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
TODAY, Increasing problem:
Tissue and Organ shortage and rejection
Regenerative Medicine

Tissue Engineering

Biosurgery

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

- Cellular biology
- Material science
- Surgery
- Pharmacology
- Engineering

Construct for transplantation

COMPUTATION CHALLENGES
So which are the challenges:....
Need to:

- monitor the course of experimental procedures;

- gather, smooth, and record data and signals;

- provide an effective medium through 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.
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 cycle, 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

PROGENITOR CELL
(e.g., myeloid progenitor cell)

SPECIALIZED CELL
(e.g., neutrophil)

SPECIALIZED CELL
(e.g., red blood cell)

SPECIALIZED CELL
(e.g., neuron)
“Potency” of Stem Cells

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

Stem Cell Potency

Embryonic

“Pluripotent”

Bone marrow

“Multipotent”

Satellite cell

“Unipotent”

“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
Amnion

- Amniotic Epithelial Cell
- Amniotic Mesenchymal Cell

Chorion

- Chorion mesenchymal cell
- Chorionic Trofoblastic cell nuclei

Question: What are the layers of the Amnion and Chorion? Please list them.

Answer: The Amnion layers include:
- Amniotic Epithelium
- Compact Layer
- Cellular Layer
- Spongy Layer

The Chorion layers include:
- Chorionic Mesoderm
- Chorionic Trofoblast

Additionally, there is a basal membrane between the Amnion and Chorion.

Image: "Reflected fetal membranes" showing the relationship between the Amnion and Chorion.
Amniotic membrane enzymatic digestion

AEC = amniotic epithelial cells
AMSC = amniotic mesenchymal stromal cells

Enzymes:
- Dispase
- Collagenase + Dnase
- Trypsin

AMSC
AEC
Differentiation Potential of AMC and CMC

**OSTEOGENIC LINEAGE**
(alizarin red staining)

**ADIPOGENIC LINEAGE**
(oil red staining)

**CHONDROGENIC LINEAGE**
(toluidine blue staining)

Soncini M. et al  J TERM 2007
IMMUNOMODULATORY FEATURES OF AMNIOTIC DERIVED CELLS
R: PBMNC from subject A
S*: PBMNC from subject B after irradiation
A: Amnion derived cells
AMSC inhibit 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
INFLAMMATION

FIBROSIS

bleomycin
ALLO-TRANSPLANTATION

BALB/c mice

C57BL/6 mice

GFP mice

C57BL/6 mice

XENO-TRANSPLANTATION

C57BL/6 mice
FIBROSIS

Bleomycin

- day 3
- day 7
- day 14

Bleomycin + allo-transplantation
Allo-transplantation

Intra-tracheal delivery

Xeno-transplantation

Intra-peritoneal delivery

Cargnoni et al, Cell Transplantation 2009
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

**Inflammation severity**

- **Inflammation extent** (% of area involved)
- *n* = 19
- *n* = 8
- *n* = 3
- *n* = 3
- *n* = 7

**Fibrosis severity**

- **Fibrosis extent** (% of area involved)
- *n* = 19
- *n* = 8
- *n* = 7
- *n* = 3

“**Bleo**”

- IP route
- IT route

“**Bleo+Cells**”

- IP route
- IT route

“**Cells**”

- IP route
- IT route

Cargnoni et al, Cell Transplantation 2009
Xeno-transplantation

Inflammation severity (score units)

Fibrosis severity (score units)

n=19  n=19
n=8  n=8
n=7  n=7
n=4  n=4
n=3  n=3

0  1  2  3  4  5  6  7

0  25  50  75  100

day 14

Inflammation extent (% of area involved)

Fibrosis extent (% of area involved)

“Bleo”  “Bleo+Cells”  “Cells”

