THE ORTHOPEDIC APPLICATIONS OF STEM CELLS

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You can be anything you want to be when you grow up.

STEM CELL PARENTAL ADVICE
Caution: The results of stem cells injections depend on the physicians specialty

SnowWoman by Plastic Surgeon

SnowMan by Urologist
Definitions

**Stromal Vascular Fraction (SVF)** – freshly isolated heterogeneous cell fraction, isolated from native adipose tissue or liposuction aspirates.

**Adipose-derived stem cells = adipose-derived stromal cells (ASCs) = adipose derived MSC** – homogeneous, plastic adherent cell population, derived from SVF and propagated in culture.
Composition of Stromal Vascular Fraction (SVF)

SVF contains many types of:
- Nucleated (Mature, Progenitor, Stem) cells,
- and
- Non-Nucleated cells, (RBC, ECM) Microvascular Elements, and non-cell objects.
Composition of Stromal Vascular Fraction (2)

Composition of SVF can Vary Significantly!

Adipose-derived stromal (stem) cells represent up to 10% (2-10%) of SVF
Cell Counters

Fluorescent automated count of nucleated and non-nucleated cells and viability assessment:
1) Concentration of cells (in cells/ml), and 2) viability (in%)
Adipose Tissue MSC proliferation was greater in cells isolated from donors aged less than 30 years old compared to donors over 50 years old.
I’ll have a double-shot!
Minimal Criteria for defining human Multipotent Mesenchymal Stromal Cells (MSC) by ISCT 2006 criteria

1. **Adherent to plastic**
   MSC must be plastic-adherent when maintained in standard culture conditions

2. **Specific surface antigen (Ag) expression**
   Level of purity. ≥95% of the MSC population must express CD105, CD73 and CD90, as measured by flow cytometry. Additionally these cells must lack expression (≤2% positive of CD45, CD34, CD14, etc.)

3. **Must be able to differentiate**
   to osteoblasts (Alizarin Red staining), adipocytes (Oil Red) and chondroblasts (Alican blue or immunohistochemically staining for Collagen type II)
Minimal Criteria for defining human Multipotent Mesenchymal Stromal Cells (MSC) by ISCT 2006 criteria

1. Adherence to plastic in standard culture conditions
2. Phenotype  
   Positive (≥ 95% +)  
   CD105  
   CD73  
   CD90  
   Negative (≤ 2% +)  
   CD45  
   CD34  
   CD14 or CD11b  
   CD79α or CD19  
   HLA-DR
3. In vitro differentiation: osteoblasts, adipocytes, chondroblasts  
   (demonstrated by staining of in vitro cell culture)
Matrix Metalloproteinase (MMP) as a Biomarker in Osteoarthritis

MMPs are a family of enzymes that play a crucial role in the degradation of extracellular matrix proteins.

<table>
<thead>
<tr>
<th>MMP Type</th>
<th>Distribution</th>
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</thead>
<tbody>
<tr>
<td>MMP-1</td>
<td>chondrocytes</td>
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<tr>
<td>MMP-2</td>
<td>osteoblasts</td>
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<tr>
<td>MMP-3</td>
<td>adipocytes</td>
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</tbody>
</table>

The specific MMP-1 distribution is chondrocytes, MMP-2 is osteoblasts, and MMP-3 is adipocytes.

The degree of heterogeneity depends, in part, on the adipose tissue depot site and the digestion protocol.

References:

### Clinical Findings in Humans (1)

<table>
<thead>
<tr>
<th>Ref.</th>
<th>Study type</th>
<th>Pathology</th>
<th>Cell type and source</th>
<th>Injection/implantation</th>
<th>Study design</th>
<th>Number of patients</th>
<th>Follow-up</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pak 2011, <em>J Med Case Reports</em> [50]</td>
<td>Case report</td>
<td>Knee OA</td>
<td>SVF Abdominal area</td>
<td>1 injection</td>
<td>(i) ADSCs + PRP + HA + dexamethasone</td>
<td>2</td>
<td>3 months</td>
<td>Cartilage volume increased at MRI. Both improved function</td>
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<tr>
<td>Koh and Choi 2012, <em>The Knee</em> [19]</td>
<td>Comparative study</td>
<td>Knee OA</td>
<td>Infrapatellar fat pad</td>
<td>1 injection after debridement</td>
<td>(i) ADSCs + PRP (ii) Only PRP (control)</td>
<td>Study group: 25 Control group: 25</td>
<td>16.4 months</td>
<td>Significant improvement in all clinical scores. Study versus control: n.s. at final follow-up, but study group had lower basal</td>
</tr>
<tr>
<td>Pak et al. 2013, <em>BMC Musculoskeletal Disorders</em> [20]</td>
<td>Case series</td>
<td>Knee OA</td>
<td>SVF Abdominal area</td>
<td>1 injection</td>
<td>(i) ADSCs + PRP + HA</td>
<td>91</td>
<td>30 months</td>
<td>VAS improved 50–60%. No major complications</td>
</tr>
<tr>
<td>Pak et al. 2013, <em>PLoS One</em> [21]</td>
<td>Case series</td>
<td>Chondromalacia patellae</td>
<td>SVF Abdominal area</td>
<td>1 injection</td>
<td>(i) ADSCs + PRP + HA</td>
<td>3</td>
<td>12 months</td>
<td>Pain improved: 50–70% at 1 m 80–90% at 3 m</td>
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<td>Ref.</td>
<td>Study type</td>
<td>Pathology</td>
<td>Cell type and source</td>
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<tr>
<td>Koh et al. 2013, <em>Knee Surg Sports Traumatol Arthrosc</em> [51]</td>
<td>Case series</td>
<td>Knee OA</td>
<td>SVF Buttocks</td>
<td>1 injection</td>
<td>(i) ADSCs + PRP</td>
<td>30</td>
<td>24 months</td>
<td>Significant clinical improvement 14/16 (87.5%) of 2nd look arthroscopy within 24 m improved or maintained cartilage status Further clinical improvement 24 versus 12 m</td>
</tr>
<tr>
<td>Koh et al. 2013, <em>Arthroscopy</em> [49]</td>
<td>Case series</td>
<td>Knee OA</td>
<td>Infrapatellar fat pad</td>
<td>1 injection after debridement</td>
<td>(i) ADSCs + PRP</td>
<td>18</td>
<td>24.3 months</td>
<td>Significant improvement of the clinical and MRI scores at final follow-up</td>
</tr>
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<td>Bui et al. 2014, <em>Biomedical Research and Therapy</em> [50]</td>
<td>Case series</td>
<td>Knee OA</td>
<td>SVF Abdominal area</td>
<td>1 injection</td>
<td>(i) ADSCs + PRP</td>
<td>21</td>
<td>6 months</td>
<td>Function improvement in all patients at 8.5 m. Increased cartilage thickness on MRI</td>
</tr>
<tr>
<td>Jo et al. 2014, <em>Stem Cells</em> [60]</td>
<td>Case series</td>
<td>Knee OA</td>
<td>Expanded Abdominal area</td>
<td>1 injection</td>
<td>(i) Phase I: 3 doses of ADSCs; the low-, mid-, and high-dose group with 3 patients each (ii) Phase II: 9 patients: receiving the high dose of ADSCs</td>
<td>18</td>
<td>6 months</td>
<td>Clinical improvement and hyaline-like regenerative tissue only in high-dose group, without adverse events</td>
</tr>
<tr>
<td>Koh et al. 2014, <em>Am J Sports Med</em> [52]</td>
<td>Case series</td>
<td>Knee OA</td>
<td>SVF Buttocks</td>
<td>1 injection after debridement</td>
<td>ADSCs local adherent technique</td>
<td>35 (37 knees)</td>
<td>12.7 months</td>
<td>Clinical improvement; 94% patients excellent or good satisfaction 76% abnormal or severely abnormal repair tissue at 2nd look</td>
</tr>
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<td>Koh et al. 2014, <em>Arthroscopy</em> [53]</td>
<td>Comparative study</td>
<td>Knee OA</td>
<td>SVF Buttocks</td>
<td>1 injection after debridement</td>
<td>(i) HTO + PRP + ADSCs ($n = 23$) (ii) HTO + PRP ($n = 21$)</td>
<td>44</td>
<td>24 months</td>
<td>Better clinical improvement in PRP + ADSCs group (some KOOS subgroups) Better tissue healing at 2nd look for PRP + ADSCs</td>
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<tr>
<td>Kim et al. 2015, <em>Am J Sports Med</em> [54]</td>
<td>Comparative study</td>
<td>Knee OA</td>
<td>SVF Buttocks</td>
<td>1 injection after debridement</td>
<td>(i) ADSCs local adherent ($n = 37$) (ii) ADSCs on FG ($n = 17$)</td>
<td>54 (56 knees)</td>
<td>12.3 m (second look)</td>
<td>Overall clinical improvement Comparable for both groups Better IKSS scores at 2nd look for ADSC-PG group Lower BMI and smaller size positively correlate with outcomes</td>
</tr>
</tbody>
</table>
Osteoarthritis of the knee

**Stage 3**

- Stage 3 OA is classified as “moderate” OA. The cartilage between bones is showing obvious damage, and the space between the bones is narrowing. People with stage 3 OA of the knee are likely experiencing frequent pain when walking, running, bending, or kneeling. They also may experience joint stiffness after sitting for long periods of time or when waking up in the morning. Joint swelling may be present after extended periods of motion, too.

- X-rays will often show narrowing of the joint space (the gap between the bones) and loss of the smooth surface of the bones with some osteophyte formation - bony knee spurs.
Intra-articular injection of AD MSC for treatment of Osteoarthritis of the Knee (Jo et al. 2014 Seoul, Korea)

- **Injected**: 3 patients with low dose (1.0x10^7 cells), 3 patients with mid-dose (5.0x10^7 cells) and 12 patients with high-dose (1.0x10^8 cells) in 3 ml of saline. Cells (culture-expanded hAdMSC) were prepared from the abdominal subcutaneous fats under GMP and tested for cell number, viability, purity, identity, sterility, endotoxin and mycoplasma before shipping.

