FONDAZIONE POLIAMBULANZA
Istituto Ospedaliero

COMPUTATIONAL CHALLENGES in REGENERATIVE MEDICINE

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Talking in front of experts in computation…..

…….

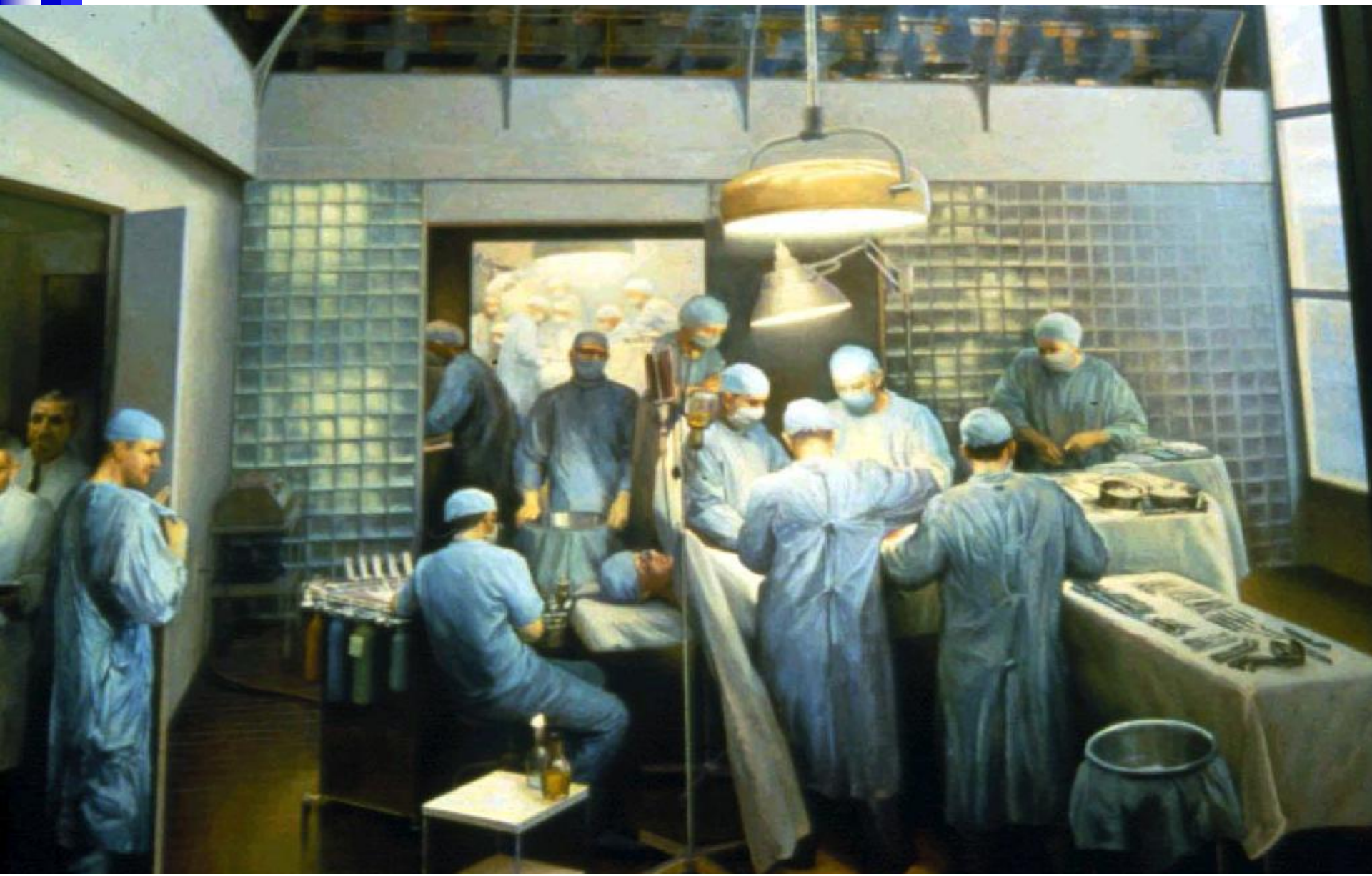
I believe I will find many collaborations for solving the problems of a biologist….

- Introduction on regenerative medicine tissue engineering and stem cells

And needs for computational applications

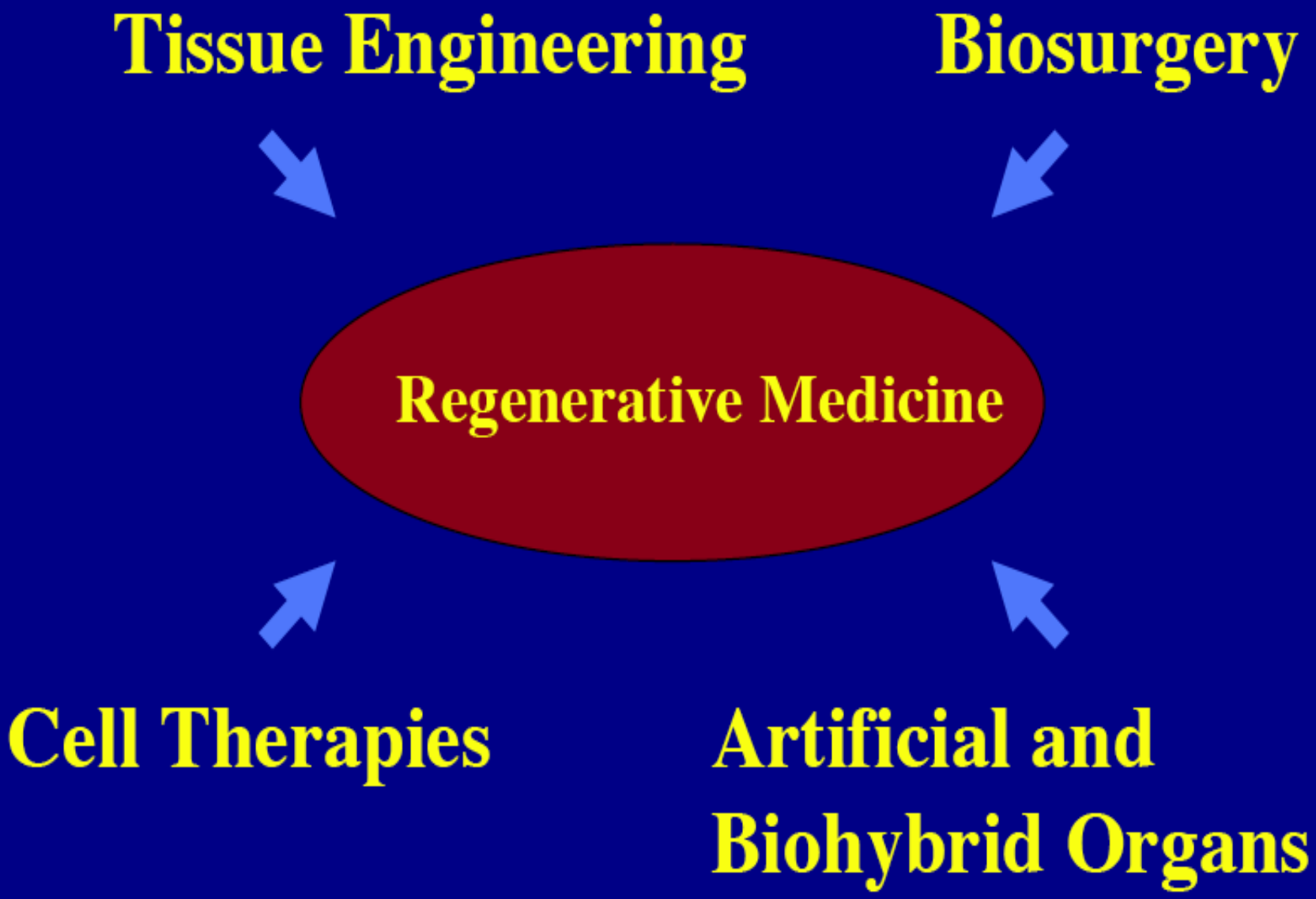
- Why an interest in cells derived from human term placenta?
- *In vitro* studies using placenta derived cells
- *In vivo* studies using placenta derived cells

1954: FIRST ORGAN TRANSPLANTATION



TODAY, Increasing problem:

Tissue and Organ shortage and rejection


Tissue Engineering
Biosurgery
Regenerative Medicine
Cell Therapies
Artificial and
Biohybrid Organs

The end goal:

- To create products that improve tissue function or heal tissue defects. Replace diseased or damaged tissue

- Because……

  - Donor tissues and organs are in short supply

  - We want to minimize immune system response by using our own cells or novel ways to protect transplant.

Regenerate, repair and replace

- Regenerate
  - Identify the cues that allow for regeneration, i.e. transplant cells that could differentiate
- Repair
  - Stimulate the tissue at a cell or molecular level, even at level of DNA, to repair itself.
- Replace
  - A biological substitute is created in the lab that can be implanted to replace the tissue or organ of interest

Cell-based therapies

Aimed at certain diseases

Uses mostly only cells and no materials

- Type I diabetes transplant of new pancreas cells
- Adult stem cells for heart disease
- Neuronal transplants for Parkinson's disease
- Bone marrow transplant for various blood cancers
- Muscular dystrophy

Tissue Engineering

Using well designed scaffolds and optimized cell growth, we can create tissues such as:

- Skin
- Bone
- Cartilage
- Intestine

These have been successfully engineered to some extent

More complex organs

Not very far in development

Complex metabolic functions

Require multiple types of cells and intricate scaffolds

- Liver
- Heart
- Lung
- Kidney

Tissue-engineered products contain mixtures of the following:

- Biological components--cells
  - Can be genetically modified to behave a specific way
- Chemicals
  - that tell the tissue to regenerate
- A non-biological component
  - Polymer scaffold
    - Fibers, plastic, other natural components
  - Gels

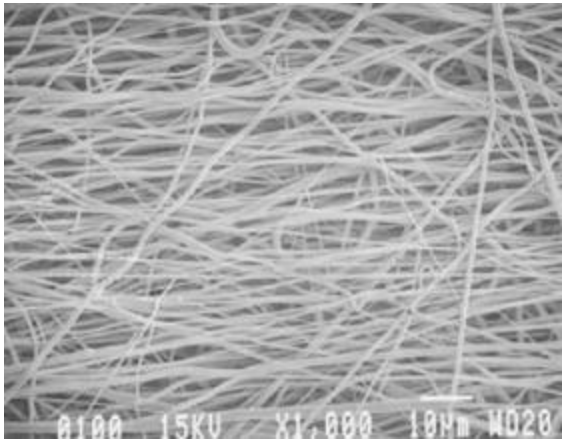
Scaffolds

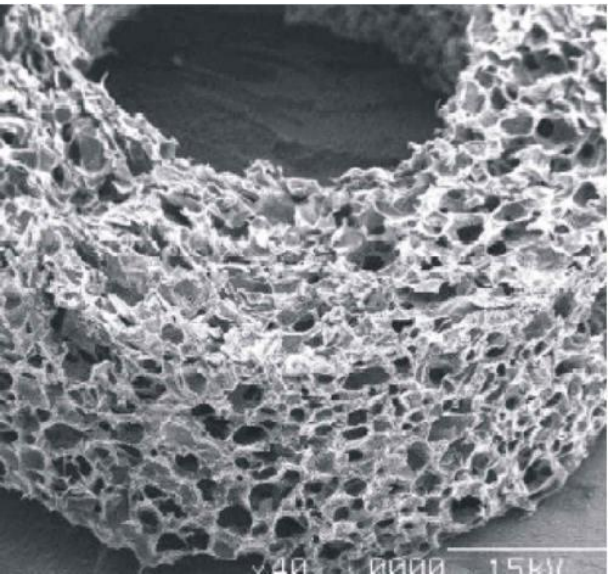
Various textures and materials

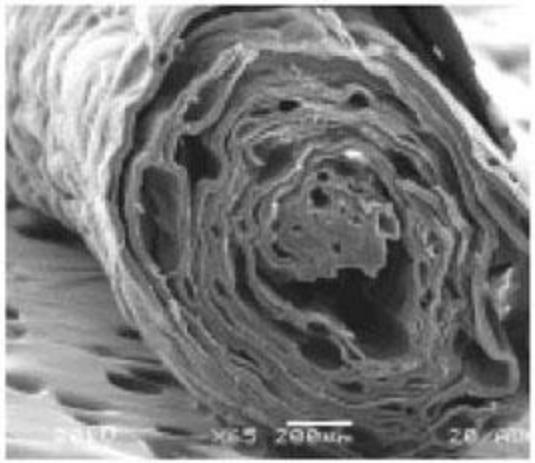
Encourage cells to grow

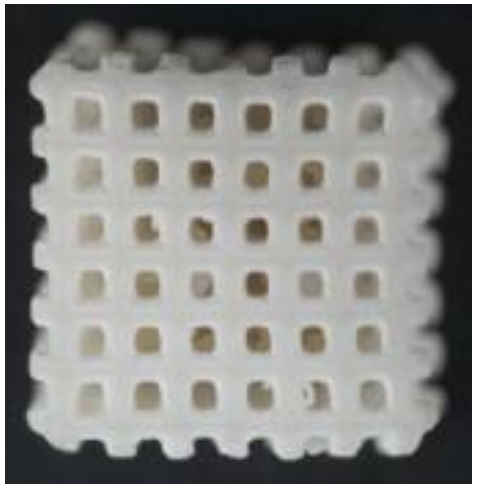
Allow nutrients to permeate

Won’t harm the patient





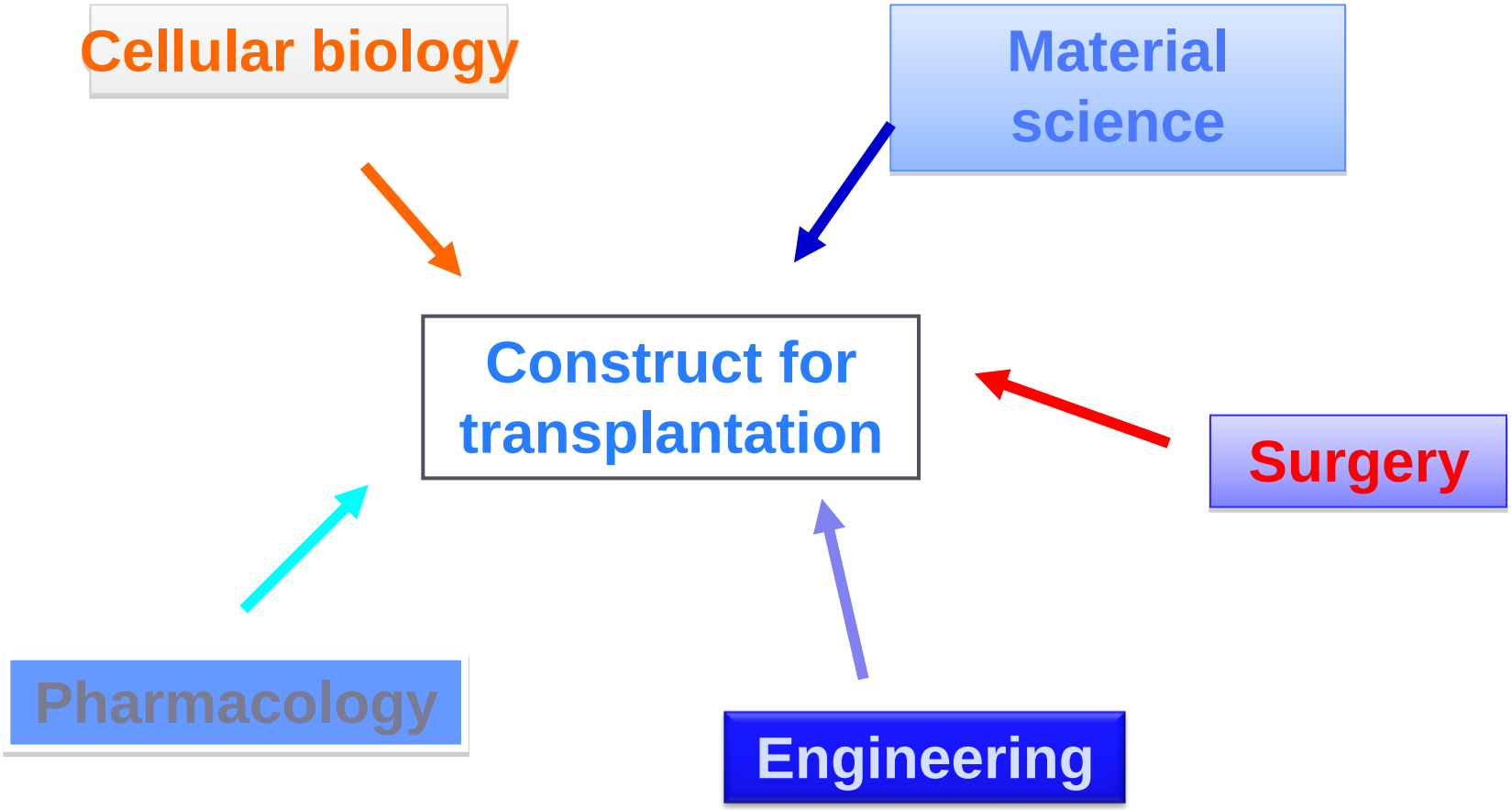




Transplants that match the patient

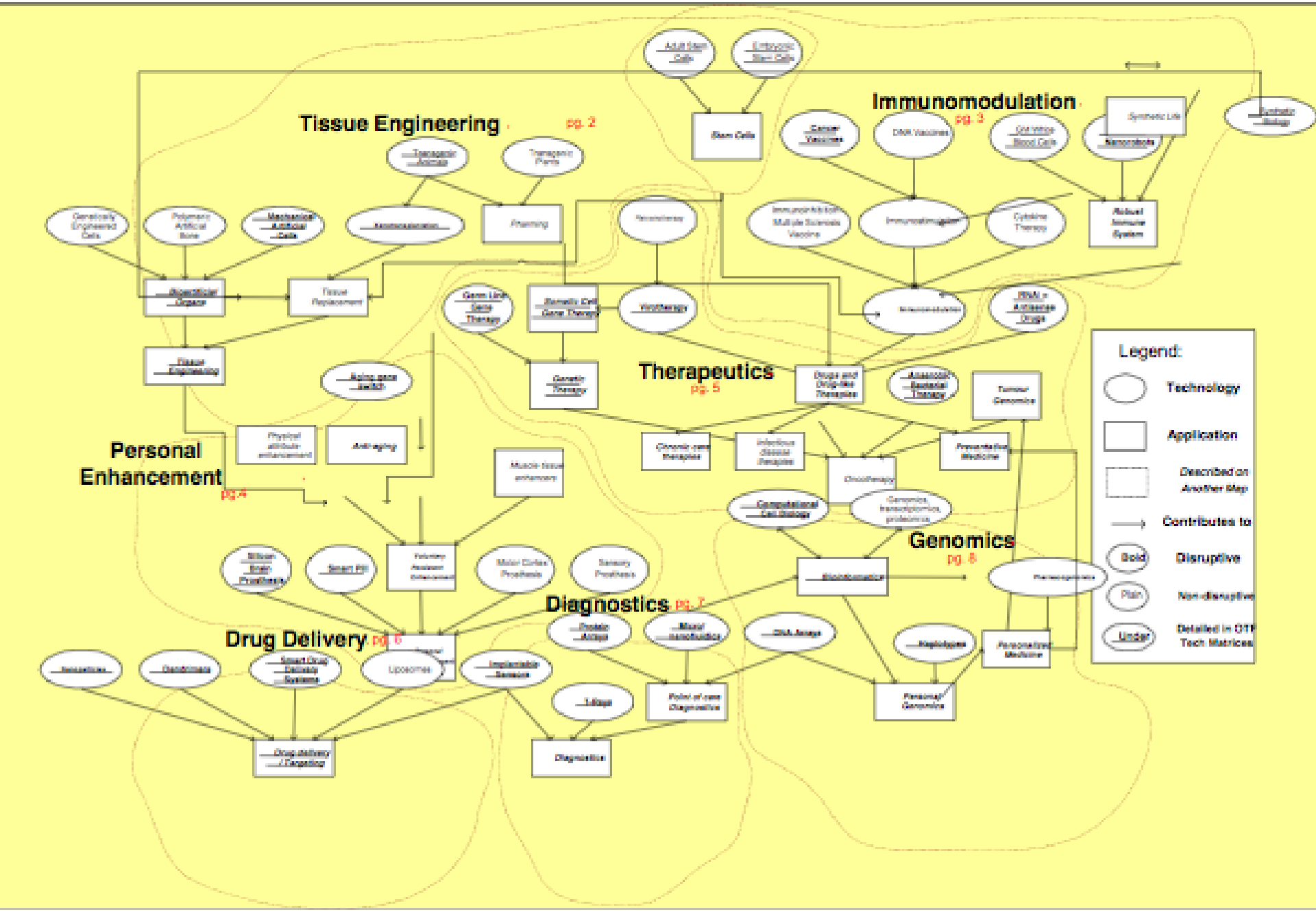
- Isolate cells from patient
- Identify a matched compatible donor
- Grow in culture with or without biomaterials
- Give appropriate “factors” to make cells do what is needed
- Replace into patient

Multidisciplinary Nature of Tissue Engineering/Regenerative Medicine



COMPUTATION CHALLENGES

So which are the challenges:…


Tissue Engineering
pg. 2
Immunomodulation
pg. 3
Therapeutics
pg. 5
Personal Enhancement
pg. 4
Genomics
pg. 8
Diagnostics
pg. 7
Drug Delivery
pg. 6
Adult Stem Cells
Embryonic Stem Cells
Stem Cells
Cancer Vaccines
DNA Vaccines
Cell Therapy
Synthetic Life
Nanomedicine
Synthetic Biology
Transgenic Animals
Transgenic Plants
Genetically Engineered Cells
Polymeric Artificial Bone
Mechanical Actuator Cells
Xenotransplantation
Pharming
Neurosciences
Immunosuppression
Cytokine Therapy
Natural Immune System
Bioartificial Organs
Tissue Replacement
Germ Line Gene Therapy
Somatic Cell Gene Therapy
Virotherapy
Tissue Engineering
Genetic Therapy
Drugs and Drug-like Therapies
Advanced Bacterial Therapy
Tumor Genomics
Chronic care therapies
Infectious disease therapies
Preventative Medicine
Oncotherapy
Computational Cell Biology
Genomics, transcriptomics, proteomics
Bioinformatics
Pharmacogenomics
Personalized Medicine
Personal Genomics
Point-of-care Diagnostics
Diagnostics
Drug delivery / Targeting
Liposomes
Implantable Sensors
Protein Arrays
Mixed nanofluidics
DNA Arrays
Haplotypes
Aging gene switch
Physical attribute enhancement
Anti-aging
Muscle-tissue enhancers
Silicon Brain Prosthesis
Smart Pill
Voluntary Movement Enhancement
Motor Cortex Prosthesis
Sensory Prosthesis
Nanotechnology
Drug Delivery Systems
Legend:
Technology
Application
Described on Another Map
Contributes to
Bold
Disruptive
Plain
Non-disruptive
Under
Detailed in OTF Tech Matrices

Need to:

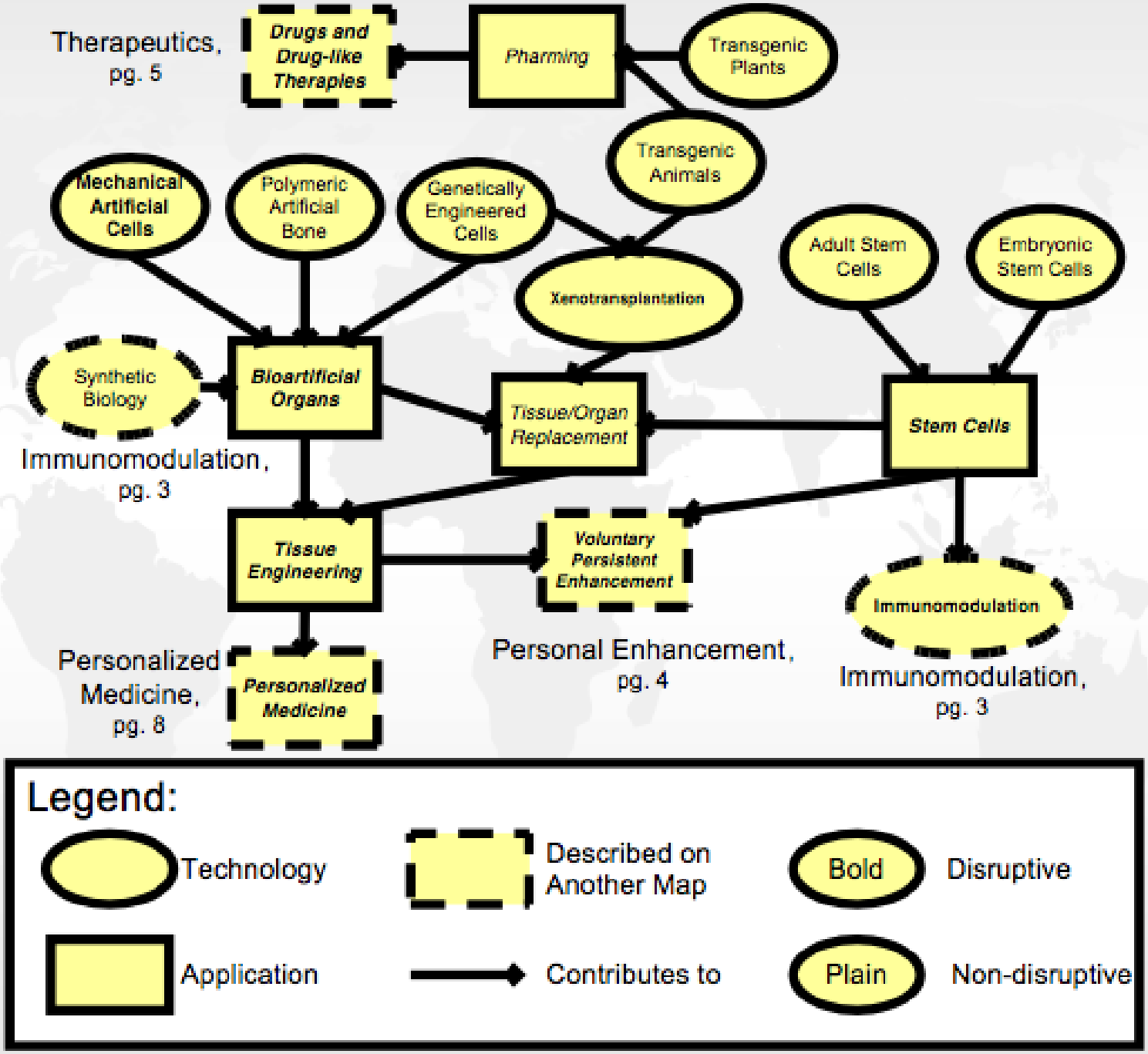
-monitor the course of experimental procedures;

-gather, smooth, and record data and signals;

-provide an effective medium though which data can be analyzed, visualized, communicated, and disseminated widely by means of databases connected to electronic networks.

