Therapeutic Potential of Stem Cells
## Types of Stem Cells

<table>
<thead>
<tr>
<th>Attributes</th>
<th>Embryonic Stem Cells</th>
<th>Adult Stem Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>In Vitro Fertilization</td>
<td>Nuclear Transfer</td>
</tr>
<tr>
<td>Attributes</td>
<td>can produce all cell types</td>
<td>can produce all cell types</td>
</tr>
<tr>
<td></td>
<td>relatively easy to identify, isolate, maintain, and grow in the laboratory</td>
<td>relatively easy to identify, isolate, maintain, and grow in the laboratory</td>
</tr>
<tr>
<td></td>
<td>large source of “excess” blastocysts from IVF clinics</td>
<td>stem cells may be genetically matched to patient</td>
</tr>
<tr>
<td>Ethical Concerns</td>
<td>destruction of human blastocysts</td>
<td>destruction of human blastocysts</td>
</tr>
<tr>
<td></td>
<td>donation of blastocysts requires informed consent</td>
<td>donation of eggs requires informed consent</td>
</tr>
<tr>
<td></td>
<td>concern about misapplication for reproductive cloning</td>
<td>concern about misapplication for reproductive cloning</td>
</tr>
</tbody>
</table>
Stem Cell from various sources

Source:
- Blastula
- Skin fibroblasts
- Heart
- Blood
- Bone marrow
- Fat

Cell type:
- Embryonic stem cells
- Cardiac stem cells
- Endothelial progenitor cells
- Mesenchymal stem cells

Potential mechanisms of action:
- Differentiation into cardiomyocytes
- Differentiation into endothelial cells
- Differentiation into smooth muscle cells
- Paracrine effects

Potential effects:
- Direct contribution to contractility
- Remodelling of electrical properties
- Remodelling of infarcts
- Angiogenesis
- Remodelling of the extracellular matrix
- Contribution to mechanical properties of the scar
- Activation of endogenous stem cells
Various Stem Cell Therapies

**Bone Marrow**

- GVHD 3%
- Bone Regeneration 4%
- Reconstruction of Blood System 5%
- Spinal Cord Regeneration 7%
- Chondrocytes 2%
- Others 1%

**Adipose**

1. Harvest
2. Separate
3. Activate
4. Return

- A small amount of fat (~200cc) is taken from your waist area.
- Stem Cells are separated from fat cells.
- Your Stem Cells are activated with natural proteins.
- The activated Stem Cells are returned back to you through an IV to repair damaged tissue.

**Diabetes** 48%

- Cardiovascular Disorders 27%
- HIV 4%
- Mesenchymal Stem Cell

**Confidential**
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Bone Marrow</th>
<th>Fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Source /Technique</td>
<td>Bone Marrow aspiration</td>
<td>Lipoaspiration</td>
</tr>
<tr>
<td>Technique time</td>
<td>3 day Hospital admittance</td>
<td>Day care center</td>
</tr>
<tr>
<td>OT requirement</td>
<td>General/Lumbar anesthesia</td>
<td>Local anesthesia/ mild sedation</td>
</tr>
<tr>
<td>Degree of Pain</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Types of cells</td>
<td>HSCs: 0.6% MSCs: 0.002% WBCs,RBCs etc</td>
<td>HSCs: 20-30% MSCs: 30-40% Adipocytes, WBCs,RBCs etc</td>
</tr>
<tr>
<td>Max Vol of aspiration</td>
<td>100 ml</td>
<td>16000 ml</td>
</tr>
<tr>
<td>Manipulation of Stem Cells</td>
<td>Yes - Culture</td>
<td>No - use directly</td>
</tr>
</tbody>
</table>
Original article

Transplantation of autologous adipose-derived stem cells ameliorates cardiac function in rabbits with myocardial infarction

ZHANG Duan-zhen, GAI Lu-yue, LIU Hong-wei, JIN Qin-hua, HUANG Jian-hua and ZHU Xian-yang

Research article

Human multipotent adipose-derived stem cells restore dystrophin expression of Duchenne skeletal-muscle cells in vitro


Adipose-Derived Stem Cells: Characterization and Current Application in Orthopaedic Tissue Repair

Hazel Tapp, Edward N. Hanley Jr, Joshua C. Patt, and Helen E. Gruber

STEM CELLS

The Immunogenicity of Human Adipose-Derived Cells: Temporal Changes In Vitro

KEVIN McINTOSH, SANJEEV ZYONIC, SARA GARRETT, JAMES B. MITCHELL, Z. ELIZABETH FLOYD, LORA HAMMILL, AMY KLOSTER, YUAN DI, HALSVOSEN, JENNY P. TING, ROBERT W. STORMS, BRIAN GOH, GAIL KIERON, XIANG WU and JEFFREY M. GIMBLE