- **Results**: High-dose injection improved function and pain of the knee without causing adverse events, and reduced cartilage defects by regeneration of hyaline-like articular cartilage.
Articular cartilage regeneration in the high-dose (100 M cells) group as seen on MRI (Jo et al. 2014)

Medial femoral condyle - green arrow and Medial tibial condyle - yellow arrow.

Regenerated cartilage became thicker, smoother and more mature. The area of the cartilage defect in both condyles was decreased 3 and 6 months after injection.
Articular cartilage regeneration in the **low-dose** (10 M cells) group as seen on MRI *(Jo et al. 2014)*

Medial femoral condyle - green arrow and Medial tibial condyle - yellow arrow.

In the low-dose group no significant changes are identified after injection at 3 months. Small cartilage island is barely noticed in MFC at 6 months.
Arthroscopic evaluation of articular cartilage in the high dose (100 M cells) group after AD MSC injection (Jo et al. 2014)

MFC – medial femoral condyle
MTC – medial tibial condyle

Absence of articular cartilage in both condyles before the injection.

Thick, glossy white and firm hyaline-like regenerated cartilage covers the majority of cartilage defects.
Arthroscopic evaluation of articular cartilage in the low-dose (10 M cells) group after AD MSC injection (Jo et al. 2014)

MFC – medial femoral condyle
MTC – medial tibial condyle

Large denuded medial femoral and tibial condyles before injection.

After 6 months, while small cartilage islands are newly formed in both condyles, the majority of denuded both condyles are not changed.
Articular cartilage regeneration in the high-dose (100 M cells) group as seen on MRI (Jo et al. 2014)

Changes of the cartilage volume of the medial femoral condyle (MFC) and medial tibial condyles (MTC) after high-dose injection:

MFC - green - upper row - right knee (viewed from the above)
MTC – orange – lower row – right knee (viewed from the below)

The void seen at the baseline before injection (the left column) was gradually filled.
Changes of WOMAC, VAS, KSS knee and function during 6 months after AD MSC injection (Jo et al. 2014)

Tendency of improvement was shown in all groups, however statistically significant improvement was observed only in high-dose group.

Initial decline in KSS function (D) after 2 months in all group was due to non-weight bearing for first 2 months.
Autologous Adipose Stem Cells for treatment of Osteonecrotic Femoral Heads and Damaged Meniscus (*Pak et al. 2012, 2014; Korea*)

- **Obtained**: 1) 40 ml of adipose tissue from abdominal area by liposuction to prepare 8.5 ml of ASC solution, 2) 30 ml autologous blood to prepare 4.4 ml of platelet-rich plasma (PRP), 3) 2 ml of 0.5% hyaluronic acid was added as a scaffold, 4) 0.1 ml of 3% CaCl$_2$ was added to activate PRP.

- **Injected**: 16 M stem cells into 1) knee joint (1 female patient 32 y.o.), 2) hip joint (2 male patients 34 and 39 y.o.) using 22-gauge needle.

- **Results**: Pain reduction, functional improvement and MRI evidence of tissue regeneration.
Autologous Adipose Stem Cells for treatment of Damaged Meniscus (Pak et al. 2014; Korea)

**Image (a)**
Pretreatment MRI sagittal T2 view of the knee

A tear (arrow) within the posterior horn of the medial meniscus

**Image (b)**
Posttreatment (3 months) MRI sagittal sequential T2 view of the knee

Healed meniscus (triangle)
Potential Mechanism of action of MSC

1. Transplanted cells differentiate into de novo tissue
2. Stem cells within the transplant replenish progenitor cells in the host
3. Transplanted cells may fuse with host cells (cell fusion)
4. Paracrine and Trophic effect via neovascularization and immunosuppression. MSC (ADSC) secrete growth factors (VEGF, HGF, etc.) and cytokines. Immunosuppression of lymphocytes and peripheral blood mononuclear cells prevents overinflammation, inhibits scarring (fibrosis) and apoptosis.
Orthopedic Patients Data

- 40 patients with Osteoarthritis of peripheral joints (knee, hip, shoulder, ankle) were injected with autologous adipose tissue derived SVF/PRP between July 2014 through July 2015.
- 35 patients experienced improvement reduction of pain with corresponding increase in functionality.
- 3 patients with progressive osteoarthritis (grade 3-4) with significantly reduced joint space did not manifest any improvement.
- 2 patients with Rheumatoid Arthritis did not show signs of improvement.
- No complications were observed.
Future Directions

❖ Initiate long term Randomized Controlled Trial.
❖ Evaluate results with pre- and post-treatment MRI.
❖ Utilize more validated tools specific to orthopedic patients with osteoarthritis.
❖ Improve standardization of SVF/PRP preparation and quantification of injectate content.
Conclusions

❖ Combined SVF/PRP therapy of the Degenerative Osteoarthritis of Joints has the potential to improve quality of life in a selected category of orthopedic patients (stage 2-3 osteoarthritis).

❖ Proposed treatment may reduce the frequency of joint replacement procedures and/or delay the necessity of surgical interventions.

❖ Further studies are warranted.
Thank You