Digital procedures that will somehow **monitor**, **collate**, and **manage** the explosion of online databases across genomics, proteomics, organisms, cell lines, and tissue projects, allowing researchers **to identify and extract data essential to targeted needs**.

A related challenge is the need for data mining procedures **able to "drill down" into the layers of catalogued information and extract key discoveries** otherwise buried among terabytes of compiled results.


Therapeutics, pg. 5
Drugs and Drug-like Therapies
Pharming
Transgenic Plants
Transgenic Animals
Mechanical Artificial Cells
Polymeric Artificial Bone
Genetically Engineered Cells
Xenotransplantation
Adult Stem Cells
Embryonic Stem Cells
Synthetic Biology
Bioartificial Organs
Tissue/Organ Replacement
Stem Cells
Immunomodulation, pg. 3
Tissue Engineering
Voluntary Persistent Enhancement
Immunomodulation
Personalized Medicine, pg. 8
Personalized Medicine
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Immunomodulation, pg. 3
Legend:
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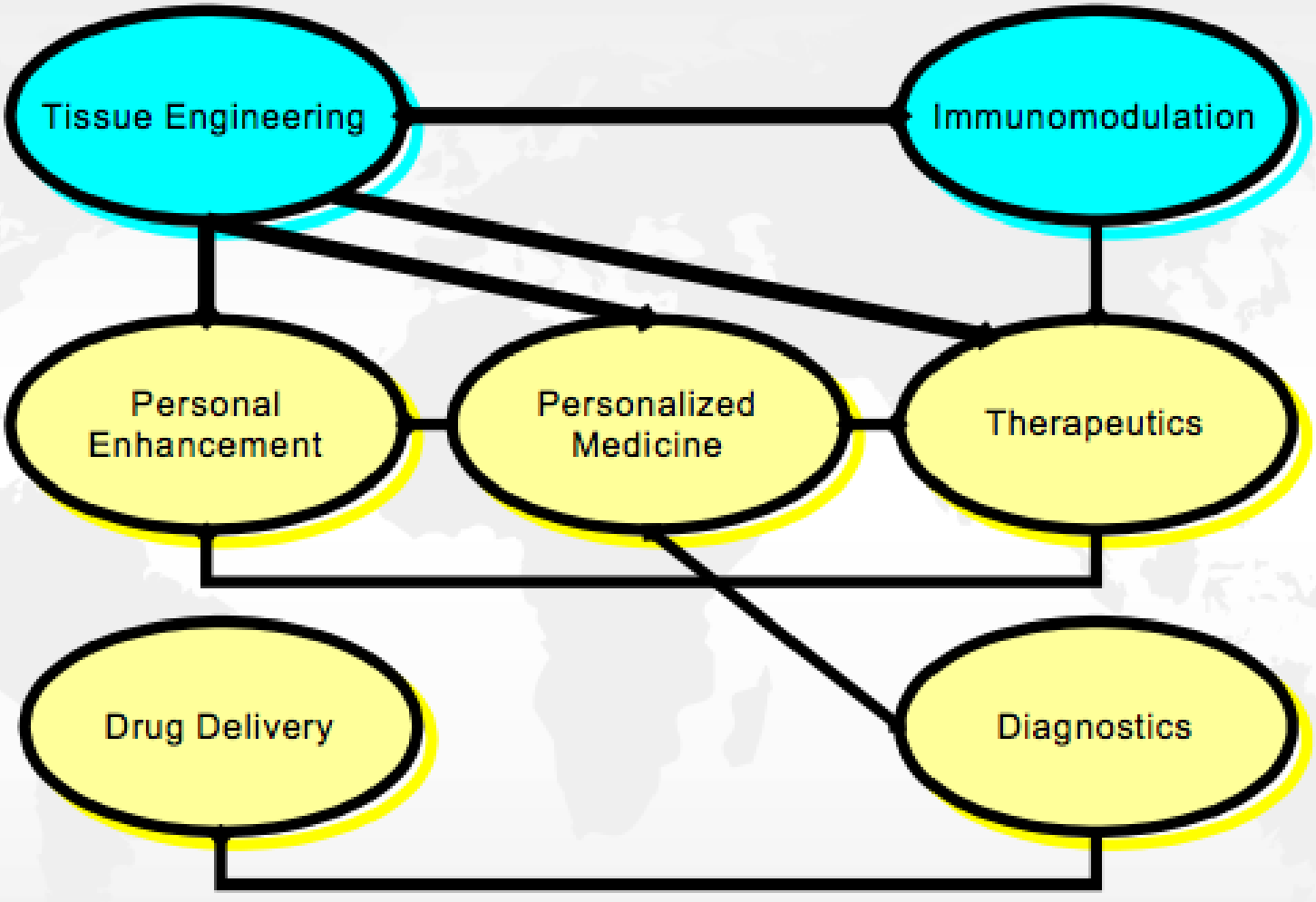
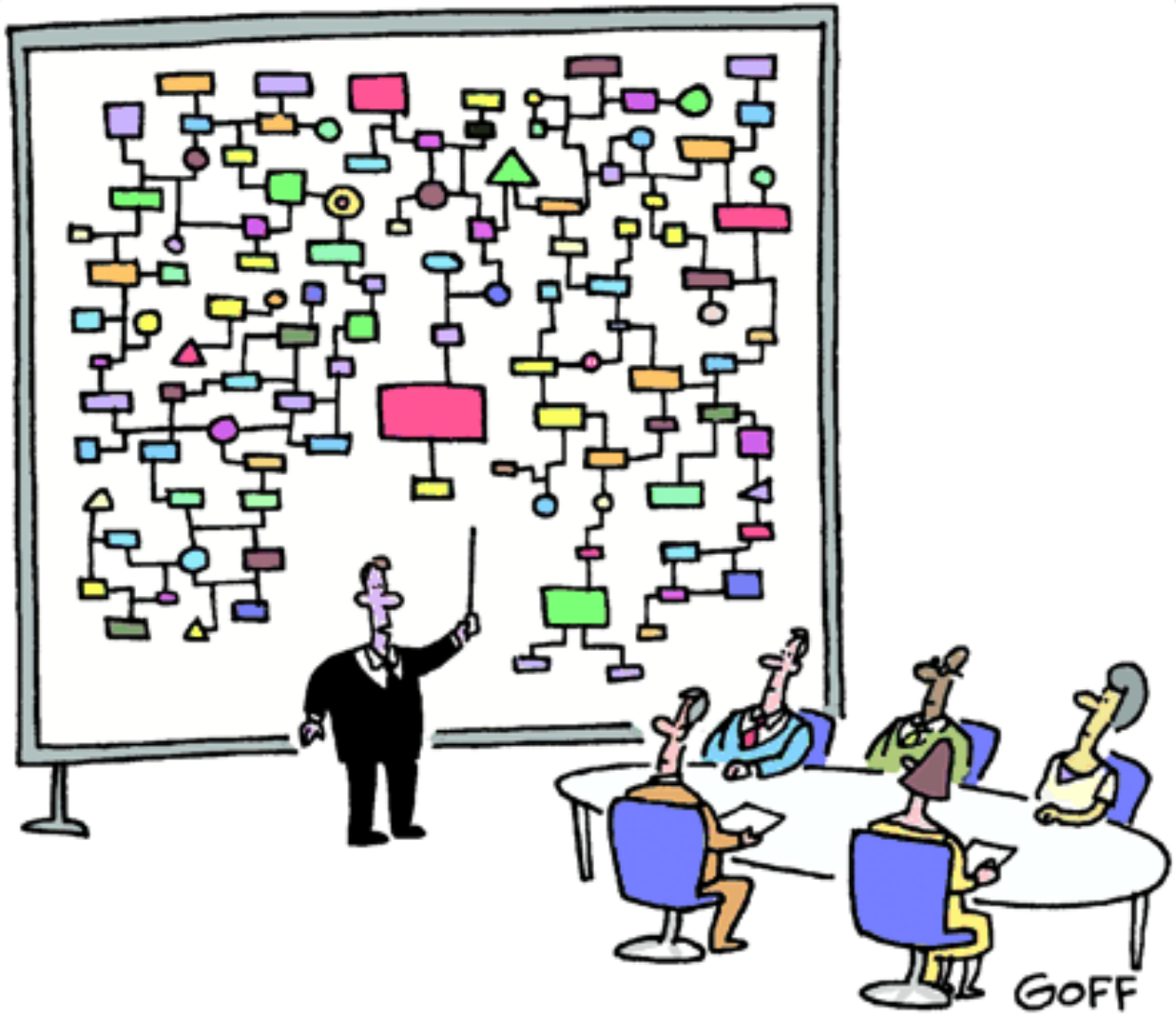

Tissue Engineering
Immunomodulation
Personal Enhancement
Personalized Medicine
Therapeutics
Drug Delivery
Diagnostics

Biostatistics and bioinformatics



"And that's why we need a computer."

Computational Modeling

- Structural and functional modeling of biological processes

- Computational and experimental frameworks for real-time mapping of biological processes
  - i.e. In tissue engineering the ability to apply accurate modelling and new cell simulation techniques can provide information and answer key questions regarding cell, tissue, and ultimately organ behavior.

Cell biology

- Visualization of cells (Flow Cytometry, **image analysis)**
- Analysis of cells and tissue (follow cell cyle, cell divisions, etc..)

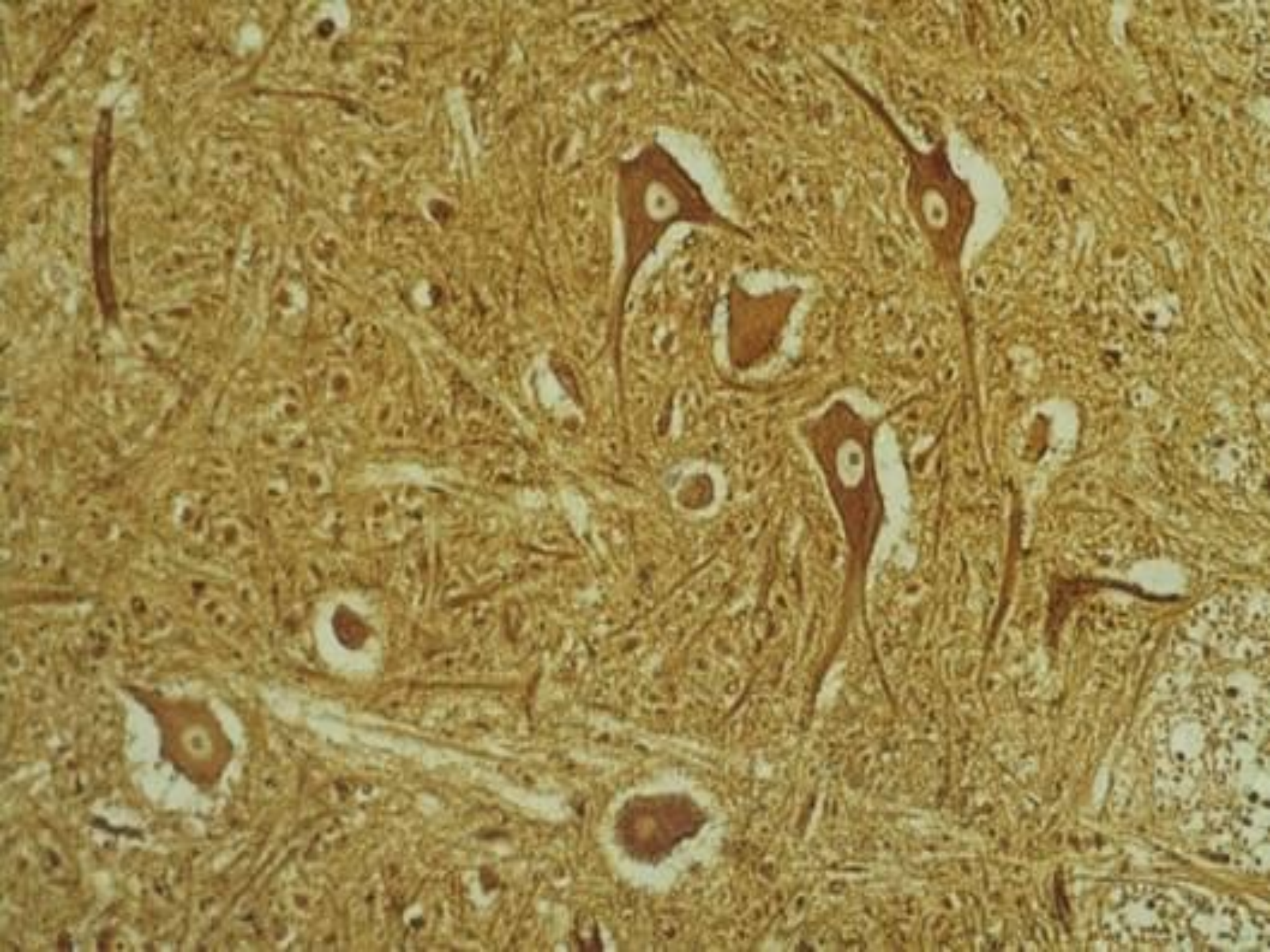
Molecular biology

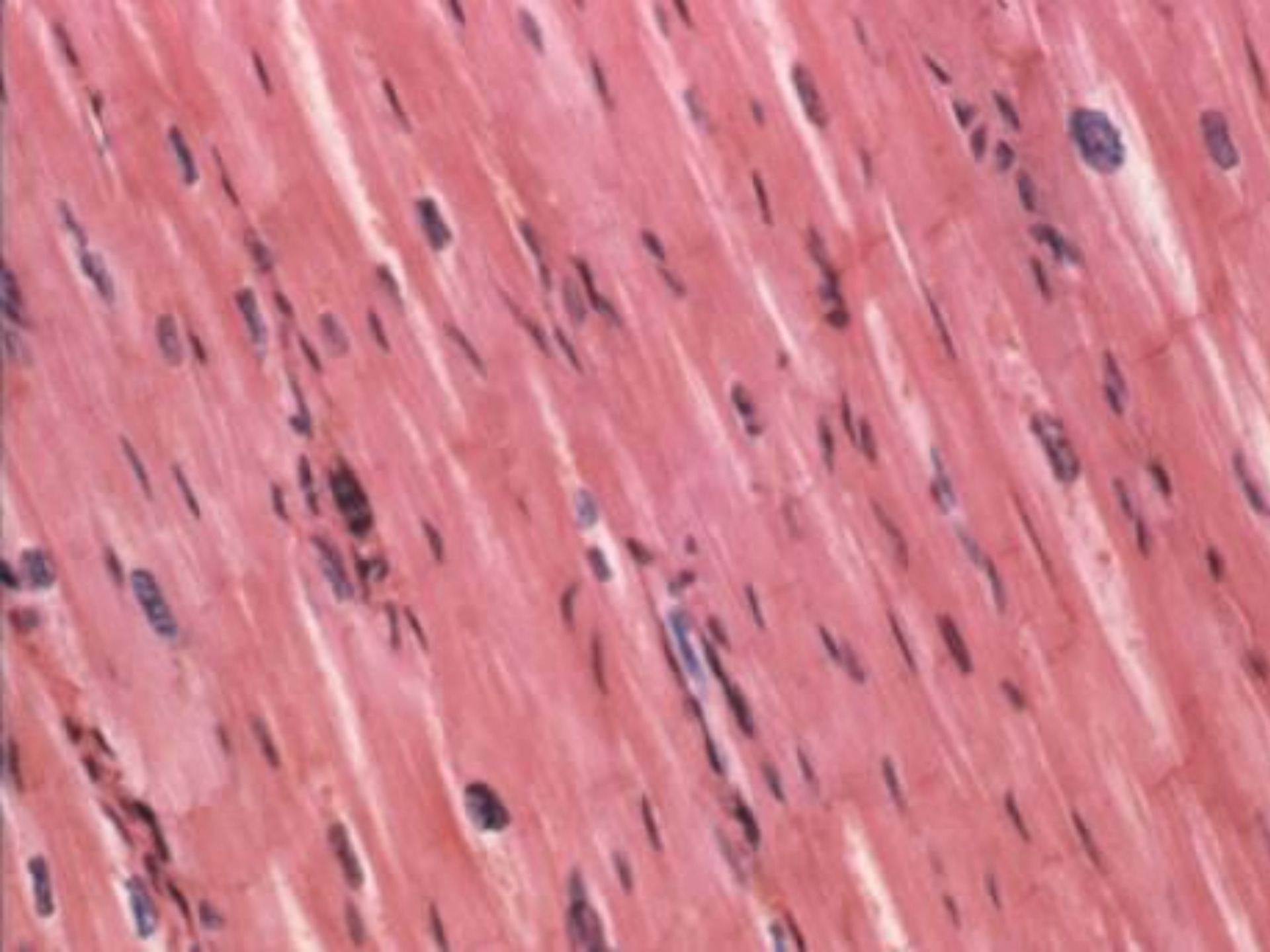
- Gene expression analysis (DNA microarray)
- Protein expression

Biochemical analysis

- Signalling Pathways

Let me tell something about stem cells…..



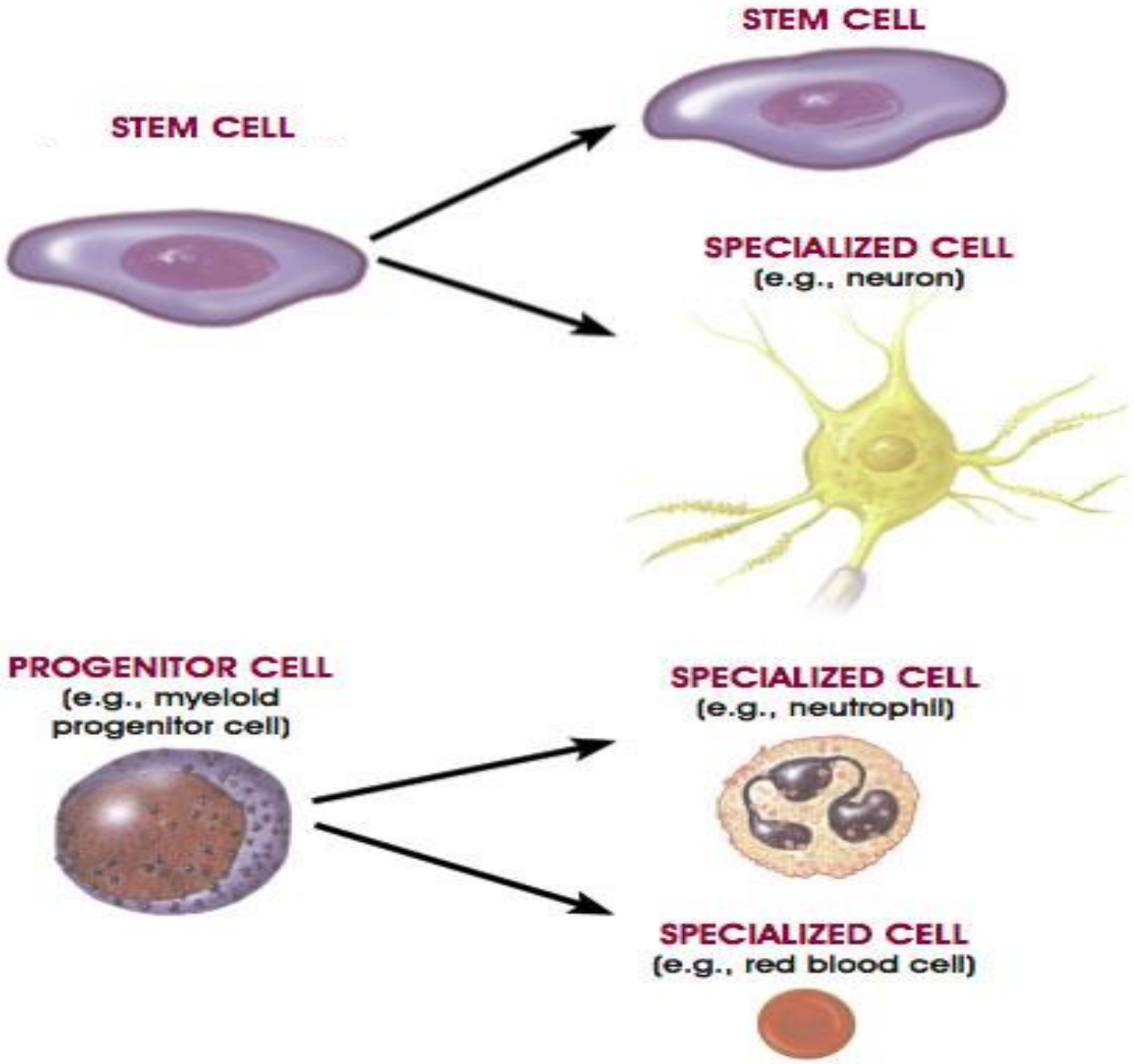


HOW IS CELL HOMEOSTASIS MAINTAINED?