The Importance of Adipose-Derived Stem Cells and Vascularized Tissue Regeneration in the Field of Tissue Transplantation

Rei Ogawa

Department of Plastic and Reconstructive Surgery, Department of Biochemistry and Molecular Biology, Nippon Medical School, Tokyo, Japan

Review Article - Übersichtsarbeiten

Clinical Protocols for the Isolation and Expansion of Mesenchymal Stromal Cells

Transfusion Medicine and Hemotherapy

Transfus Med Hemother 2008;35:286-294
DOI: 10.1159/000141507

Received: June 15, 2008
Accepted: June 19, 2008
Published online: July 17, 2008
Cell Components of Adipose Tissue
Adipocytes
Adipose Stromal Cells
Vascular Endothelial Cells
Pericytes
Fibroblasts
Extracellular Matrix

Stromal Vascular Fraction (SVF)
Stromal cells + Vascular Endothelial + Mural cells
Blood cells - Leucocytes + Erythrocytes

~ 37% Leucocytes
~ 35% ASCs
~ 15% Endothelial Cells
Tumescent Lipoaspirate

Collagenase Digestion

Lecithin Emulsification

Stromal Vascular Fraction

Resuspended for final application
Your Fat Stem Cells to Treat

1. A small amount of fat - 200cc is taken from your waist area.
2. Stem Cells are separated from fat cells.
3. Your Stem Cells are activated with natural proteins.
4. The activated Stem Cells are returned back to you through an IV to repair damaged tissue.
### Laboratory Results

<table>
<thead>
<tr>
<th>Antigen</th>
<th>SVF %</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD34</td>
<td>45.98 ± 9.82</td>
</tr>
<tr>
<td>CD45</td>
<td>39.11 ± 7.24</td>
</tr>
<tr>
<td>CD73</td>
<td>36.84 ± 5.76</td>
</tr>
<tr>
<td>HLA DR</td>
<td>23.28 ± 2.18</td>
</tr>
</tbody>
</table>

Data are presented as the mean ± standard deviation obtained from the number of samples indicated in parentheses.

<table>
<thead>
<tr>
<th>Culture Type</th>
<th>Count (n=18)</th>
<th>Viability (n=18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacterial Culture</td>
<td>&lt; 2 (EAPC) CFU/ml = Not detected = ND</td>
<td></td>
</tr>
<tr>
<td>Fungal Culture</td>
<td>&lt; 10 (EAPC) CFU/ml = Not Detected = ND</td>
<td></td>
</tr>
</tbody>
</table>
Aspirated VS Intact Fat
Cell-Assisted Lipotransfer (CAL)

- **Excised whole fat (progenitor-rich fat)**
- **Aspirated fat (progenitor-poor fat)**
- **Centrifuged fat**
- **SVF-supplemented centrifuged fat (progenitor-rich fat)**

Steps:
1. Liposuction
2. Centrifugation
3. Collagenase digestion
4. Freshly isolated SVFs
30 yr woman, CAL, 310 ml in each breast
Top - Preoperative
Bottom - Post Operative 24 months
Mammogram 24 months showed no abnormality
Case Study

33 y woman
210 ml saline implants
CAL 260 ml
Mammograms at 12 months

Saline

CAL
Idiopathic Pulmonary Fibrosis

- **IPF 0.04% of total population (0.4Mn India incidence)**
  - Disease attributable to smoking and air pollution
  - No available drug has consistently shown any effect in improving lung function or reducing the number of deaths
  - **IPF is a death sentence**
  - Life expectancy is for around 2 years from the time of disease onset depending on the severity

Understanding IPF

Normal Lung

IPF Lung

Pulmonary Fibrosis

Inflammatory Cells
- Eosinophil
- Mast cell
- Macrophage
- Lymphocyte

Parenchymal Cells
- Epithelial Cells
- Endothelial Cells

Mediators
- PDGF
- HB-EGF
- TGFβ
- TGFβ-1
- Endothelin-1
- IL-1
- FGF-2
- PGE2
- IFNγ
- TNF

Fibroblast

Confidential
Stem cell compartments in the lungs. The endoderm-derived epithelium subdivided into at least 4 types: whereas smooth muscle, fibroblasts, and vascular cells are derived from mesoderm.