IP route  IT route  IP route  IT route

Cargnoni et al, Cell Transplantation 2009
Pulmonary fibrosis

<table>
<thead>
<tr>
<th>DISEASE MODEL:</th>
<th>Bleomycin-induced lung fibrosis in mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>TREATMENT:</td>
<td>Xenogeneic cell transplant</td>
</tr>
<tr>
<td></td>
<td>Allogeneic cell transplant</td>
</tr>
<tr>
<td>TREATMENT ROUTE:</td>
<td>Systemic delivery= IP injection</td>
</tr>
<tr>
<td></td>
<td>Local delivery= IT injection</td>
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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**

**Time (days):**
- 0
- 7
- 30
- 60
- 90

**Ischemia induction**

**Ischemia induction + amnion application**

**Echocardiography**

**Echocardiography**

**Echocardiography**

**Echocardiography**

**Sacrifice**

**EXPERIMENTAL GROUPS:**
- Failure Group \((n=14)\)
- Amnion Group \((n=16)\)
- Control Group \((n=8)\)

**Selection of rats showing infarct size >10%**
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

**Systolic diameter (mm)**

**Diastolic diameter (mm)**

<table>
<thead>
<tr>
<th>Days</th>
<th>Control Group</th>
<th>Ischemia Group</th>
<th>Ischemia+amnion Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>5</td>
<td>6</td>
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</tr>
<tr>
<td>30</td>
<td>6</td>
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</tr>
<tr>
<td>60</td>
<td>7</td>
<td>8</td>
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</tr>
<tr>
<td>90</td>
<td>8</td>
<td>9</td>
<td></td>
</tr>
</tbody>
</table>

- p<0.05
- p>0.05

- ○○ Control Group
- ■■ Ischemia Group
- ▲▲ Ischemia+amnion Group
Cardiac dimensions: left ventricle wall thickness

**Systolic LV wall thickness (mm)**

- Control Group
- Ischemia Group
- Ischemia+amnion Group

**Diastolic LV wall thickness (mm)**

- Control Group
- Ischemia Group
- Ischemia+amnion Group

*p* < 0.05
Cardiac function parameters

**Fractional shortening (%)**

- Control Group
- Ischemia Group
- Ischemia+amnion Group

**Ejection fraction (%)**

- Control Group
- Ischemia Group
- Ischemia+amnion Group

Days: 7, 30, 60, 90

- p<0.01
- P<0.01

Legend:
- ○ Control Group
- ■ Ischemia Group
- ▲ Ischemia+amnion Group
Myocardial ischemia induced by coronary ligation in rats

DISEASE MODEL:

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

Fibrotic LIVER

Ligation
Surgery: Assessing BDL Model

A. Common Bile duct Exposed
B. Bile duct Double Ligated
C. Bile duct Cut between ligatures
Study Groups

BDL Group

Sacrifice: 2, 4, 6 weeks

BDL+AM Group

Amniotic Membrane

Sacrifice: 2, 4, 6 weeks
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: Knodell* scoring patterns for liver fibrosis

Sample Fields

**SCORE: 0**

Periportal area is morphologically normal

**SCORE: 1**

Portal tract is enlarged by the accumulation of collagen

**SCORE: 3**

Collagen infiltration forms bridging septa between portal tracts

**SCORE: 4**

Collagen deposition infiltrates lobular parenchyma
Results: Knodel semiquantitative fibrosis scoring system
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)

**Myocardial ischemia**
(Cargnoni A. et al. Cell Transplant; 2009)

**Liver fibrosis**
(Sant`Anna Barros L. et al. submitted)

- Placenta-derived cell transplantation significantly reduced bleomycin-induced lung fibrosis
- Amniotic membrane application significantly improved cardiac functions in ischemic rat hearts for at least 2 months post-injury
- Amniotic membrane application significantly reduced liver fibrosis induced in rats by BDL
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.
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.
Marta Magatti  Luciana Barros Sant`Anna
Silvia De Munari
Patrizia Bonassi
Elsa Vertua
Daniele Rossi
Anna Cargnoni
Lorenzo Ressel
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Animal Facilities: Istituto Zooprofilattico Brescia
Università di Milano Dept. Veterinaria

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