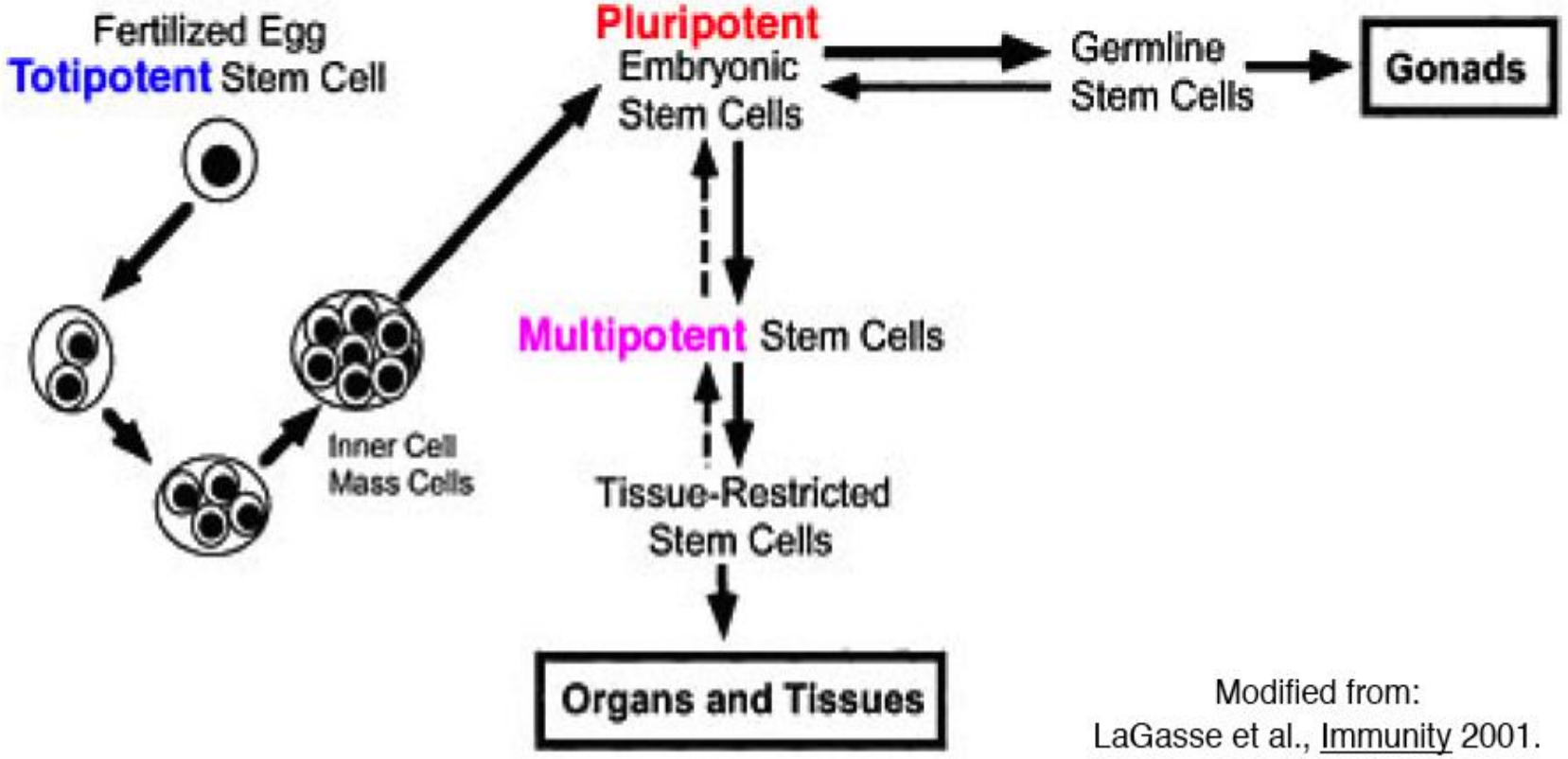
STEM CELLS WITHIN THE DIFFERENT TISSUES

WHAT ARE THE UNIQUE PROPERTIES OF ALL STEM CELLS

- Stem cells differ from other kinds of cells in the body.
- All stem cells—regardless of their source—have three general properties:
  - they are capable of dividing and renewing themselves for long periods;
  - they are unspecialized;
  - they can give rise to specialized cell types.


STEM CELL
STEM CELL
SPECIALIZED CELL
(e.g., neuron)
PROGENITOR CELL
(e.g., myeloid
progenitor cell)
SPECIALIZED CELL
(e.g., neutrophil)
SPECIALIZED CELL
(e.g., red blood cell)

"Potency" of Stem Cells

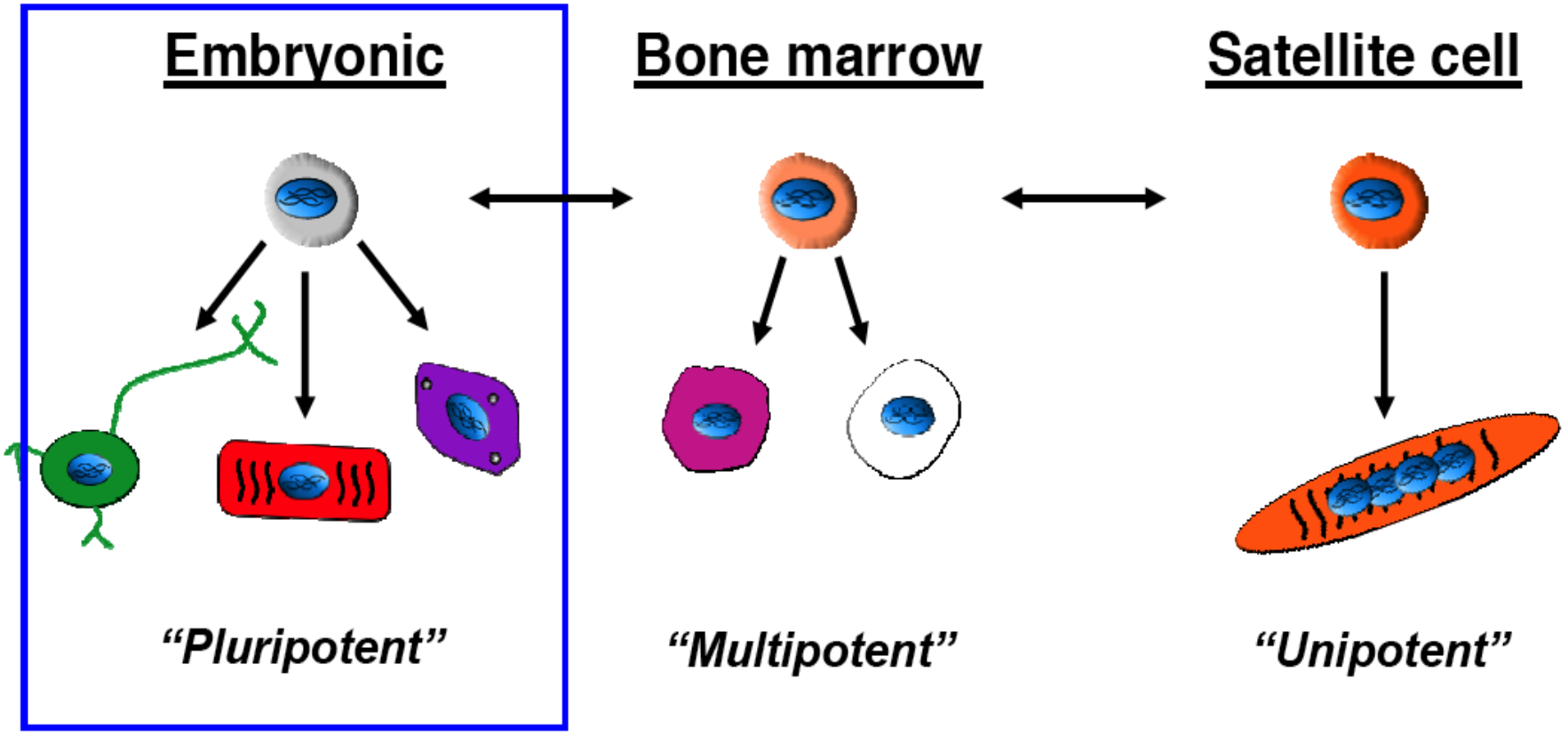


Modified from:
LaGasse et al., Immunity 2001.

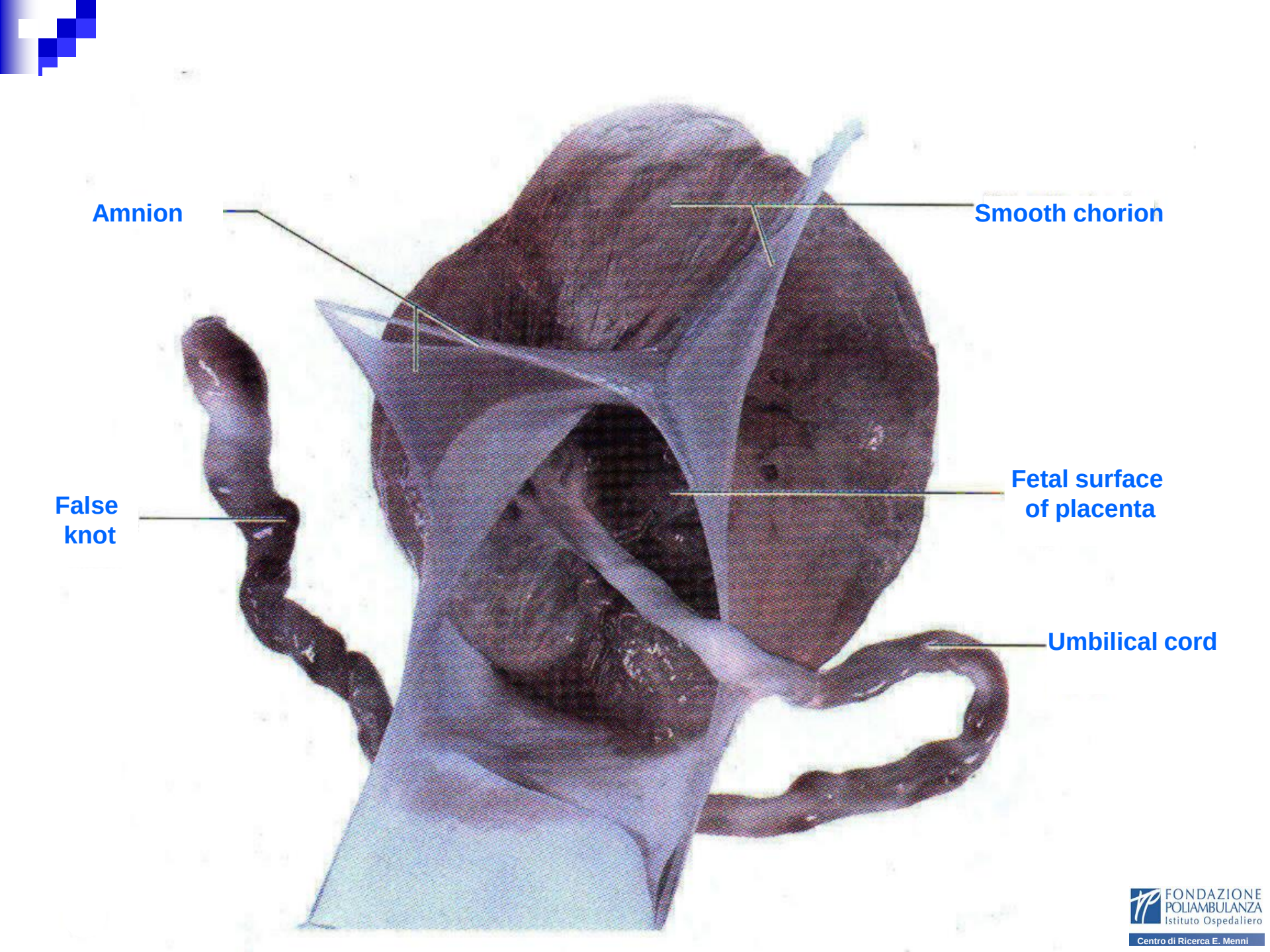
- Totipotent – all cell/tissue types
- Pluripotent – embryonic & adult cells
- Multipotent – multiple cell types

Potential

Stem Cell Potency



"Potency" affects applications & ease of manipulation

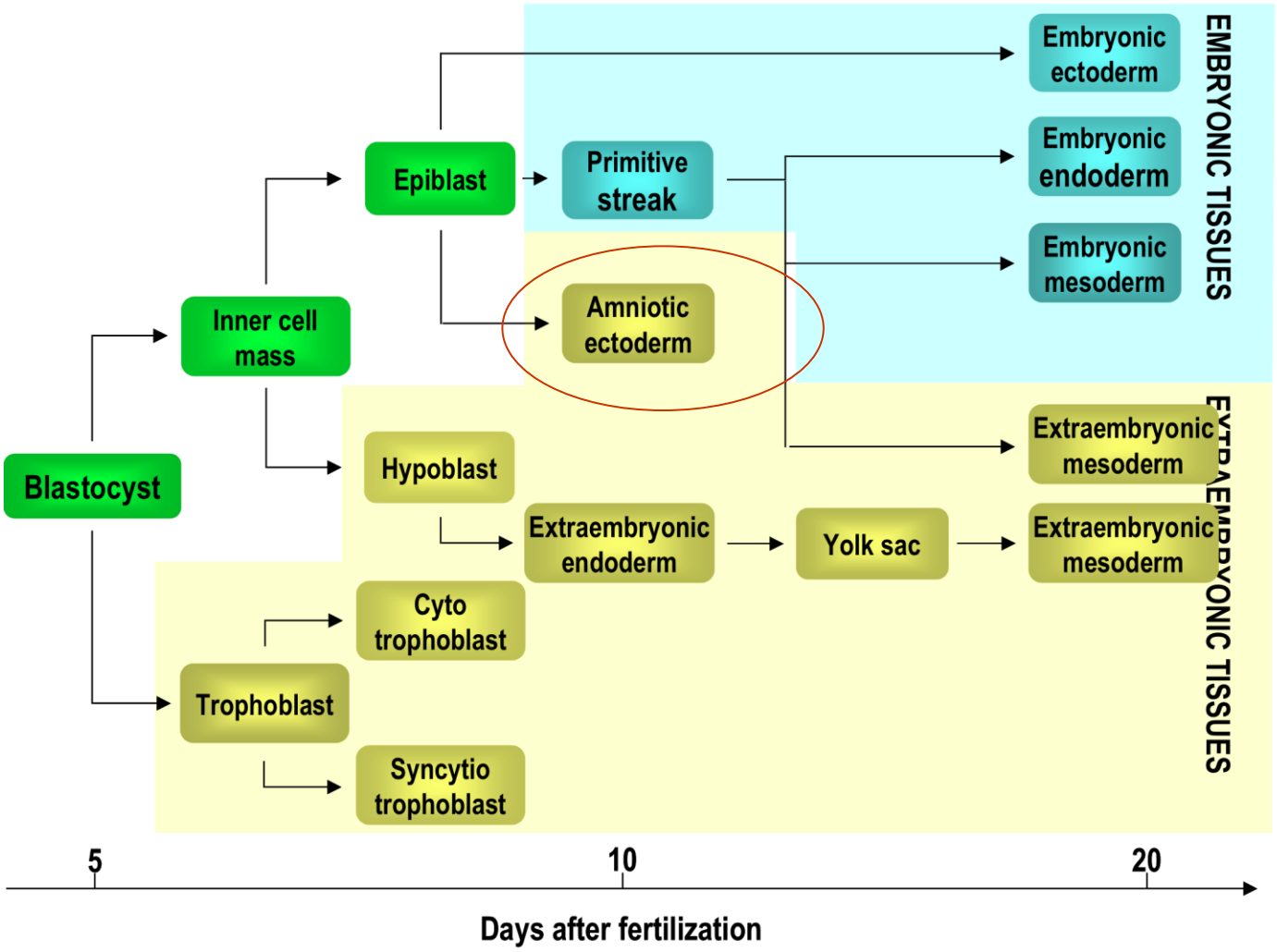


Why an interest in human placenta?

- **Identify stem cells for cell therapy approaches:**
  - **Stem cell potential**
  - **No transplant rejection**

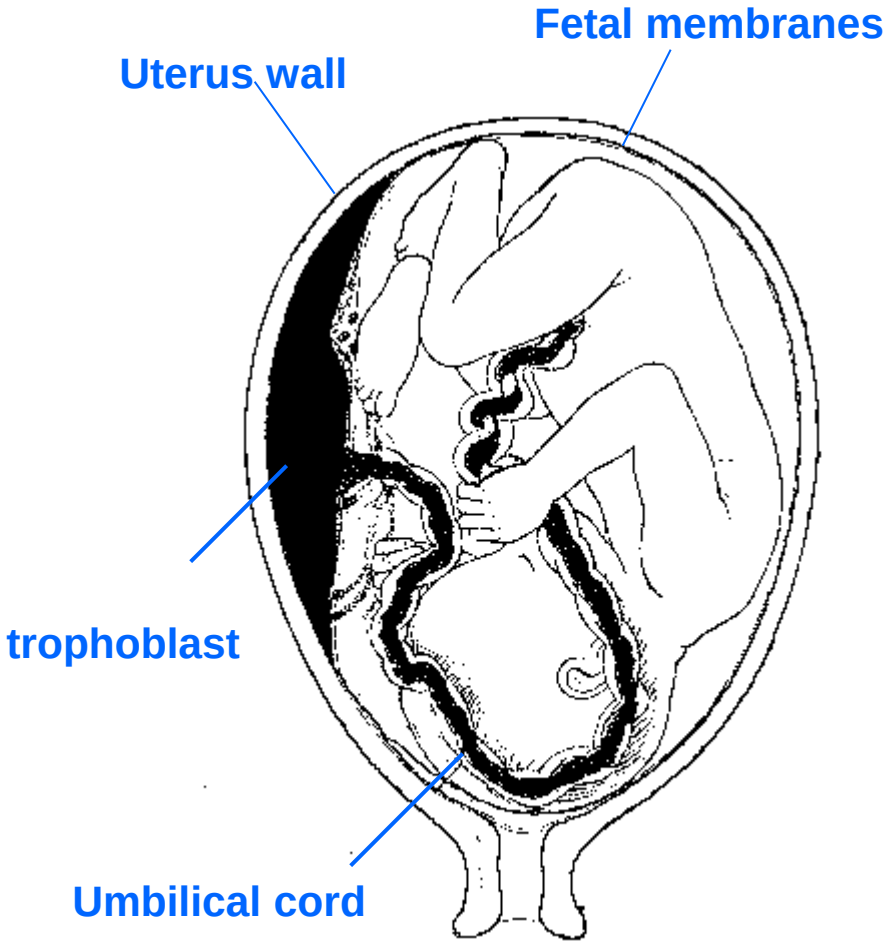
- **Placenta may combine these two essential features on the basis of:**
  - **Embryological origin**
  - **Immunological characteristics**

Embryological Origin



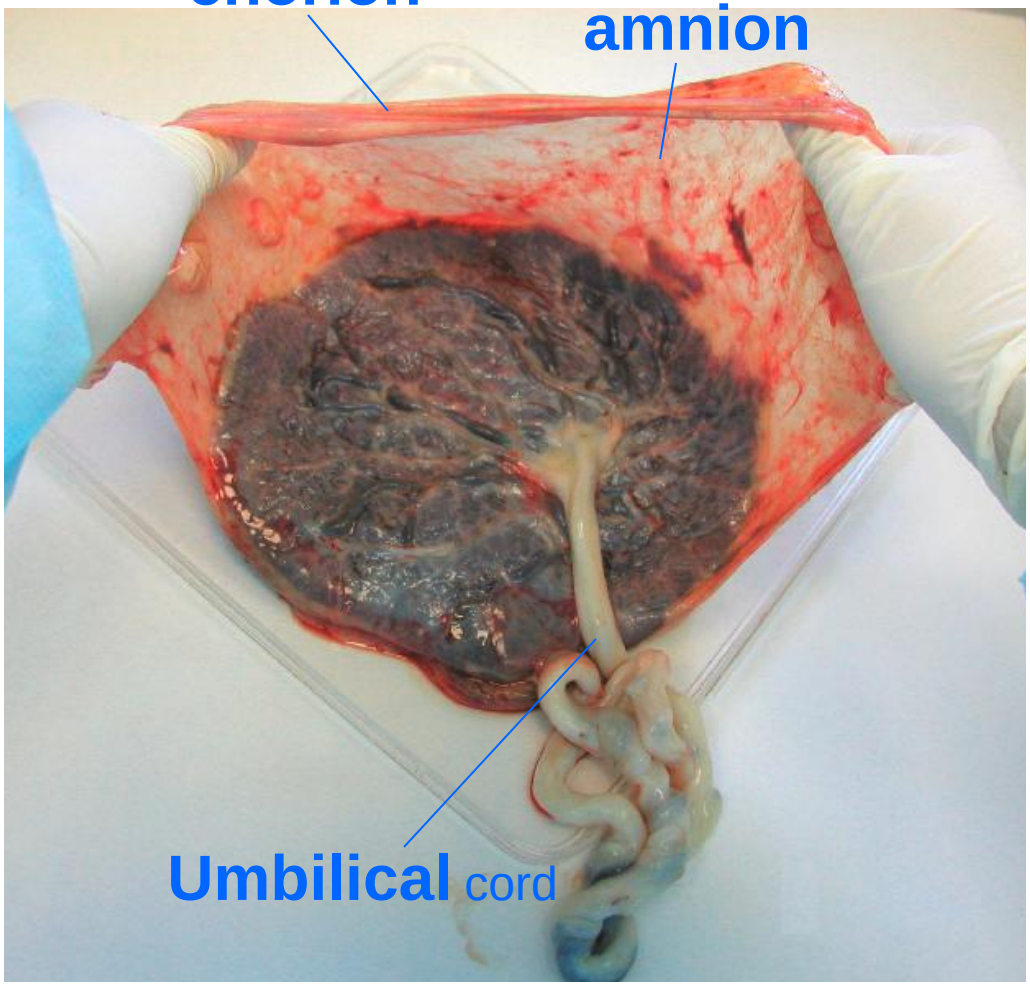
FETAL MATERNAL TOLERANCE:

Pregnancy is a unique event in which a genetically and immunologically foreign fetus survives to full term without rejection by the mother's immune system.