The coordinated interaction of multiple cell types, including alveolar epithelium, interstitial fibroblasts, myofibroblasts and pulmonary endothelium, is necessary to form alveolar septa.
Rationale for IPF
### Stem Cell Characteristics

<table>
<thead>
<tr>
<th>Parameter</th>
<th>USP</th>
<th>Benefit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activation</td>
<td>PRP</td>
<td>Faster Homing to injury</td>
</tr>
<tr>
<td>Additional cells</td>
<td>Macrophages</td>
<td>Eat away dead tissue</td>
</tr>
<tr>
<td>Cell viability</td>
<td>94%</td>
<td>More live cells to do the work</td>
</tr>
<tr>
<td>Stem Cell Properties</td>
<td>36% MSCs, 40% HSCs As per CoA</td>
<td>Proof that stem cells present</td>
</tr>
</tbody>
</table>

- Processing differently, application of rationale leading to higher efficacy and IPR.
Clinical Outcome – claudicating distance
Clinical Outcome – Forced Vital Capacity

FVC L

Clinical Outcome – FEV

FEV 1 L

Graph showing FEV 1 L over time from 14-03-09 to 8/1/2011.
Clinical Outcome – Total lung capacity

TLC L

### HbA1C

<table>
<thead>
<tr>
<th>Date</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>7th June 2010</td>
<td>HbA1C (IFCC) = 6.2%</td>
</tr>
<tr>
<td>24th August 2010</td>
<td>HbA1C (IFCC) = 5.5%</td>
</tr>
</tbody>
</table>

### Stem Cells Administration:

<table>
<thead>
<tr>
<th>Date</th>
<th>Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>27th May 2010</td>
<td>1st</td>
</tr>
<tr>
<td>28th June 2010</td>
<td>2nd</td>
</tr>
<tr>
<td>17th July 2010</td>
<td>3rd</td>
</tr>
<tr>
<td>30th July 2010</td>
<td>4th</td>
</tr>
</tbody>
</table>

### ECHO Cardiogram

<table>
<thead>
<tr>
<th>Date</th>
<th>PH – PA pressure</th>
</tr>
</thead>
<tbody>
<tr>
<td>30-11-09</td>
<td>(moderate) 57 mmHg</td>
</tr>
<tr>
<td>12-01-10</td>
<td>(moderate) 58 mmHg</td>
</tr>
<tr>
<td>08-03-10</td>
<td>(moderate) 55 mmHg</td>
</tr>
<tr>
<td>16-09-10</td>
<td>(mild) 45 mmHg</td>
</tr>
<tr>
<td>Parameter</td>
<td>Competitor</td>
</tr>
<tr>
<td>----------------------------</td>
<td>----------------</td>
</tr>
<tr>
<td>Disease specificity</td>
<td>COPD</td>
</tr>
<tr>
<td>Cells used</td>
<td>Bone Marrow-MSC</td>
</tr>
<tr>
<td>Activation of cells</td>
<td>No</td>
</tr>
<tr>
<td>Type of cell</td>
<td>Single type (MSC)</td>
</tr>
<tr>
<td>Activated Macrophages</td>
<td>No</td>
</tr>
<tr>
<td>Therapy</td>
<td>Only MSC</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>
KFT - Creat

Normal Range 0.5 - 1.6

Dose 1

Dose 2

Courtesy – Bijoy / Priya Jain
Right Hip – with broken acetabular margins with extensive lytic areas

Sclerosis suggestive of increased Ca^{2+} suggestive of reduced Osteopenia

Date: 28th July, 2010

Date: 23rd Sept, 2010

Right Hip – well defined cortical margin

Osteo applications

Courtesy – Bijoy / Priya Jain
Fat Stem Cells - Osteo

Osteo

Healing after adipose SCT
Fat Stem Cells for Multiple Sclerosis

Patient 1

Patient 2
Fat Stem Cells for Liver Cirrhosis

Pre Injection CT scan

Post Injection CT scan

Ascites
Regenerative Cellutions

Day 0

Day 7

Day 15
Moving Forward

Adipose Tissue

Clinical Trials, IPR
- IPF
- ESLD

Stromal Vascular Fraction

Cosmetology
- Cell Assisted lipotransfers

Adherent Mesenchymal Stem Cells

Forward Path

Dental Pulp Stem Cell Banking

Fibrosis
Ischemia
Neural
Allogenic Mesenchymal Stem Cells

- Ease of isolation and scale up
- Efficient large scale expansion
- Lower cost of cell culture process
- **Immune privileged**
- no ethical issues
- **No risk of teratoma formation**

