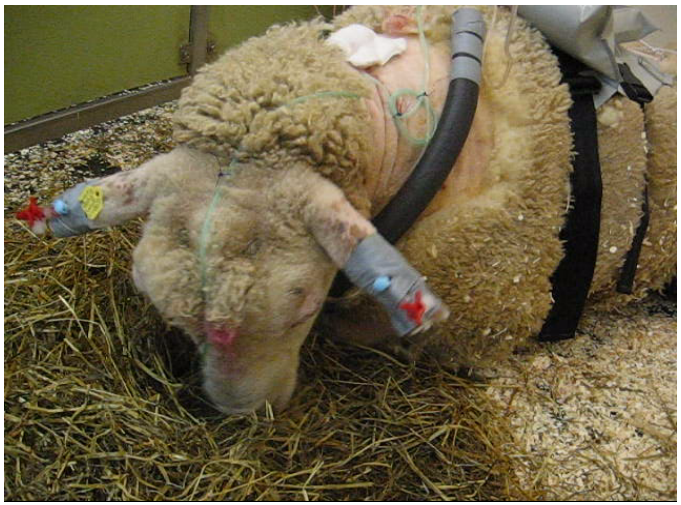


Result



One hour after operation

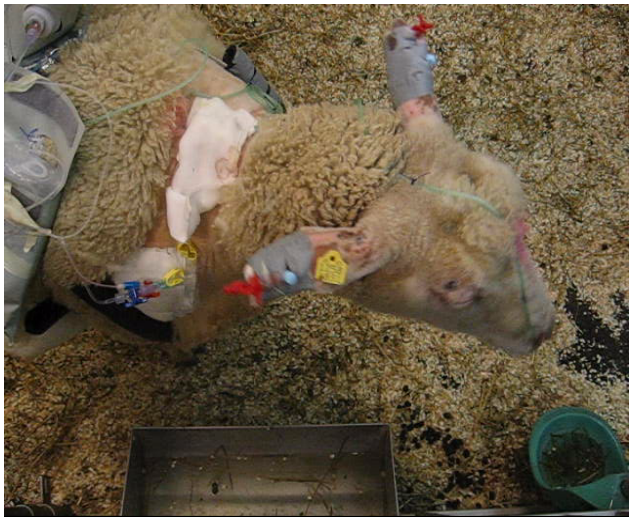
Resume from the anaesthesia
No dyspnea
No stridor



Three hours after operation

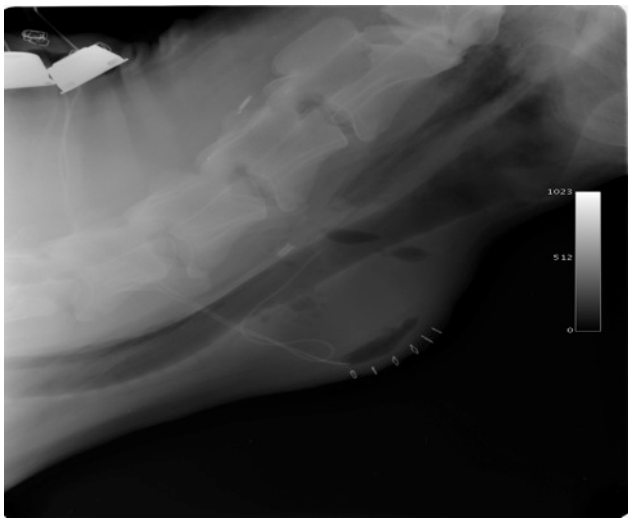
Normal food intake
No dyspnea
No stridor
Fluent perfusion
Blood inside the sucking tube

Results



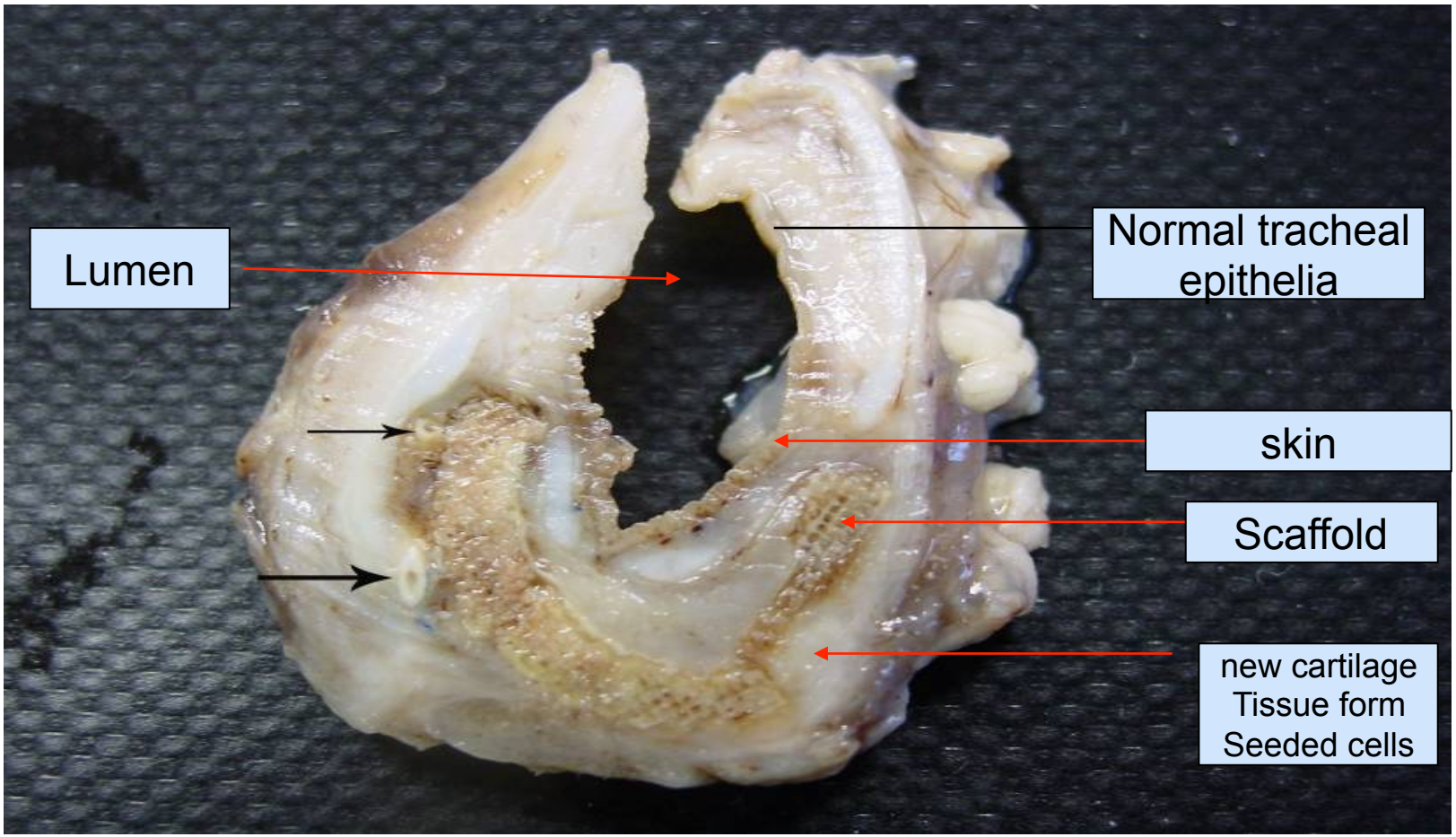
Next morning (20 hours after operaion)

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



Summary

"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!