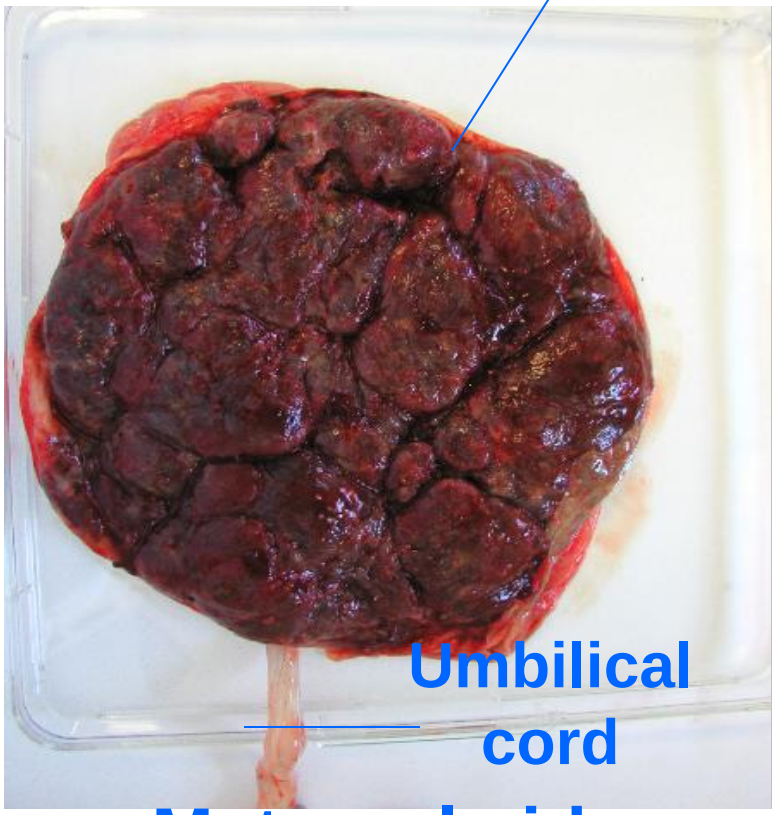


In vitro studies

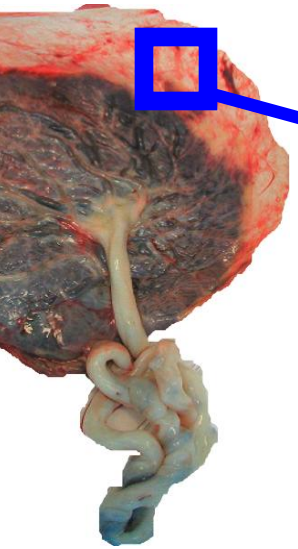
Amniotic derived cells isolation



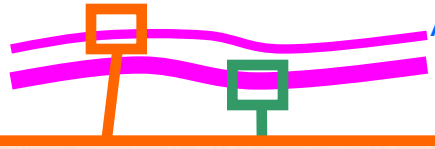
Fetal side



Maternal side

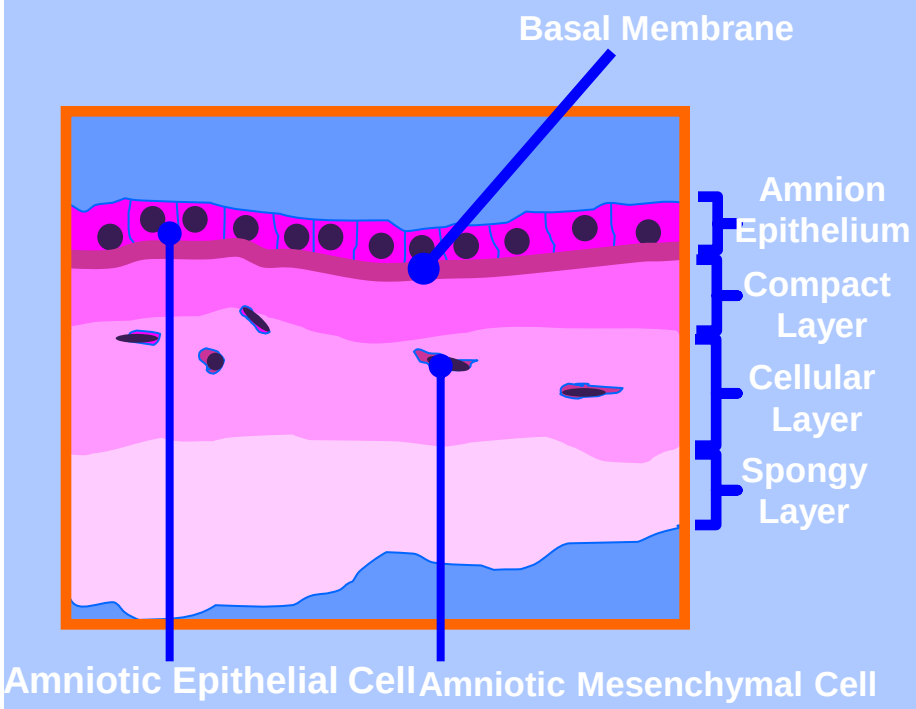


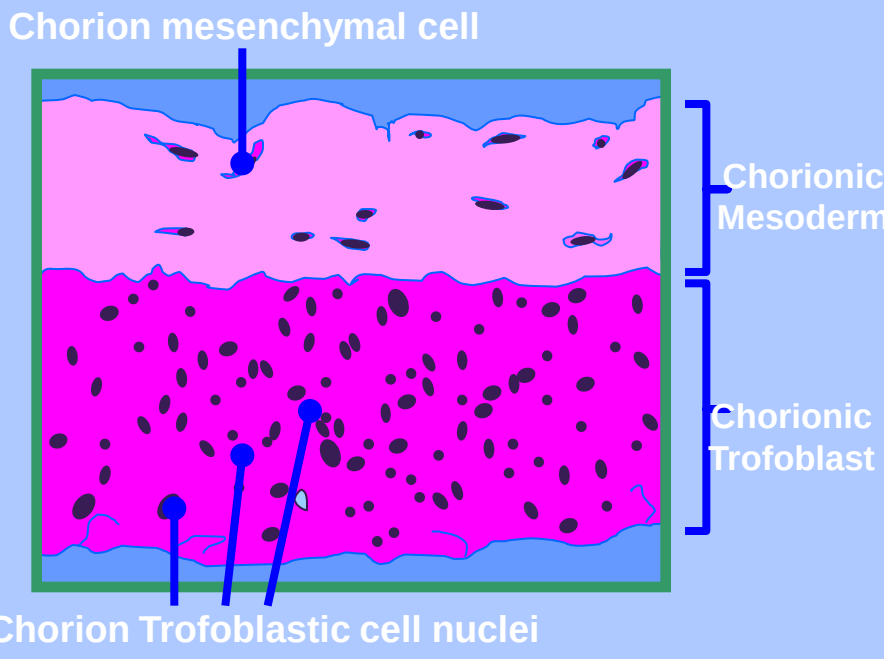
Reflected fetal membranes



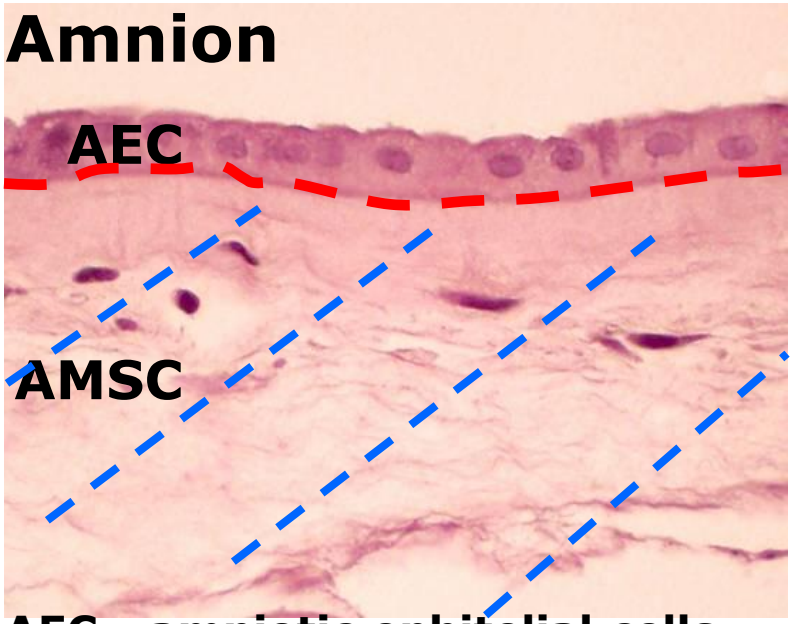
Amnion

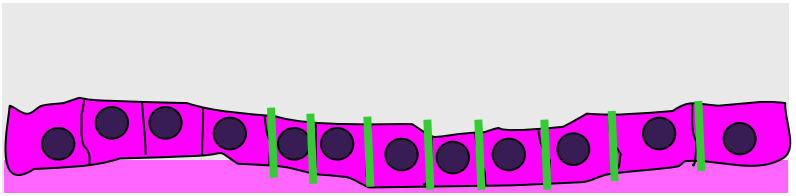
Chorion





Amniotic membrane enzymatic digestion

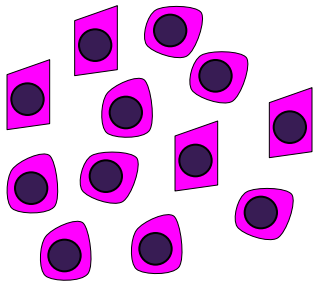




AEC= amniotic ephitelial cells

AMSC= amniotic mesenchymal stromal cells

AMSC



AEC

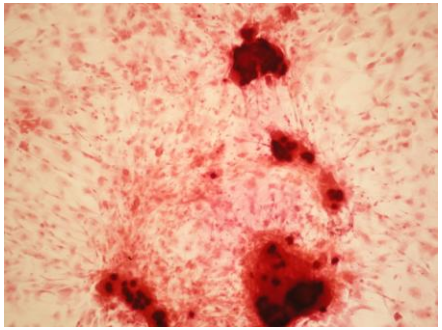
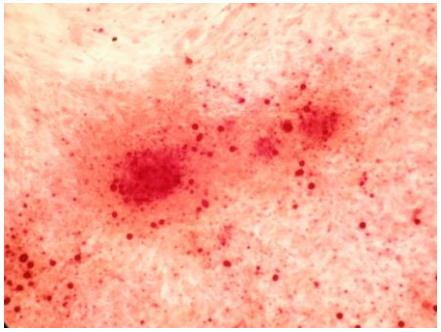
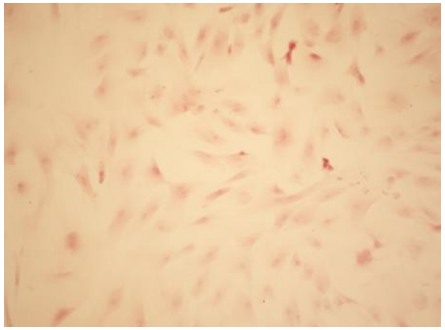
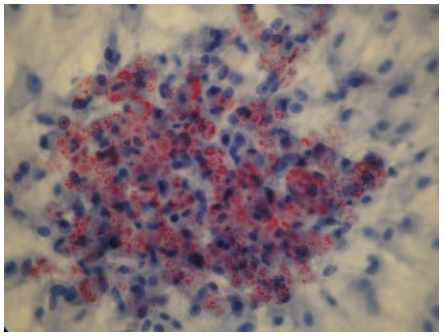
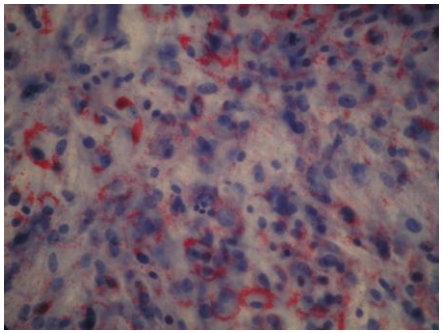
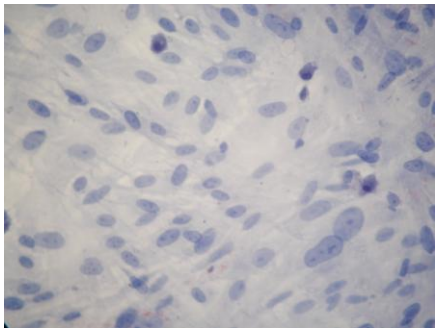
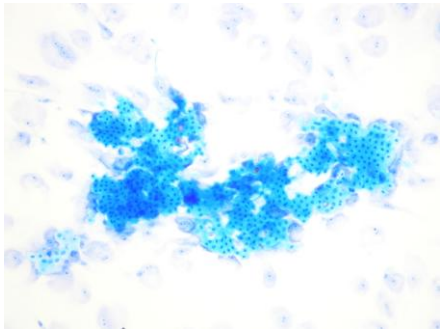
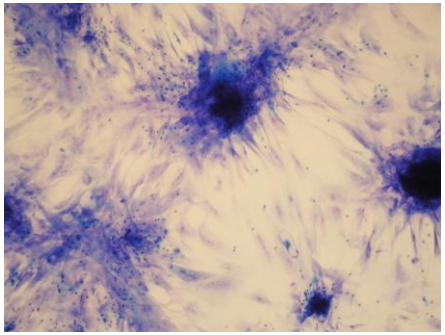
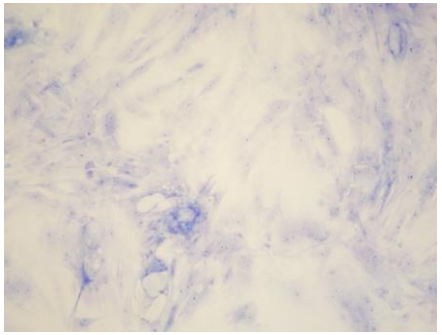
Enzymes:

Dispase

Collagenase + Dnase

Trypsin

Differentiation Potential of AMC and CMC

	AMC	CMC	control
OSTEOGENIC LINEAGE (alizarin red staining)			
ADIPOGENIC LINEAGE (oil red staining)			
CHONDROGENIC LINEAGE (toluidine blue staining)			

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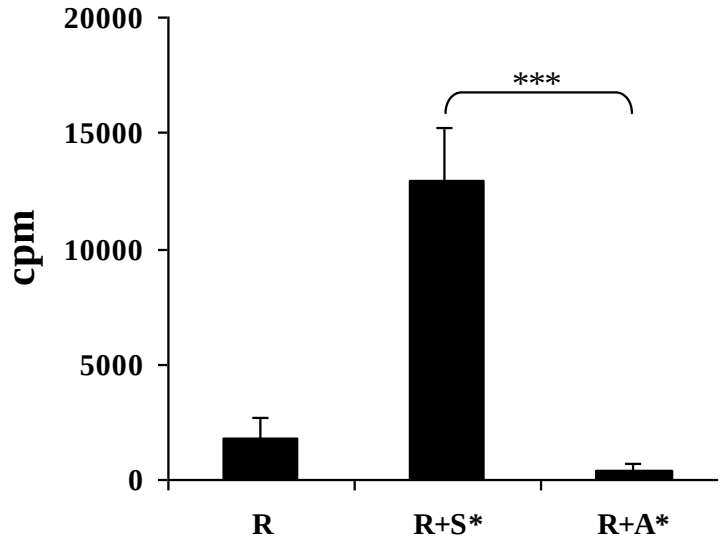
Centro Ricerca Eugenia Menni

Soncini M. et al J TERM 2007

IMMUNOMODULATORY FEATURES OF AMNIOTIC DERIVED CELLS

A

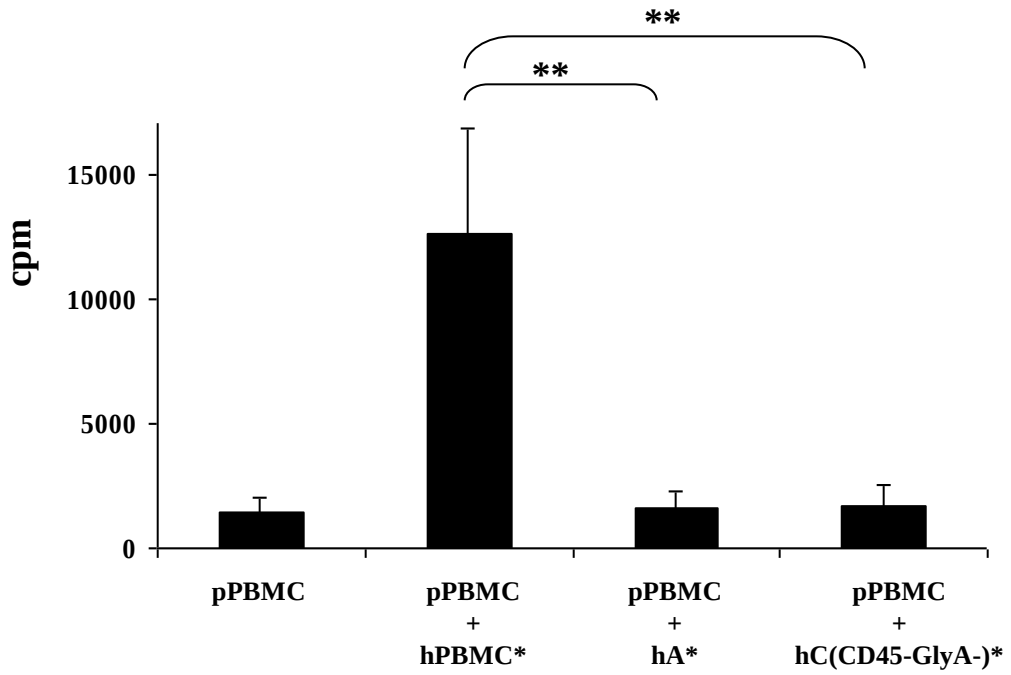


R: PBMNC from subject A

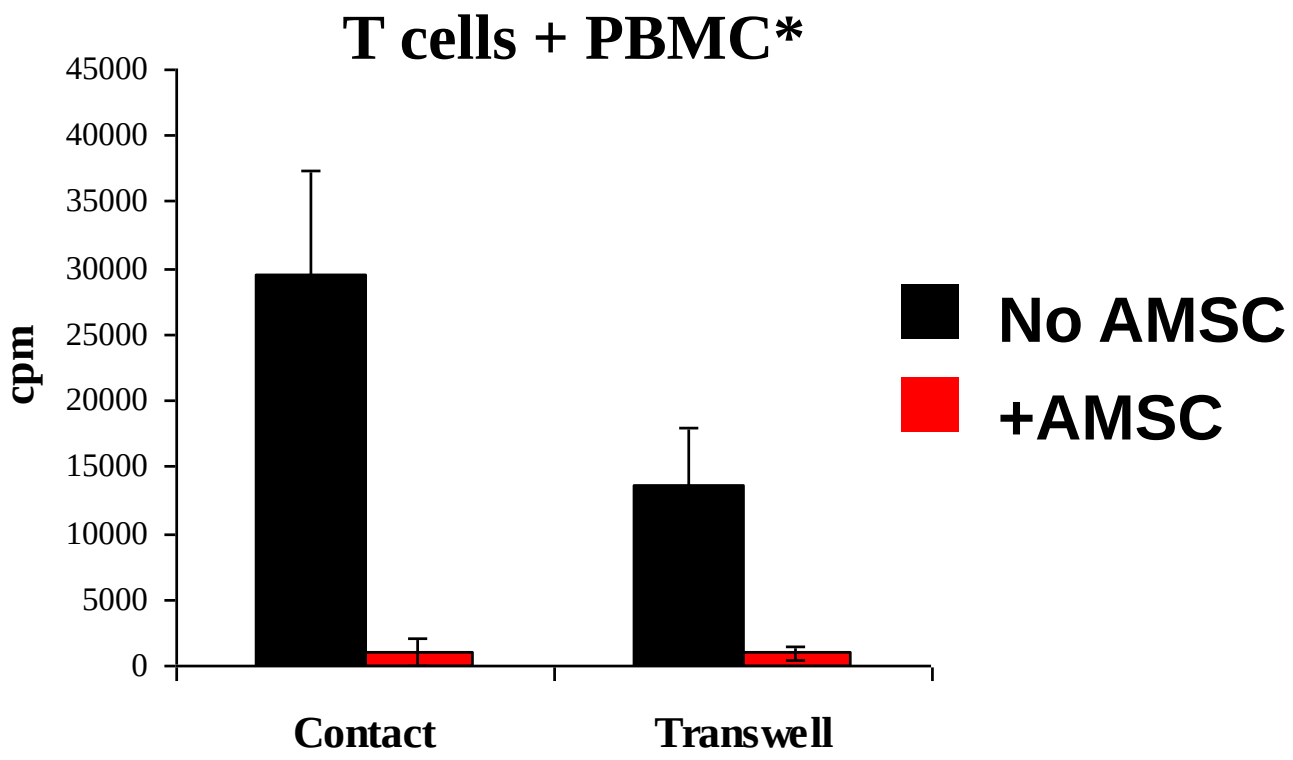
S*: PBMNC from subject B after irradiation

A: Amnion derived cells

B



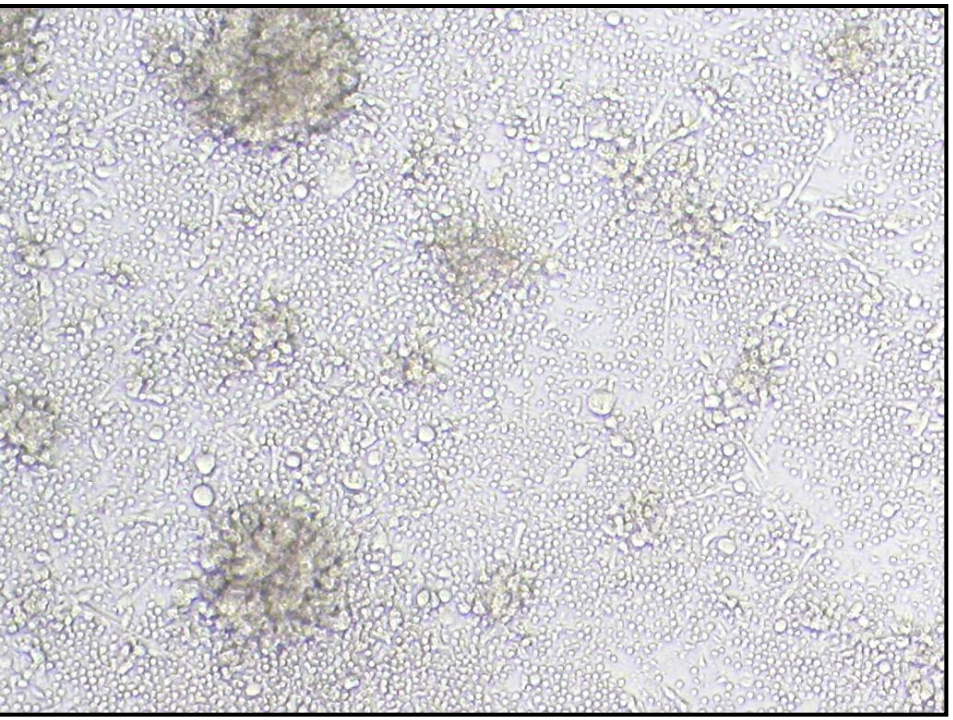
AMSC effect on lymphocyte proliferation



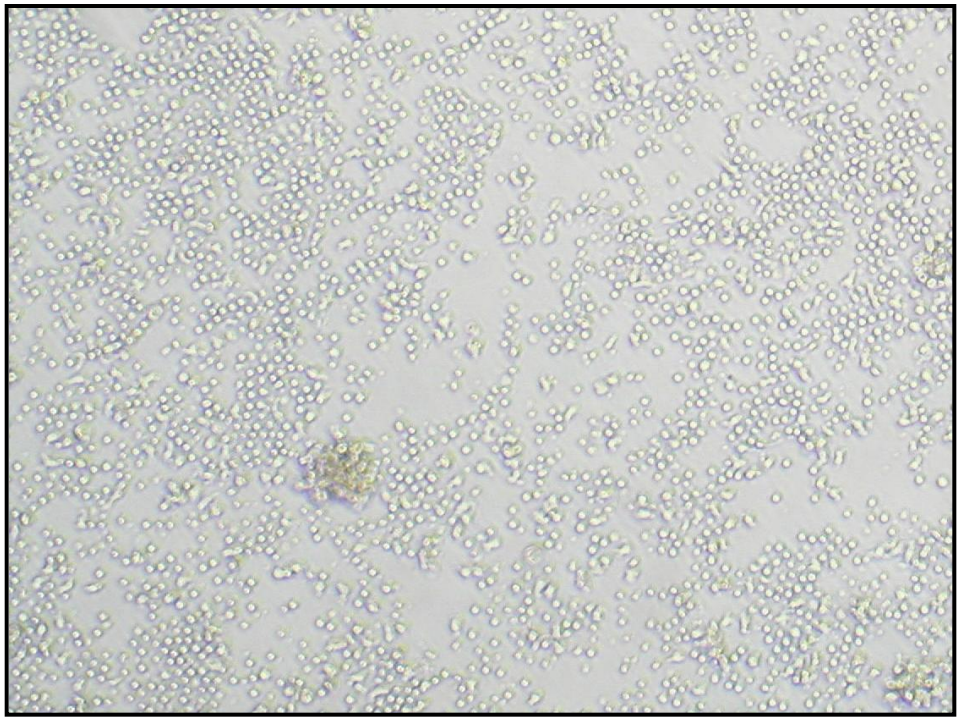
AMSC inihibit lymphocyte proliferation induced in a mixed lymphocyte reaction

AMSC effect on lymphocyte proliferation

PBMC+ allo PBMC*



PBMC+ allo PBMC* + AMSC



In vivo studies:

Transplant and engraftment potential of fetal membrane cells

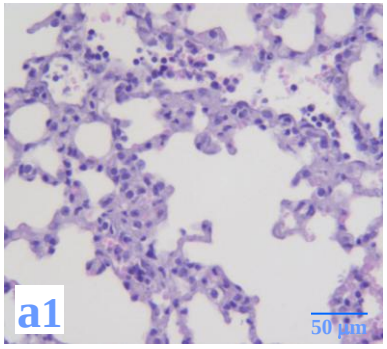
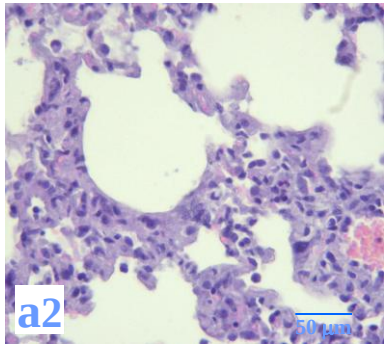
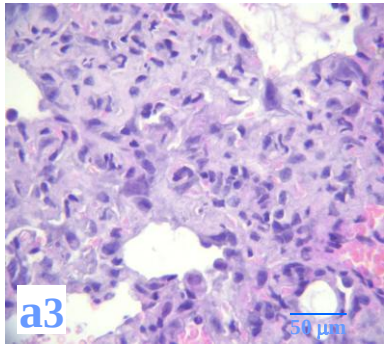
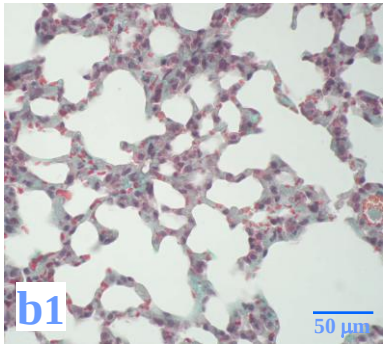
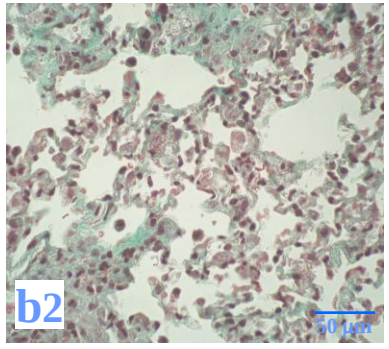
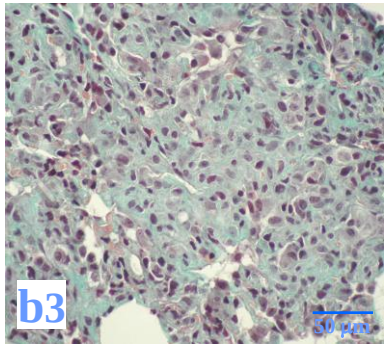
Murine model of lung fibrosis induced by intra-tracheal bleomycin instillation

Intratracheal instillation of bleomycin induces:

- **LUNG INJURY -** alveolar epithelial cell injury
- **INFLAMMATION -** migration of inflammatory cells

- **FIBROSIS -** fibroblast proliferation and extensive accumulation of collagen

BLEOMYCIN

	day 3	day 7	day 14
INFLAMMATION			
FIBROSIS			

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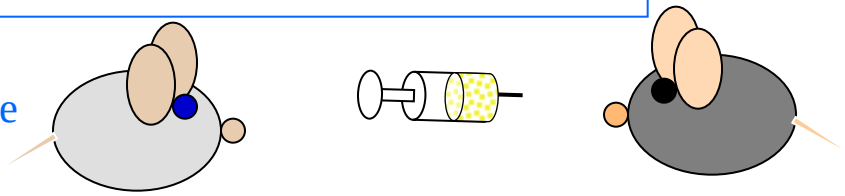
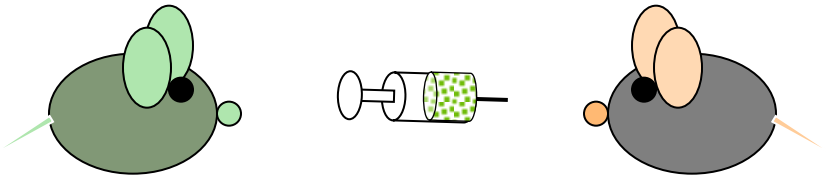
ALLO-TRANSPLANTATION

BALB/c mice



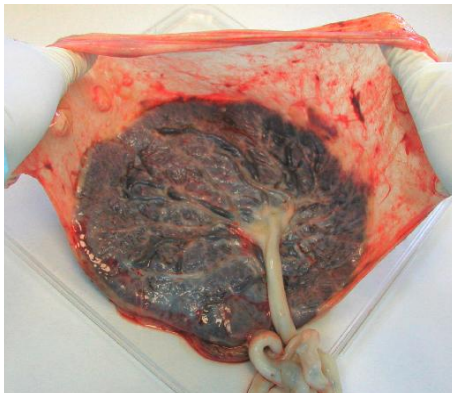
C57BL/6 mice

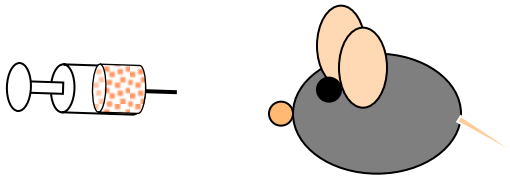
GFP mice



C57BL/6 mice

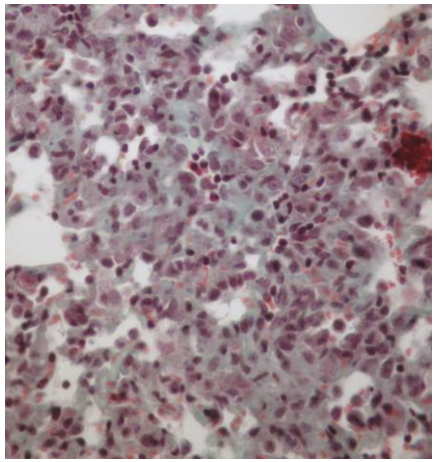
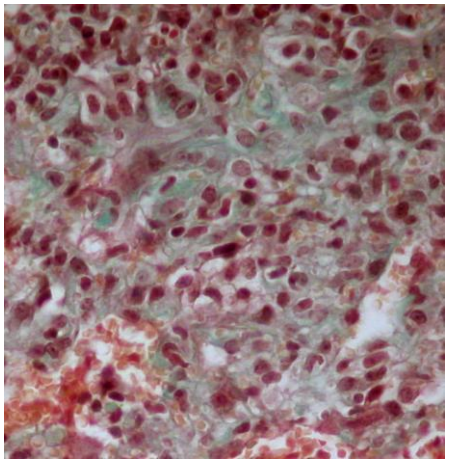
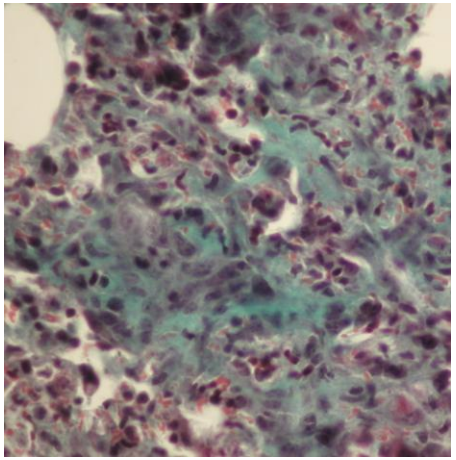
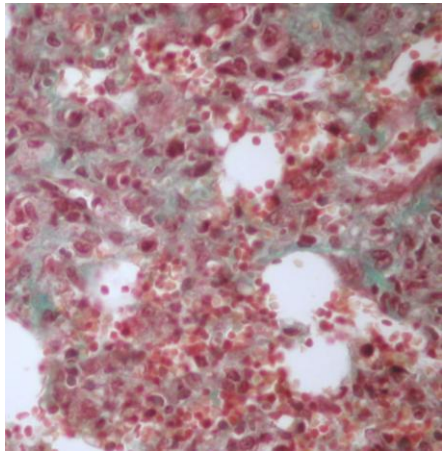
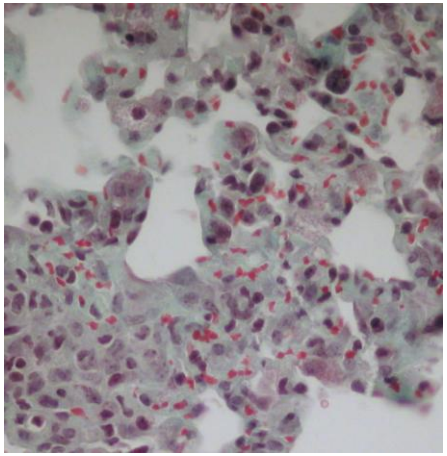
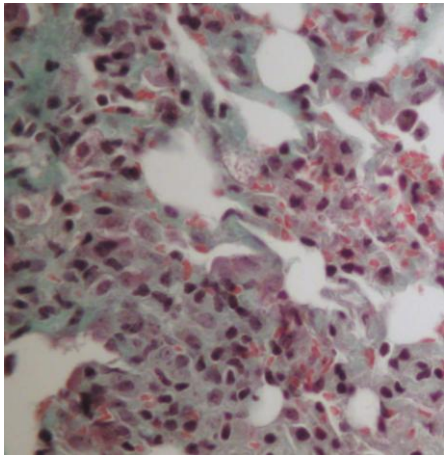
XENO-TRANSPLANTATION

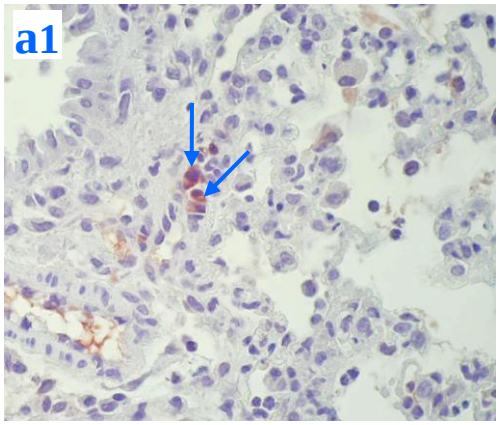
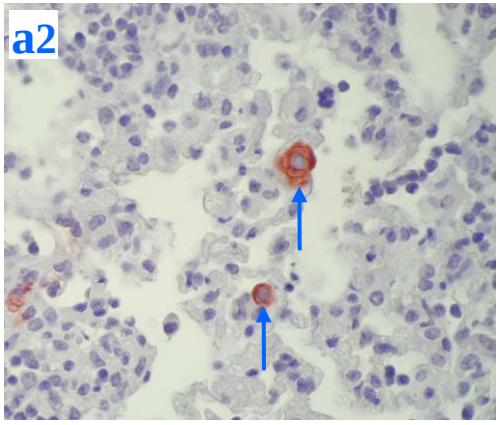
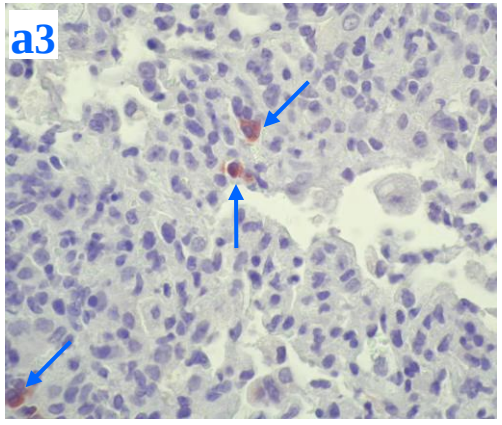
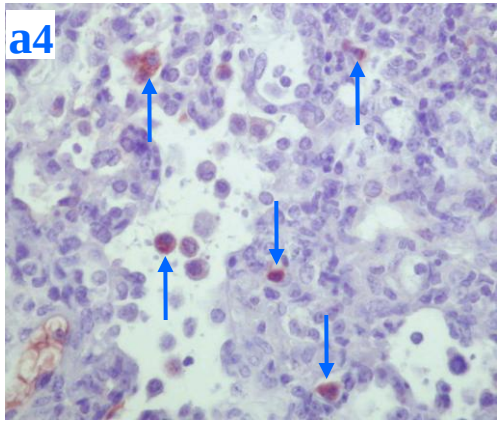




C57BL/6 mice

FIBROSIS

	day 3	day 7	day 14
Bleomycin			
Bleomycin + allo-transplantation			

	Allo-transplantation	Xeno-transplantation
Intra-tracheal delivery	 a1	 a2
Intra-peritoneal delivery	 a3	 a4

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INFLAMMATION SCORE

- **Inflammation severity**
  - *type of inflammatory cells*
  - *number of inflammatory cells*
  - *edema presence*

- **Inflammation extent**

- represents the lung area involved in the process

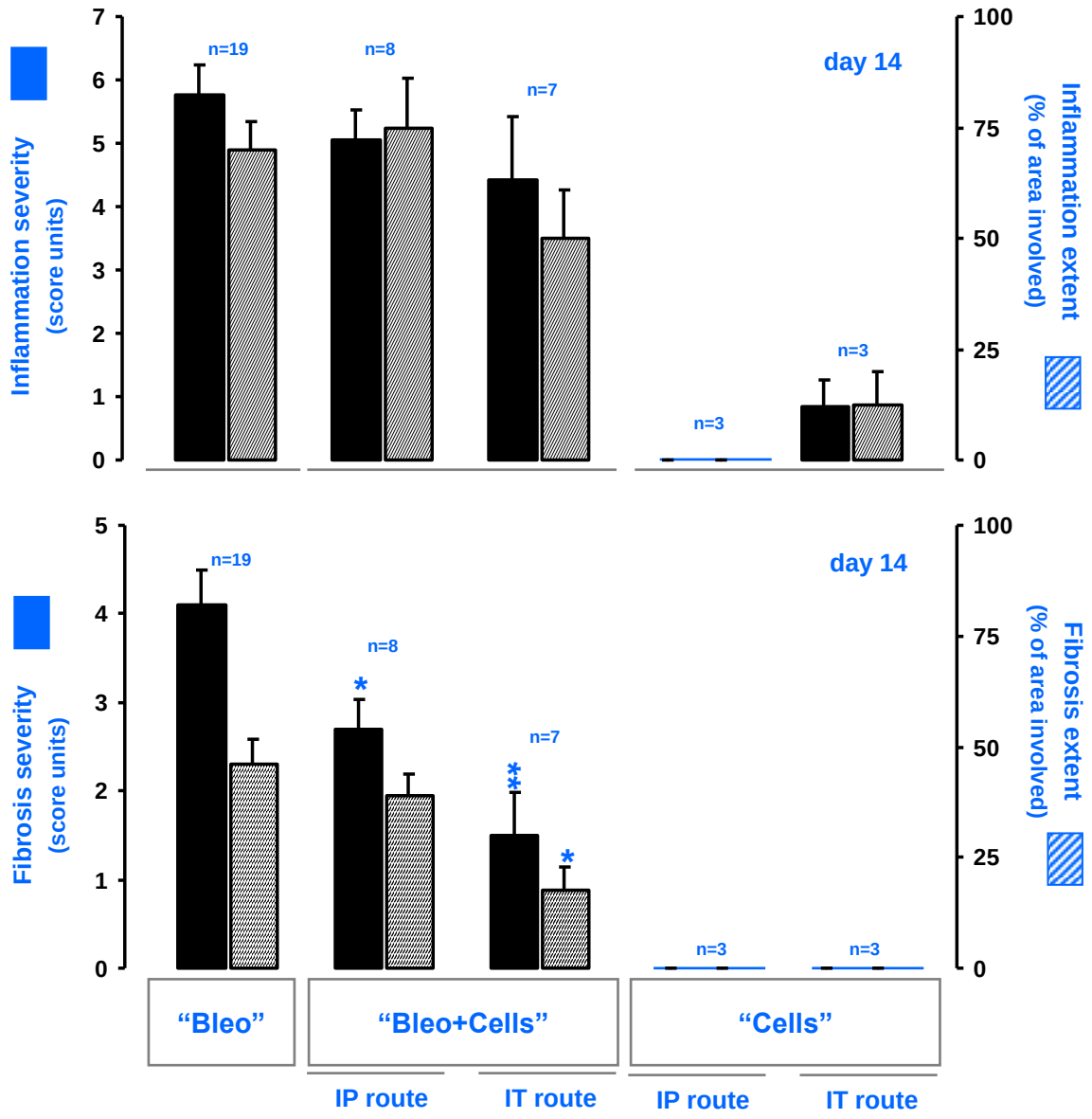
FIBROSIS SCORE

- **Fibrosis severity**
  - *Fibroblast proliferation*
  - *Collagen deposition*

- **Fibrosis extent**

- represents the lung area involved in the process

Allo-transplantation



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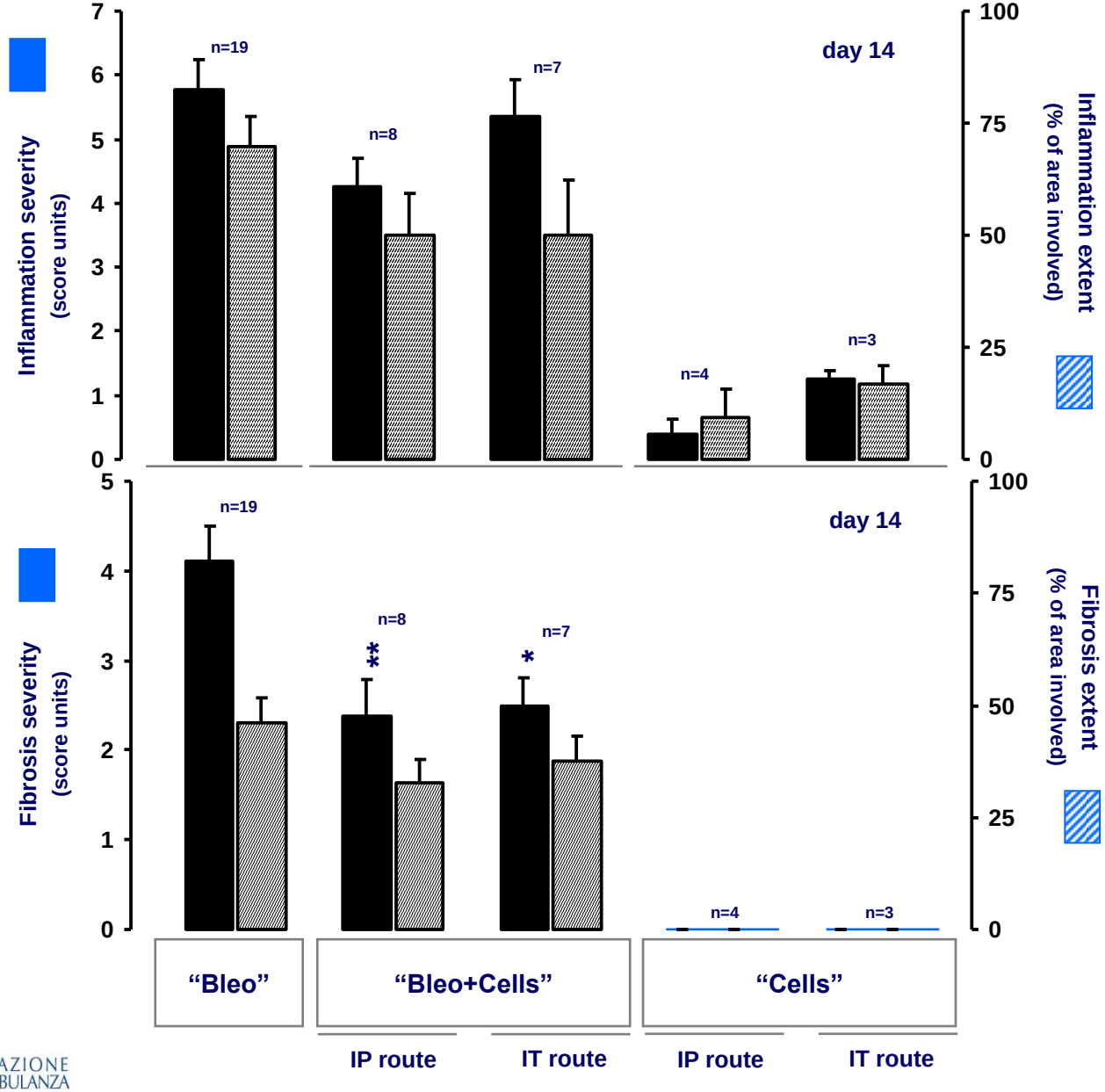
Xeno-transplantation



Cargnoni et al, Cell Transplantation 2009

Pulmonary fibrosis

DISEASE MODEL: Bleomycin-induced lung fibrosis in mice

TREATMENT:
- Xenogeneic cell transplant
- Allogeneic cell transplant

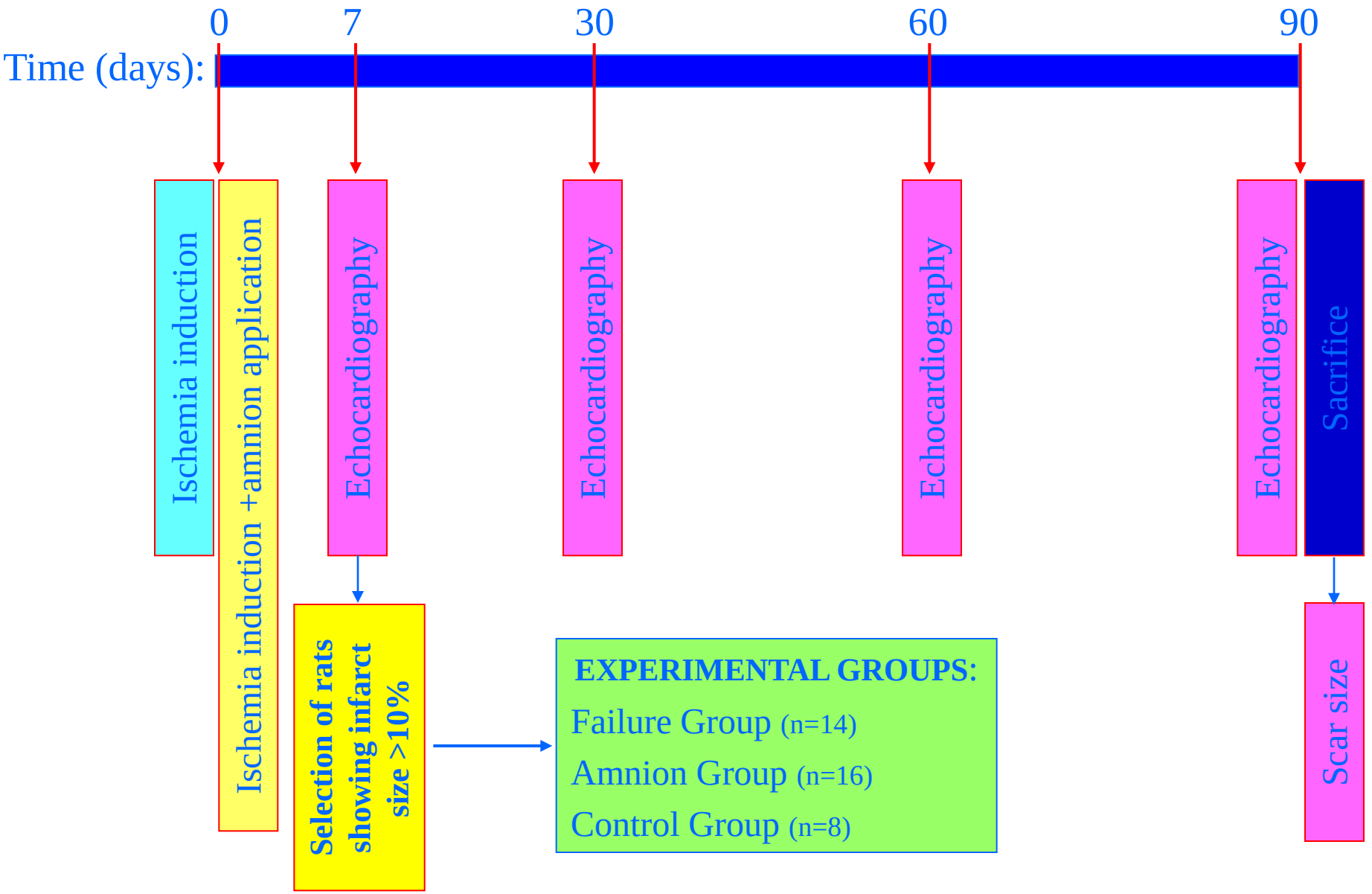
TREATMENT ROUTE:
- Systemic delivery= IP injection
- Local delivery= IT injection

Placenta-derived cell transplantation significantly reduced bleomycin-induced lung fibrosis

Effects of placenta-derived cells and amniotic membrane on cardiac injury induced by coronary ligation in rats

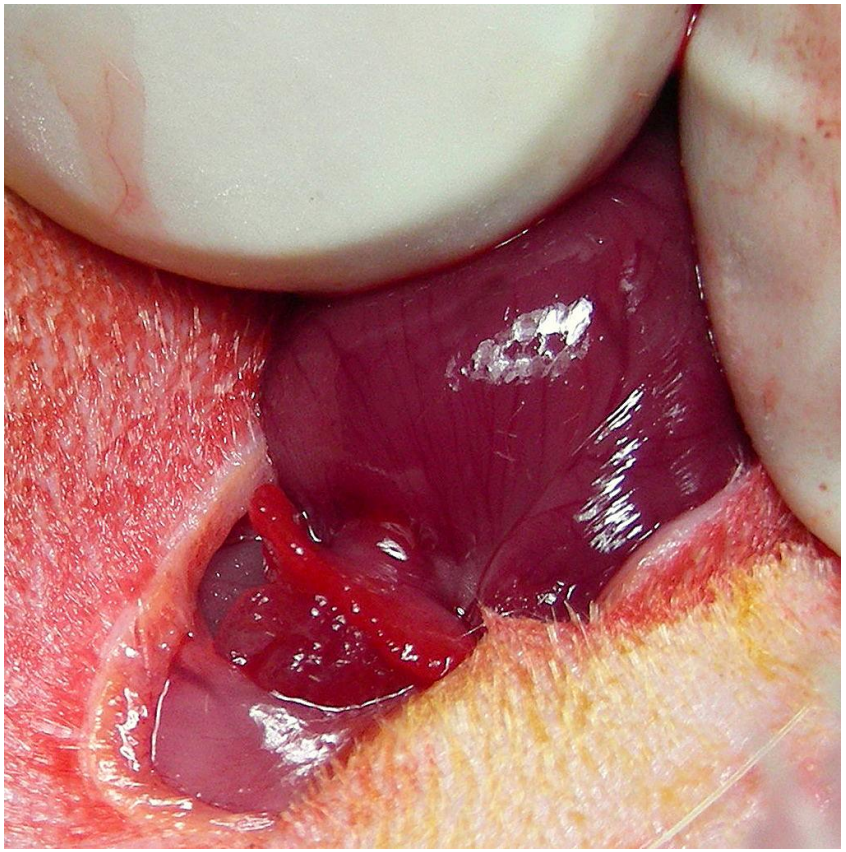
Reduction of post-ischemic cardiac dimensional alterations and improvement of myocardial function for up to at least 60 days after ischemia induction.