Critical Limb Ischemia treated with allogenic MSCs

Before treatment

After treatment
MSC’s can maintain their “stemness” over several passages

One 10 Cell Stack gives approx 750Mn Cells
**Dental Pulp Stem Cell Banking**

- Dental pulp is a rich source of Mesenchymal Stem cells
- MSCs can be isolated from falling milk teeth and extracted teeth
- Banking of Dental Pulp Stem Cells (DPSC) offers significant advantages over cord blood

<table>
<thead>
<tr>
<th>Dental Pulp</th>
<th>Cord Blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>Easy access, dentist’s clinic</td>
<td>Requires hospital setting, during childbirth</td>
</tr>
<tr>
<td>MSCs make up a large part of the cell population, i.e. better ‘quality’ stem cells</td>
<td>Very low concentration of MSCs in Cord Blood. Cord Tissue would be required</td>
</tr>
<tr>
<td>Banking requires basic regulatory approvals</td>
<td>Blood Bank license required for banking operations</td>
</tr>
<tr>
<td>Decreased risk due to multiple teeth</td>
<td>Chances of failure to culture</td>
</tr>
<tr>
<td>Untapped market, no indigenous companies (only collection centres)</td>
<td>Saturated market with &gt;10 players</td>
</tr>
</tbody>
</table>

MSC’s isolated from dental pulp can form
- Nerve and spinal cord
- Brain
- Heart
- Liver
- Bone
- Ligaments and cartilage
- Muscle
- Skin
Facility

Phase 1 (SVF)
Class 10K clean room
37°C incubators
Centrifuges
Biosafety Cabinets
LN$_2$ Dewars
-86°C Freezer
-20°C Freezer
(approx investment= Rs. 70L)

Phase 2 (MSC)
Class 10K clean room
37°C CO$_2$ incubators
Centrifuges
Biosafety Cabinets
LN$_2$ Dewars
-86°C Freezer
-20°C Freezer
(approx investment= Rs. 70L)
<table>
<thead>
<tr>
<th></th>
<th>Phase 1</th>
<th>Phase 2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Type of stem cells</strong></td>
<td>Adipose SVF</td>
<td>Adipose MSC</td>
</tr>
<tr>
<td><strong>Type of therapy</strong></td>
<td>Autologous</td>
<td>Allogenic</td>
</tr>
<tr>
<td><strong>Business model</strong></td>
<td>Service</td>
<td>Product</td>
</tr>
<tr>
<td><strong>Scalability</strong></td>
<td>Moderate to low</td>
<td>High</td>
</tr>
<tr>
<td><strong>Banking</strong></td>
<td>Service- Dental pulp stem cells</td>
<td>Mass manufacture of vialled stem cells for sales distribution</td>
</tr>
<tr>
<td><strong>Business</strong></td>
<td>Restricted by cold chain to major metros and sub metro</td>
<td>Global distribution</td>
</tr>
</tbody>
</table>

**Bottlenecks to Growth**

- **Phase 1**
  - Clinical trials
  - Infrastructure: Cold chain, scale up

- **Phase 2**
  - USFDA approved trials
  - USFDA facility
## Potential Market Sizes for Therapies

<table>
<thead>
<tr>
<th>Unmet Medical Therapies</th>
<th>Market Size - India - No. of cases</th>
<th>Market Size - Global - No. of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ischemic disorders</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td>31 Mn</td>
<td>180 Mn</td>
</tr>
<tr>
<td>Ischemic Heart Disease</td>
<td>1.5 (Deaths) Mn YoY</td>
<td>7.1 (Deaths) Mn YoY</td>
</tr>
<tr>
<td>Ischemic Stroke</td>
<td>1 Mn</td>
<td>15 Mn</td>
</tr>
<tr>
<td><strong>Fibrosis disorders</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IPF</td>
<td>400,000 (YoY)</td>
<td>2.4 (YoY) Mn</td>
</tr>
<tr>
<td><strong>Neurological disorders</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver Cirrhosis</td>
<td>5 Mn</td>
<td>17 Mn</td>
</tr>
<tr>
<td>Alzheimer’s Disease</td>
<td>4 Mn</td>
<td>24 Mn</td>
</tr>
<tr>
<td>Multiple Sclerosis</td>
<td>50,000</td>
<td>2 Mn</td>
</tr>
<tr>
<td>Parkinson’s Disease</td>
<td>1 Mn</td>
<td>6.3 Mn</td>
</tr>
</tbody>
</table>

Projected growth for Stem Cell Therapy

The market for Stem Cell therapies is growing rapidly
Differentiating Stem Cells
Fortis to set up stem cell clinical trial centres

BS Reporter / New Delhi March 15, 2011, 0:00 IST