Experimental design

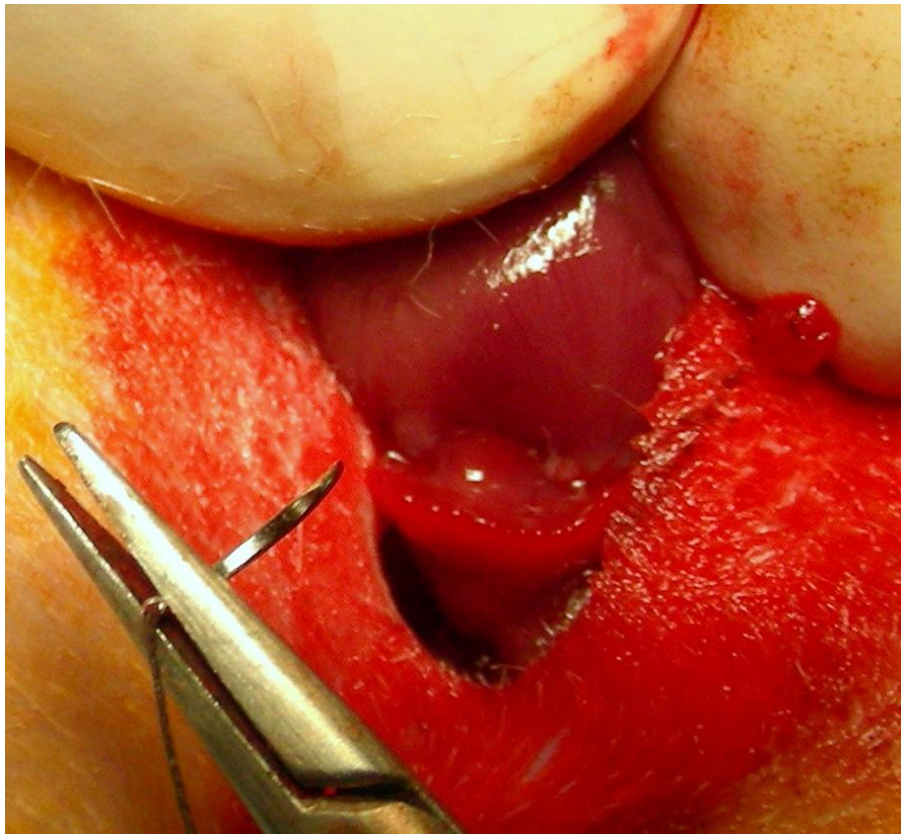


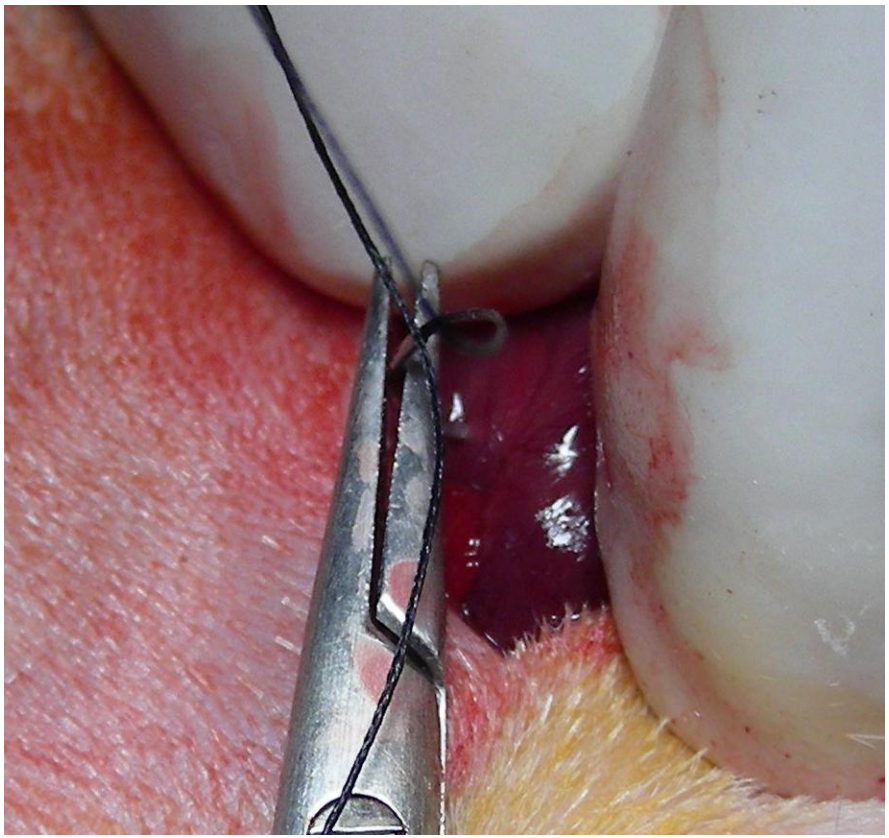
A lateral thoracotomy was performed at level of 4th-5th intercostal space

Heart was exteriorised from the thorax

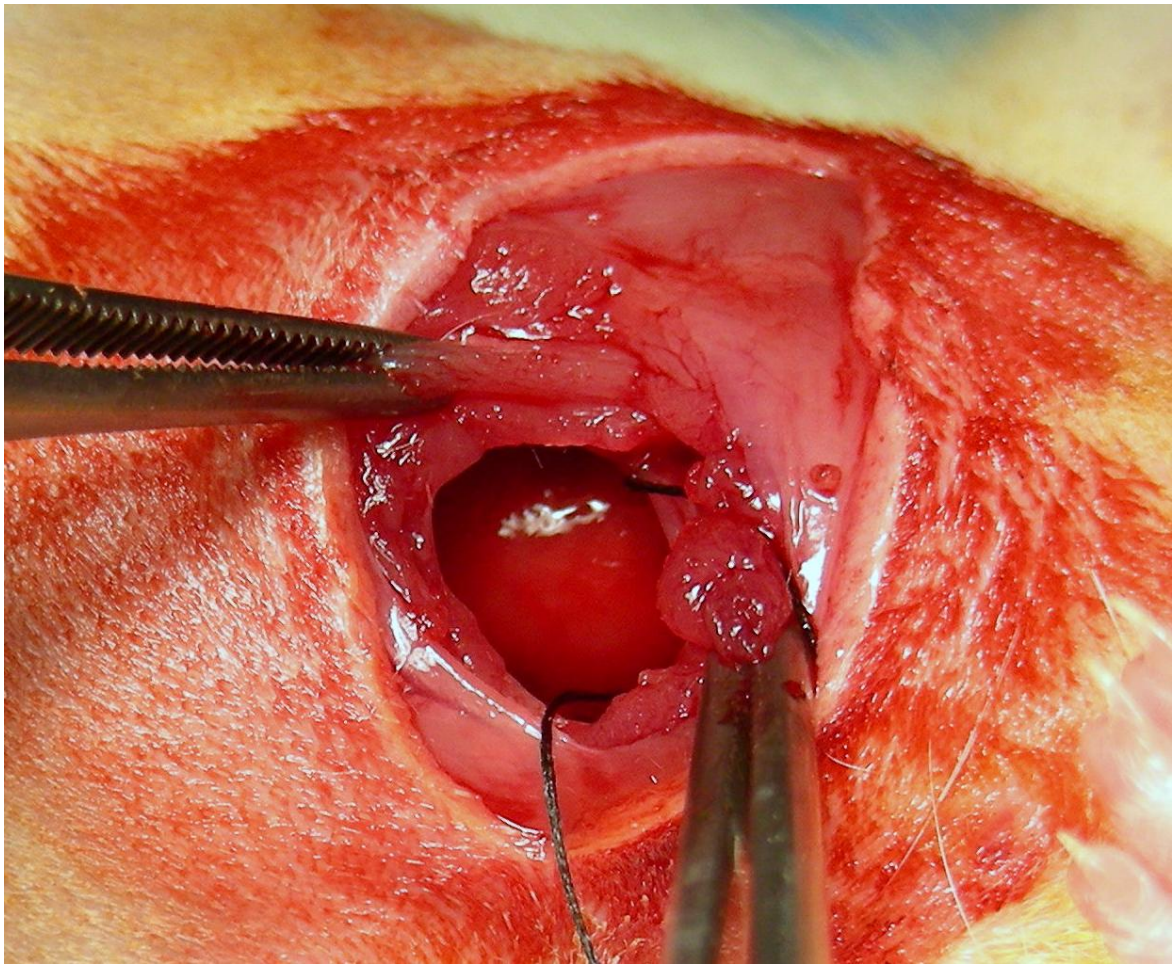


A 5-0 silk suture was passed under the LAD artery

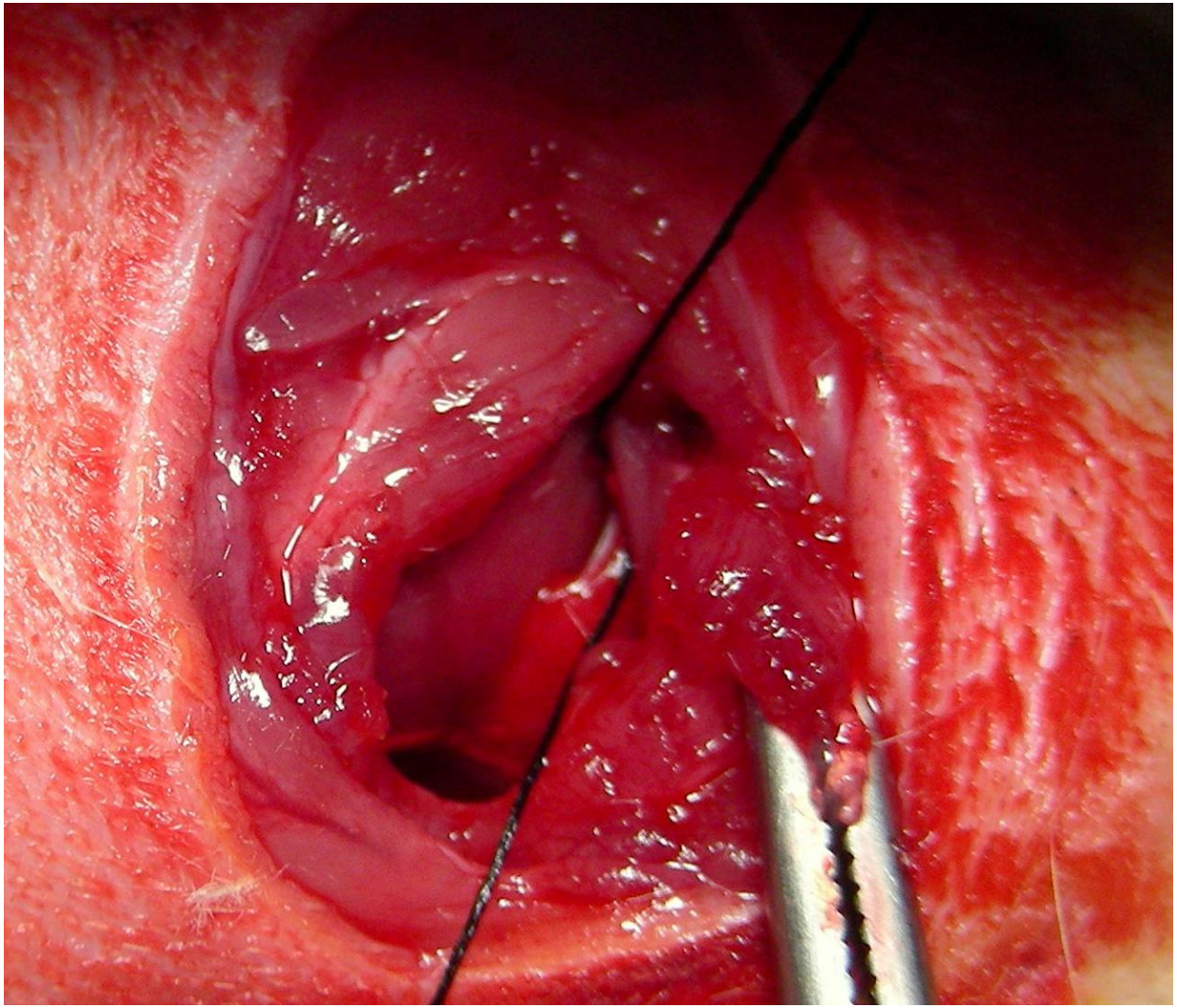




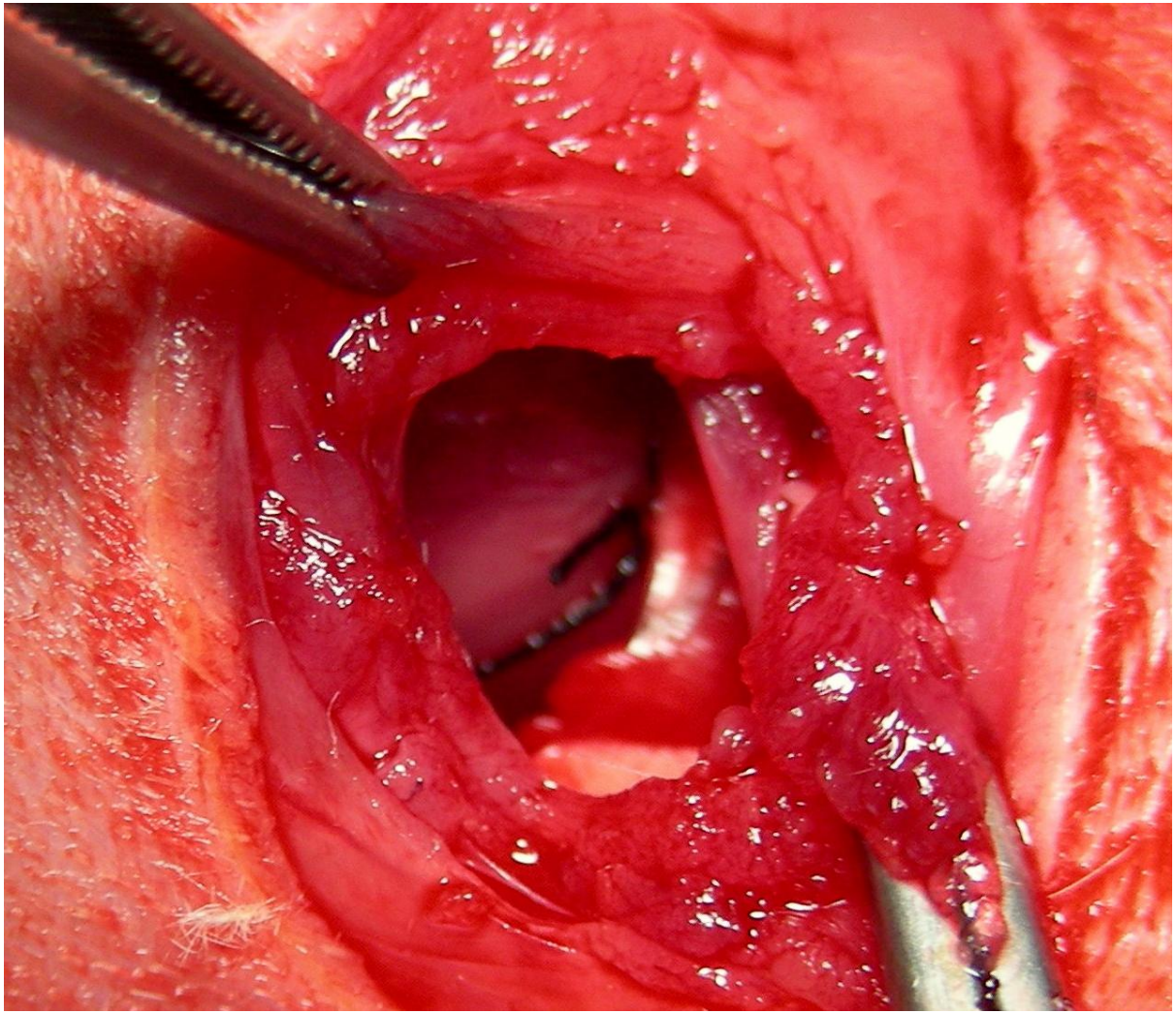
The heart was replaced into the thoracic cavity



The suture was tightened around the LAD coronary



The ischaemic cardiac area was whitening



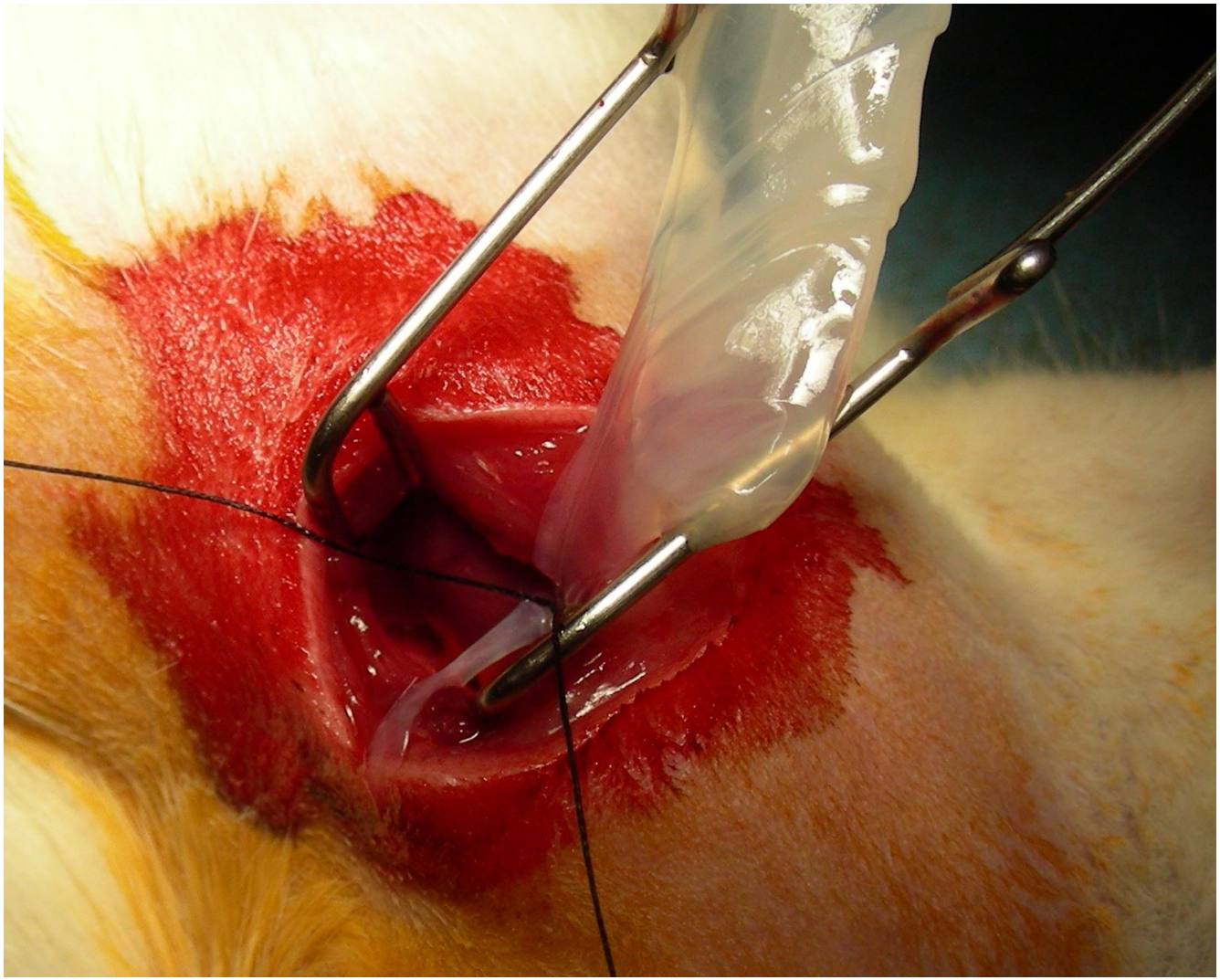
10. Thorax closure, in case of ischaemic untreated rats

In case of ischaemic treated rats

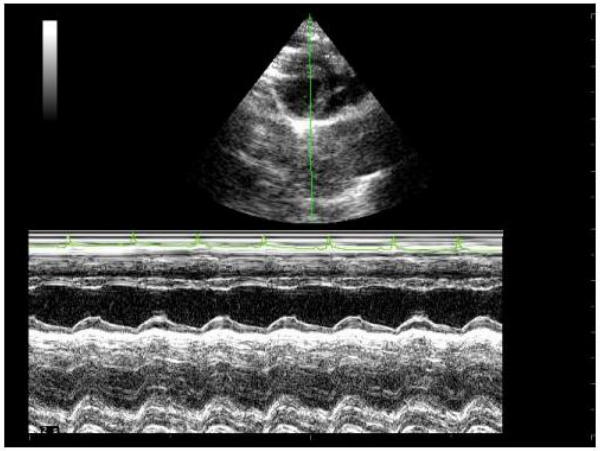
↓

Amniotic membrane application

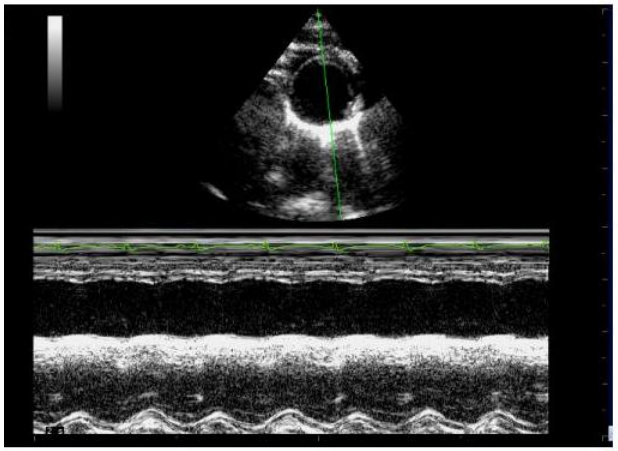
The membrane was softly applied on the left ventricle with the mesenchymal side in contact with epicardial surface



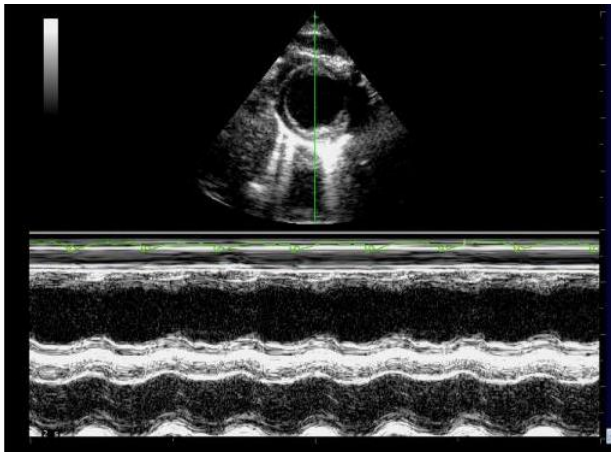
Echocardiographic analysis



Healthy rat heart

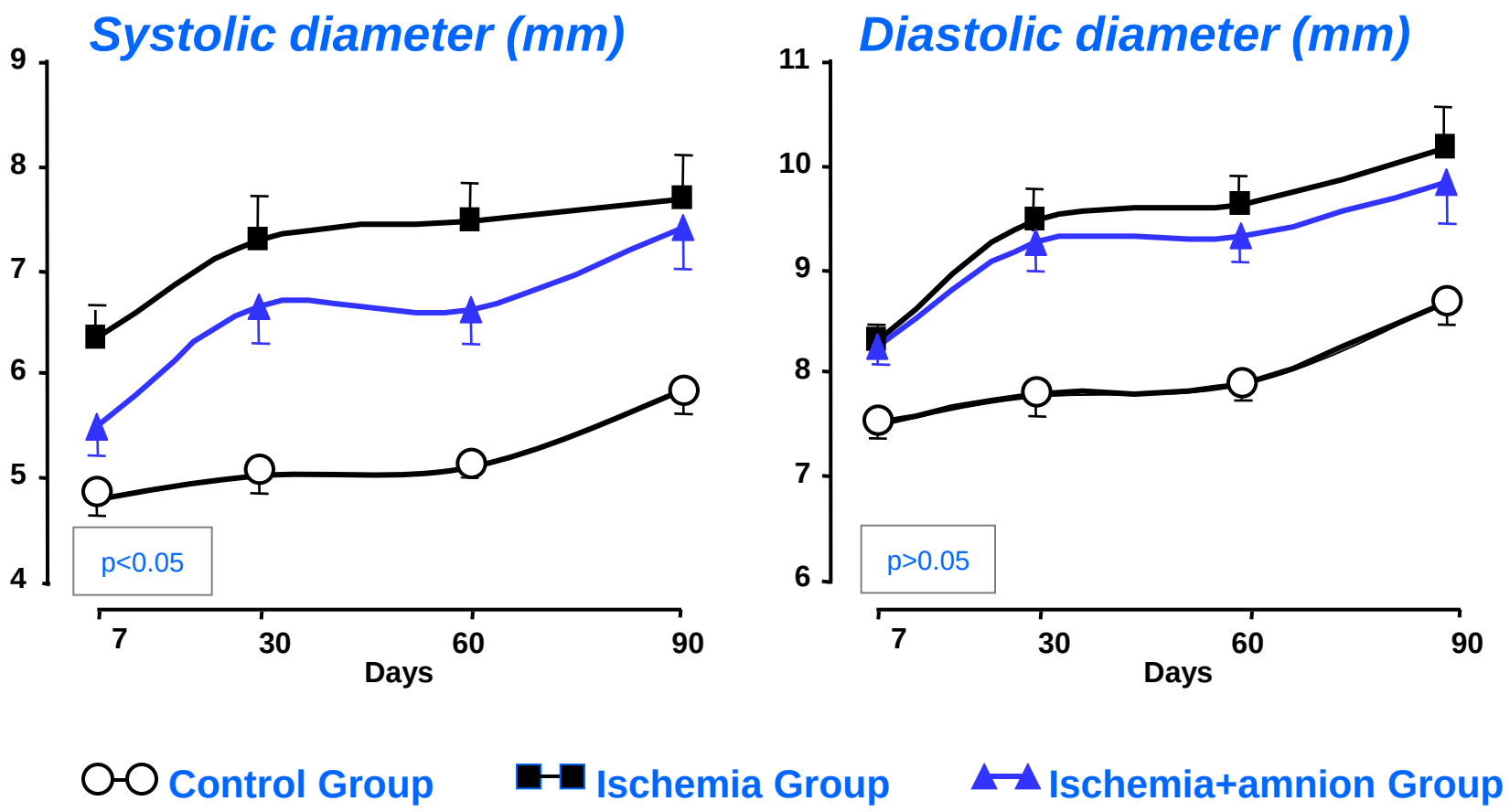


Ischemic rat heart

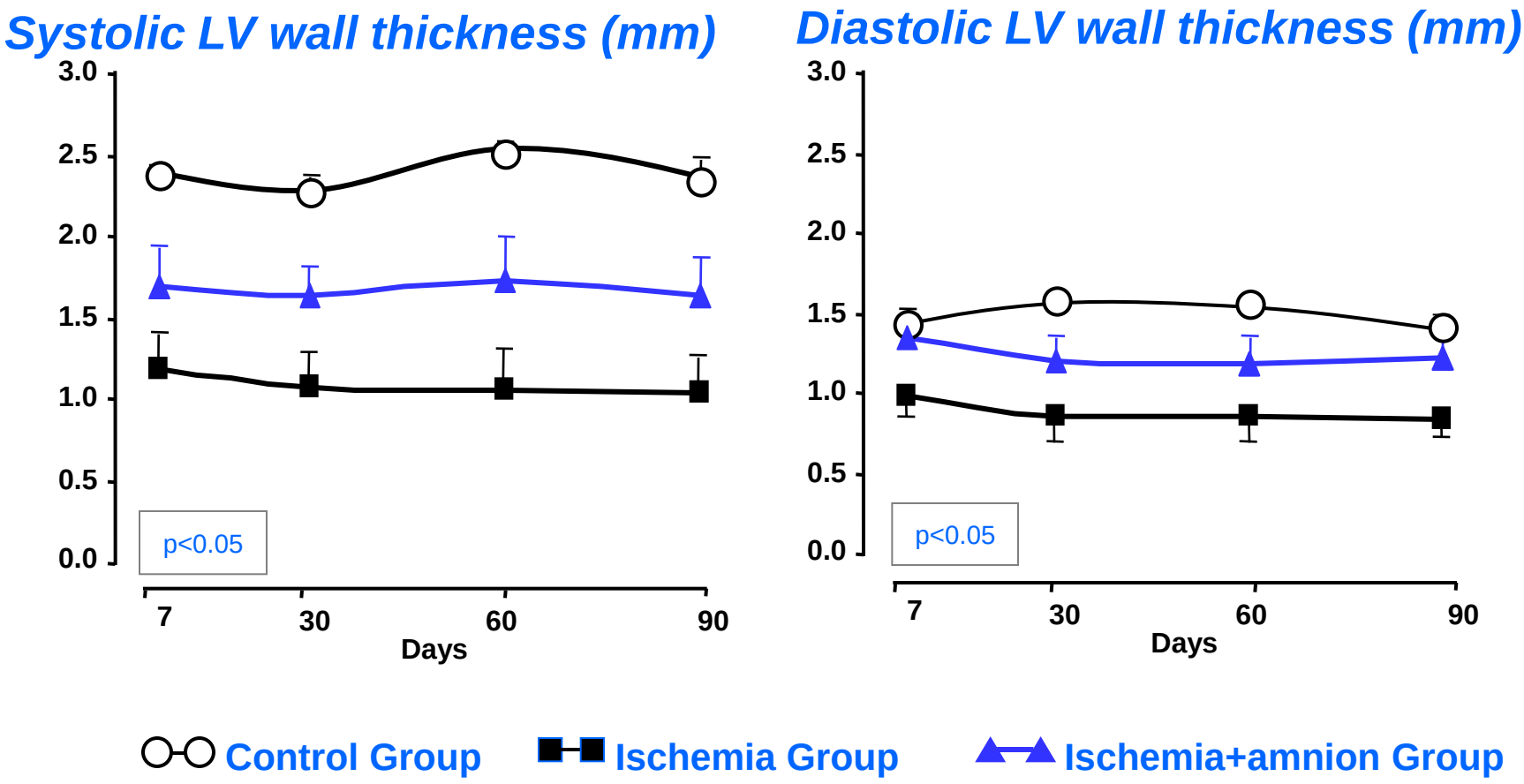


Ischemic rat heart + amnion

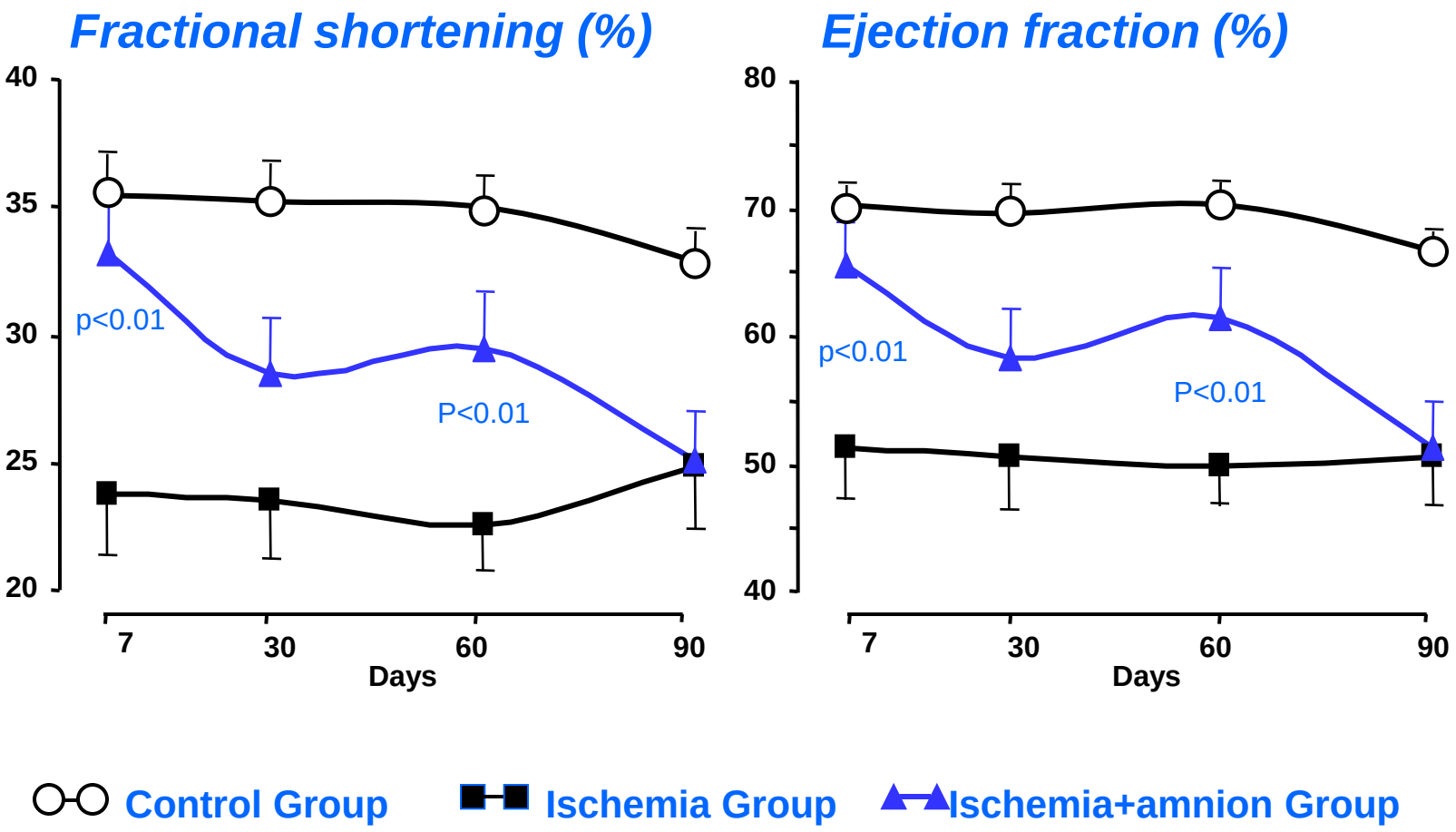
Cardiac dimensions: left ventricle diameter



Cardiac dimensions: left ventricle wall thickness



Cardiac function parameters



Myocardial ischemia

DISEASE MODEL: Myocardial ischemia induced by coronary ligation in rats

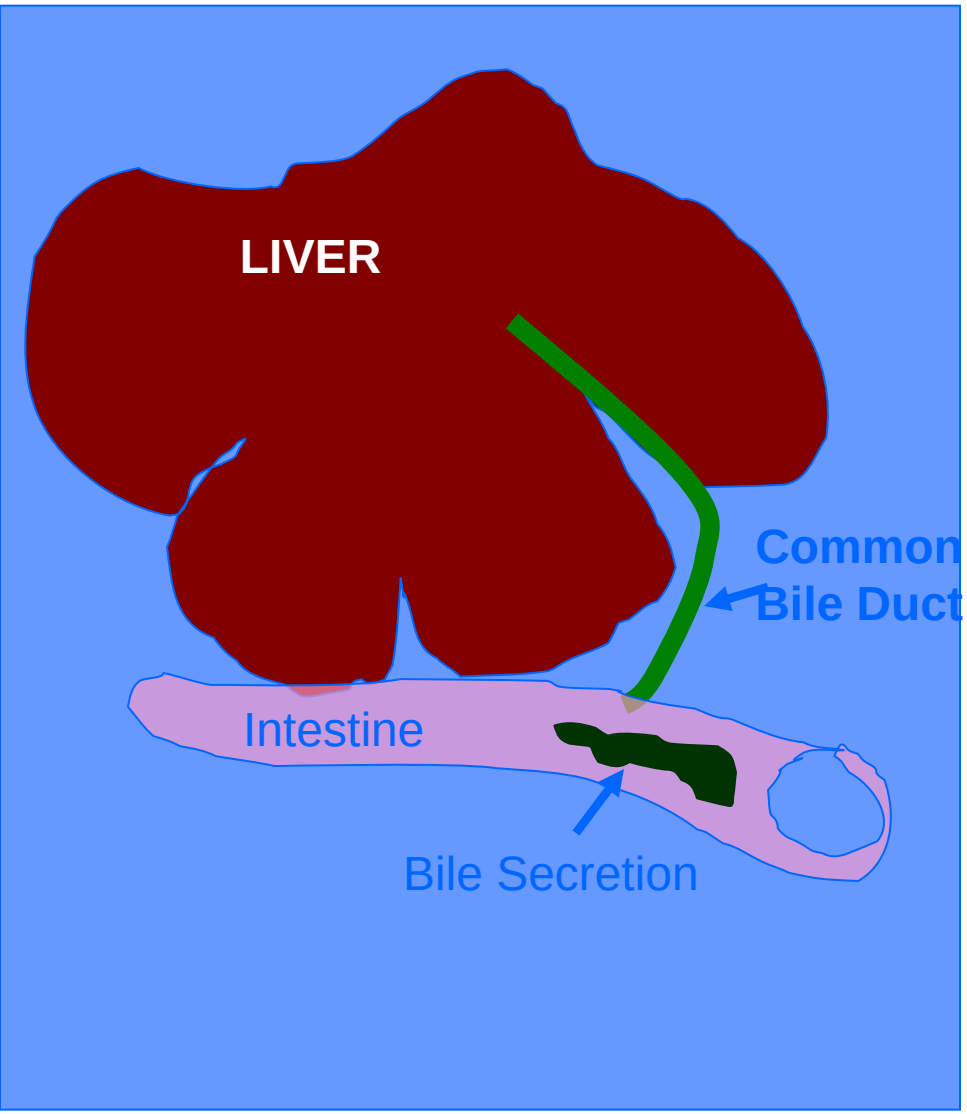
TREATMENT: Amniotic membrane application

Amniotic membrane application significantly improved cardiac functions in ischemic rat hearts for at least 2 months post-injury

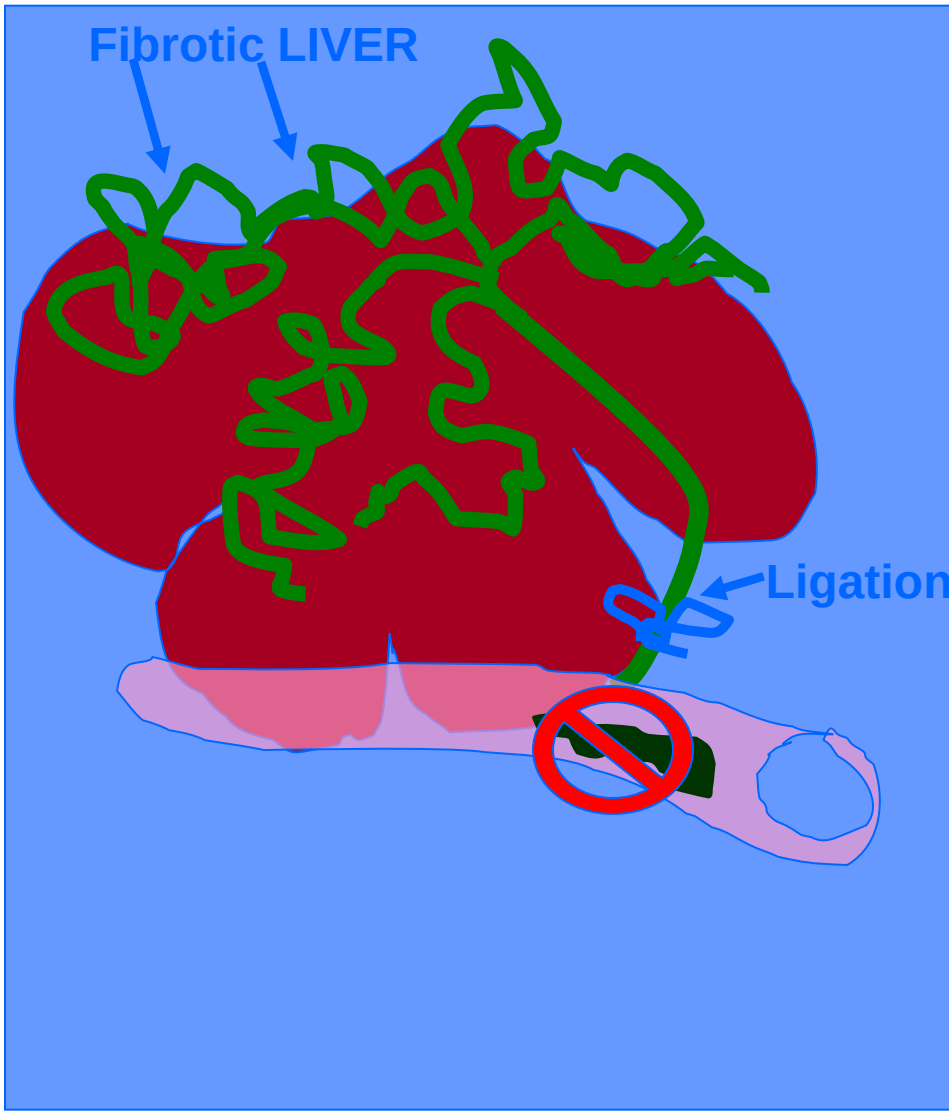
Which other models of fibrosis….

Bile duct ligation rat model

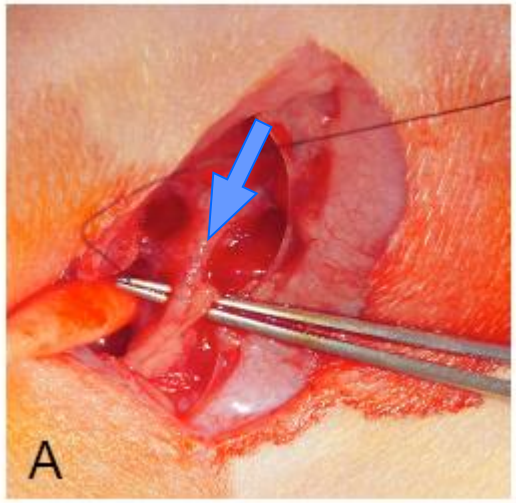
Normal Liver



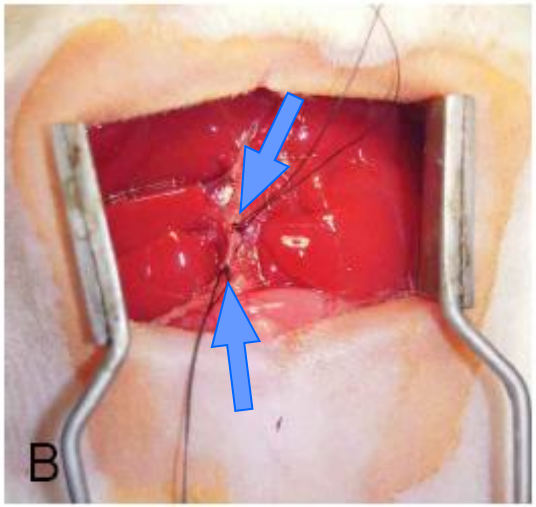
BDL model



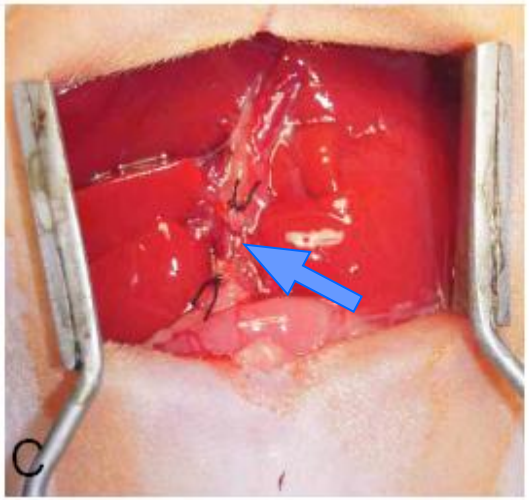
Surgery: Assessing BDL Model



Common Bile duct
Exposed



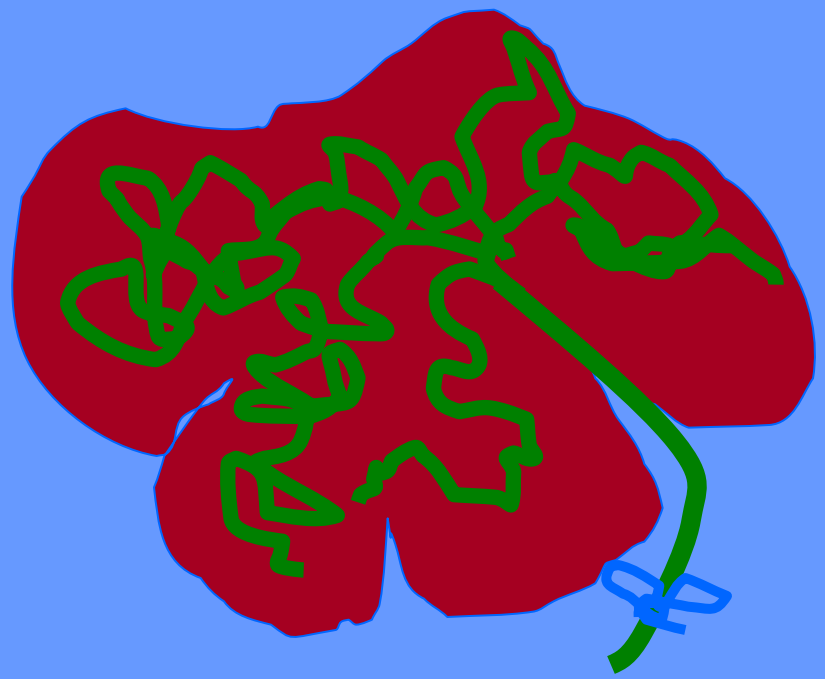
Bile duct
Double Ligated



Bile duct
Cut between ligatures

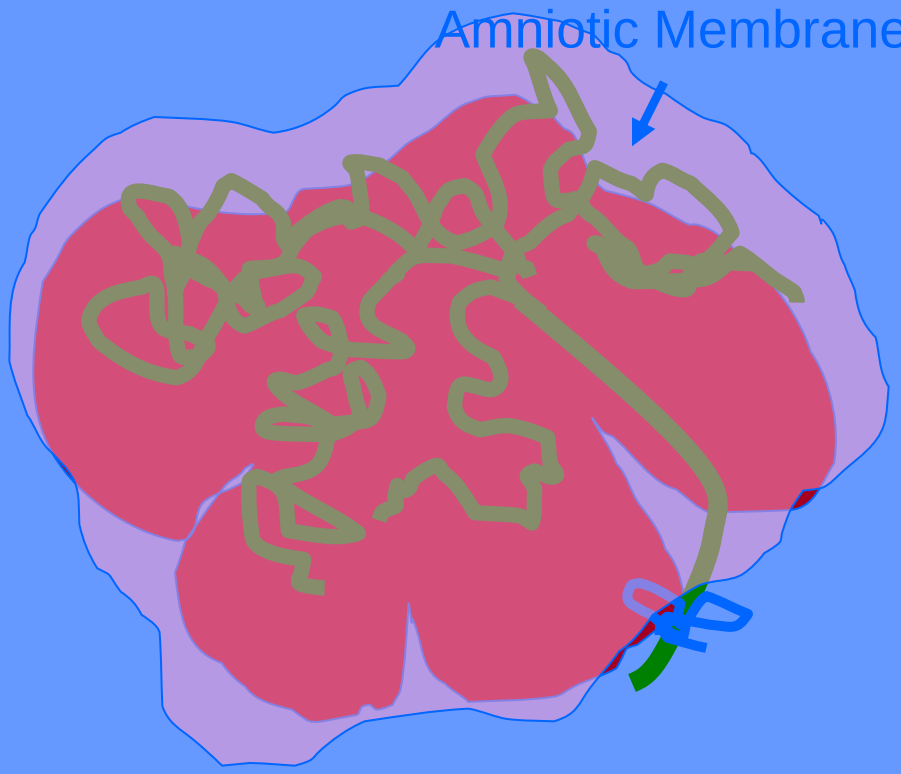
Study Groups

BDL Group



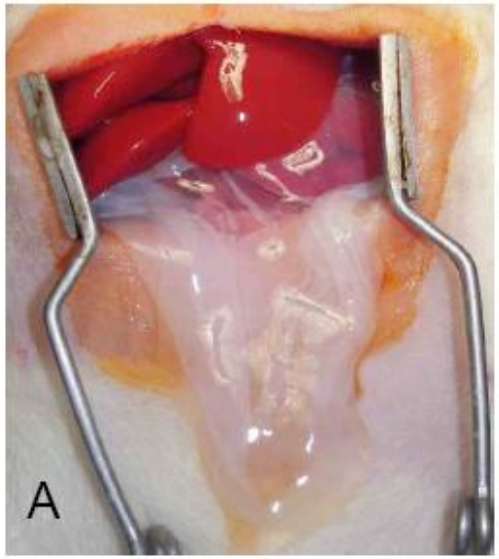
Sacrifice:
2,4,6 weeks

BDL+AM Group

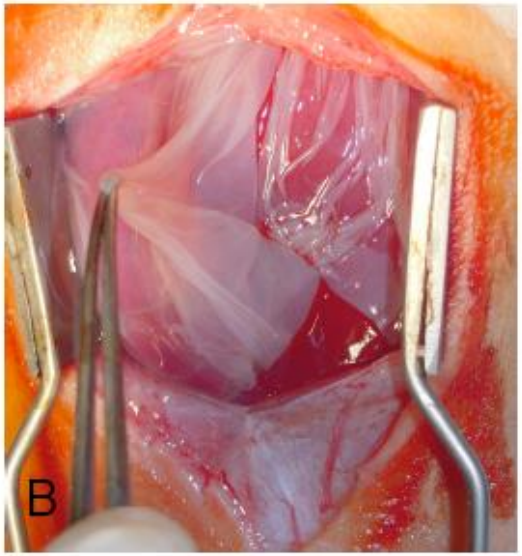


Sacrifice:
2,4,6 weeks

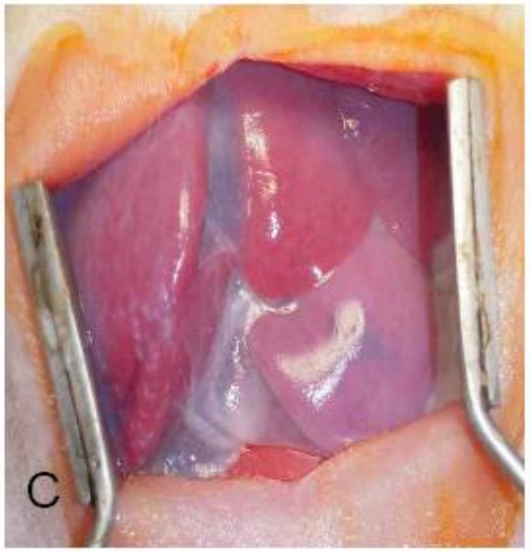
Surgery: AM application



AM fragment was inserted under the liver lobes

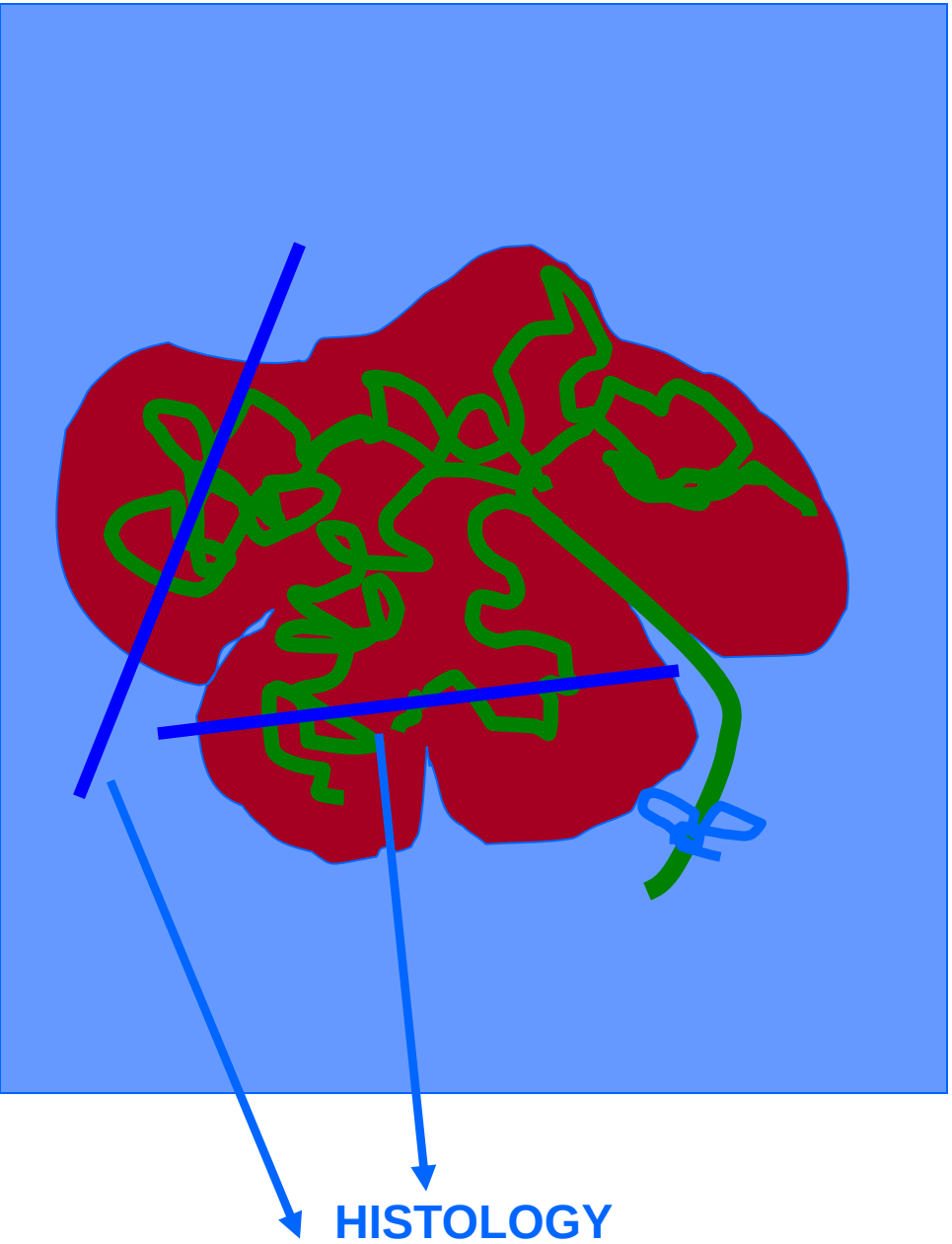


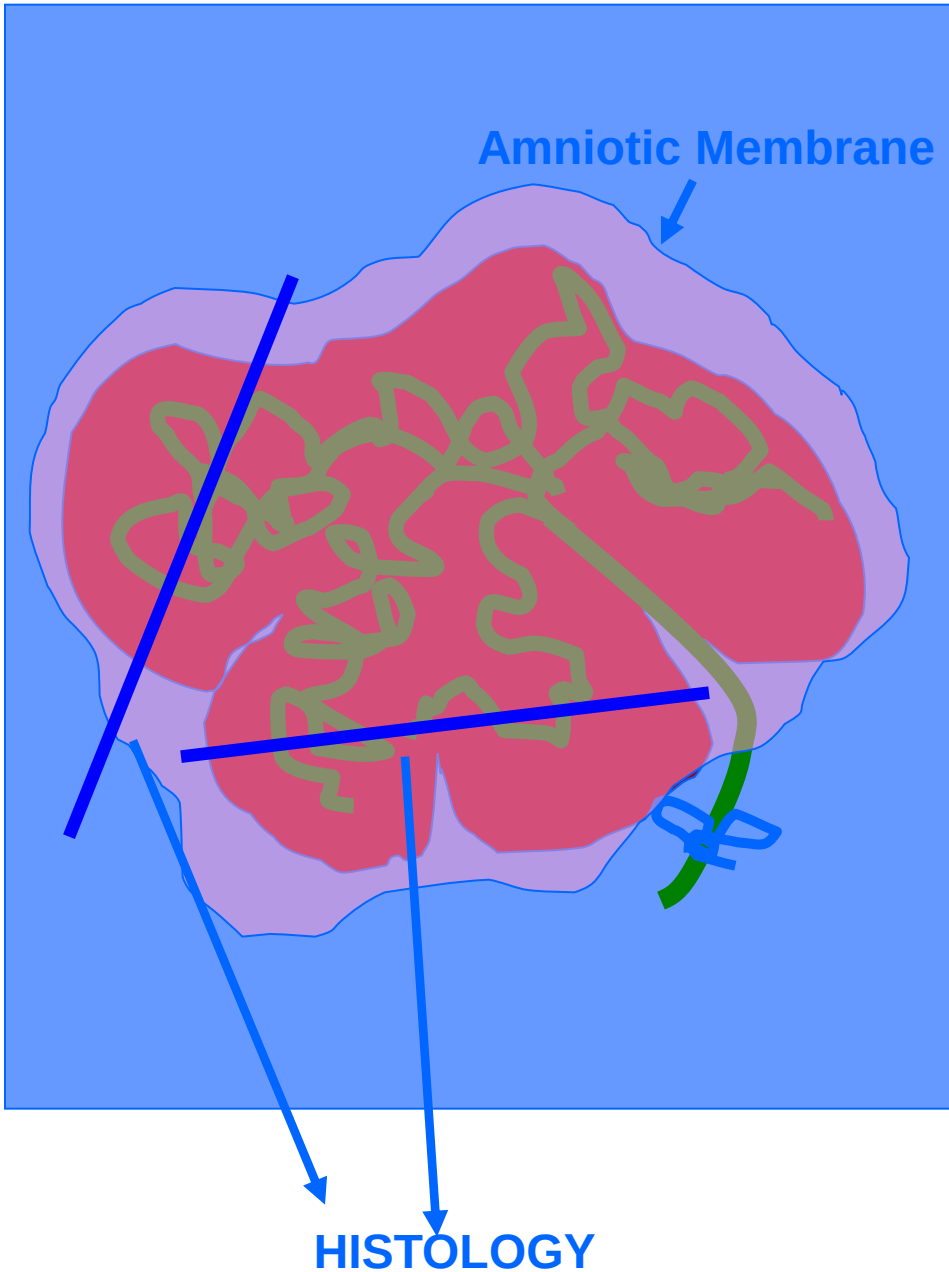
The extremities were raised…



…and fixed to cover the whole liver surface

Evaluation of Fibrosis

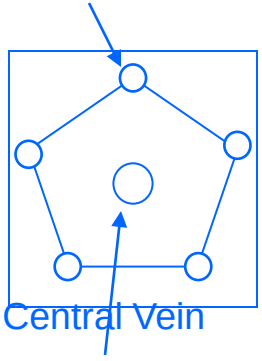


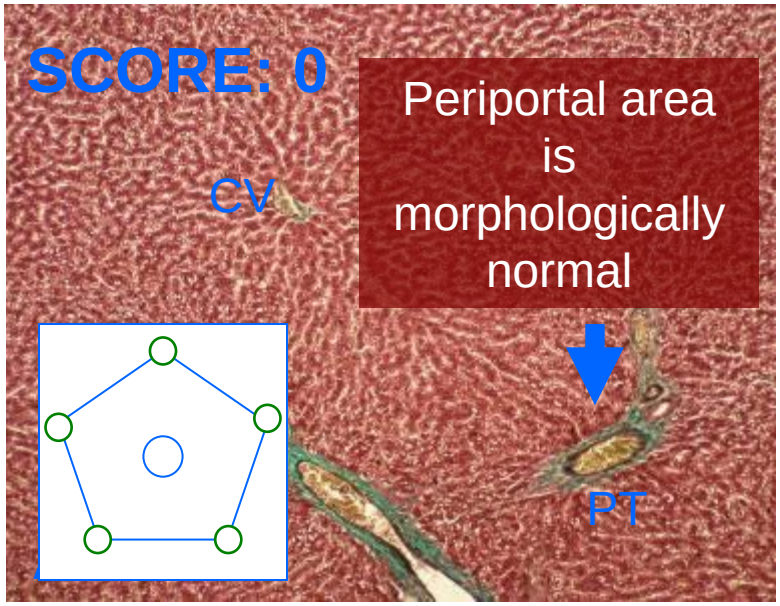


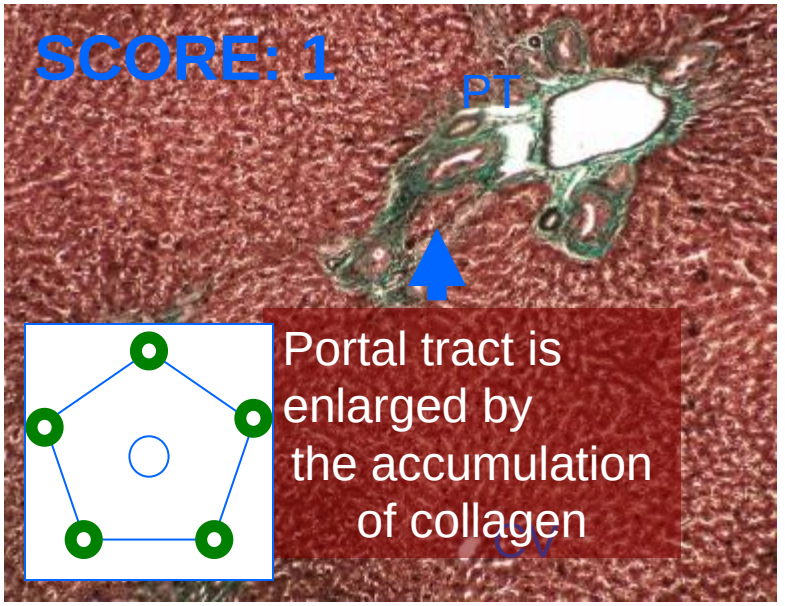
MASSON STAIN: Knodel* scoring patterns for liver fibrosis

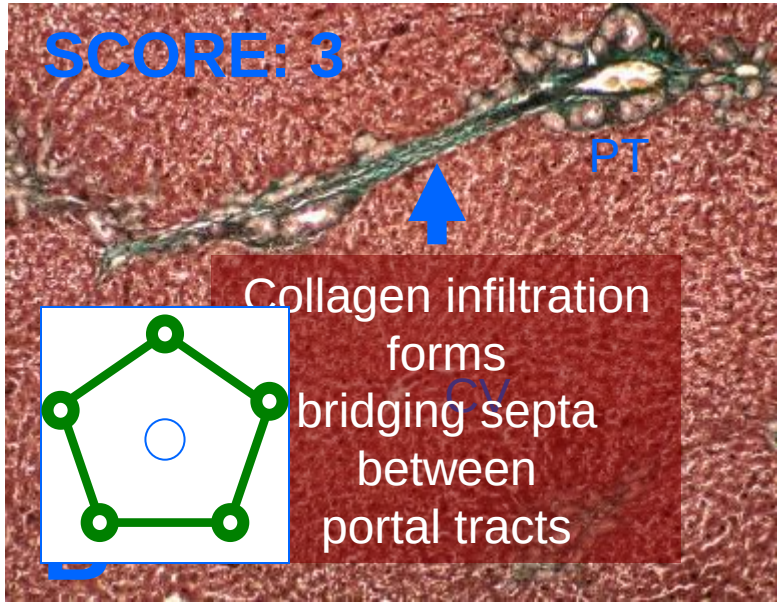
Sample Fields

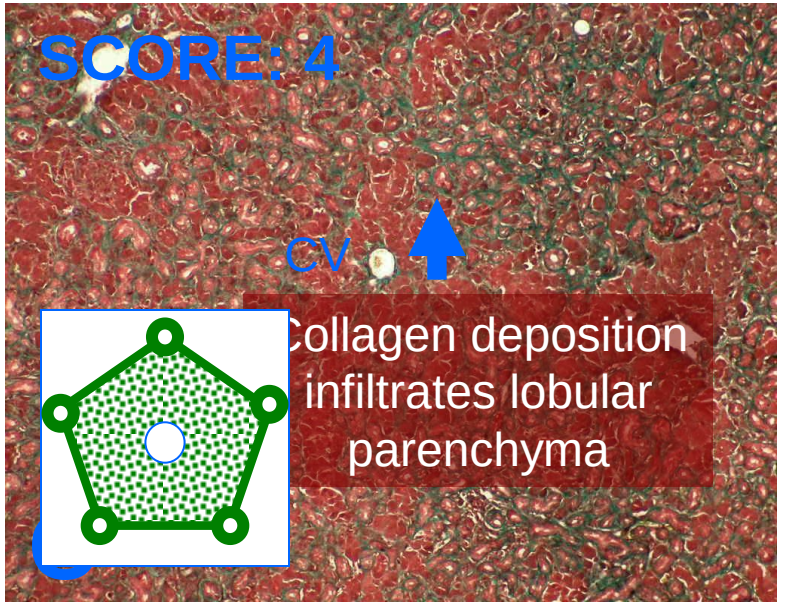
Portal Tracts



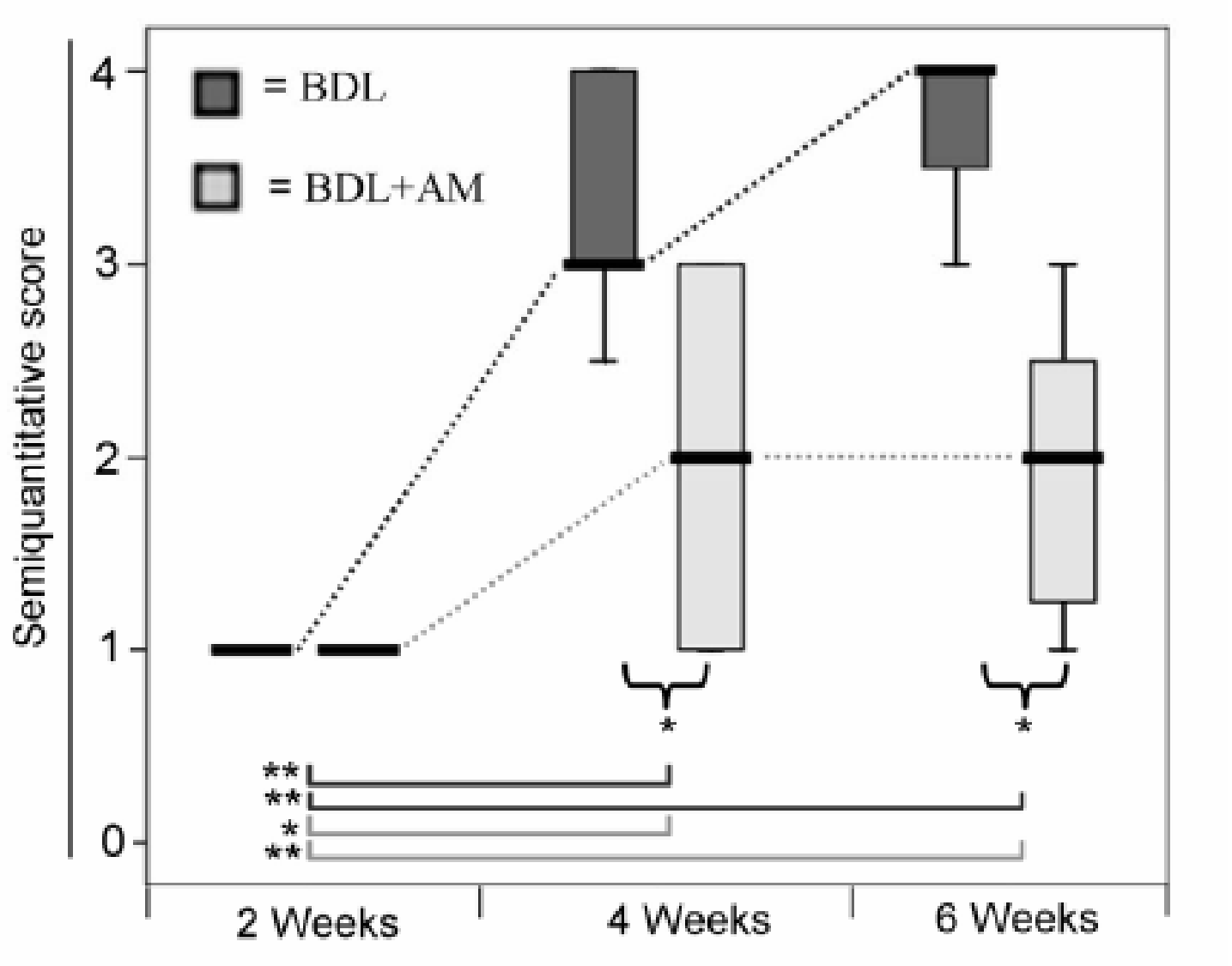








Results: Knodel semiquantitative fibrosis scroing system



UNPUBLISHED DATA

Liver fibrosis

DISEASE MODEL: liver fibrosis induced by bile duct ligation (BDL) in rats

TREATMENT: Amniotic membrane application

Amniotic membrane application significantly reduced liver fibrosis induced in rats by BDL

Steps toward clinical application of placenta : “in vivo” experiments

- Pulmonary fibrosis
- Myocardial ischemia
- Liver fibrosis

Which treatment was applied in these disease models?

Pulmonary fibrosis → **Fetal membrane-derived cells**

Myocardial ischemia → **Amniotic membrane application**

Liver fibrosis → **Amniotic membrane application**

What results were obtained?

Pulmonary fibrosis

(Cargnoni A. et al. Cell Transplant; 2009)

Placenta-derived cell transplantation significantly reduced bleomycin-induced lung fibrosis

Myocardial ischemia

(Cargnoni A. et al. Cell Transplant; 2009)

Amniotic membrane application significantly improved cardiac functions in ischemic rat hearts for at least 2 months post-injury

Liver fibrosis

(Sant`Anna Barros L. et al. submitted)

Amniotic membrane application significantly reduced liver fibrosis induced in rats by BDL


PHASE TWO: INTERPRETATION
SIDMAN The Post-Ledger
I THINK I FOUND A CORNER PIECE.
3 BILLION PIECES
GENOME

Database for the precise description of the experimental plan and the correlation between the parameters in the set up and the results

Network of databases to compare results: using different stem cells for the same clinical application.... and different application with the same cell type.

Image analysis system to quantify different type and properties of cells

Evaluation systems that are not only analysing a single slide/section, but the entire organ

REGENERATION versus REPAIR

In vivo studies demonstrate mainly the ability of amniotic cells/amniotic membrane to modify the environment and exert paracrine effects that improve local surrounding tissue favouring repair from the host cells

NEW WAY TO CONSIDER CELL THERAPY?

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