Stem Cells 101

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My background

• Began using stem cells to treat orthopedic conditions in 2005
• Have published 16 peer reviewed publications on the use of MSCs to treat orthopedic conditions
• We have a university style stem cell research lab as part of our Colorado practice
• Tracking >4,000 stem cell treated patients in a non-profit registry
Types of Stem Cells?
What are stem cells?

- Undifferentiated blank slate cells that can differentiate into other cells
- Likely do most of their work through paracrine actions, exosomes, mitochondrial transfer and other mechanisms
- Main types: adult, embryonic, induced pluripotent
- Adult stem cells are the repair men of the body
- Main adult stem cell players: mesenchymal stem cells (MSCs), hematopoietic stem cells (HSCs)
Where can you get stem cells?

- Bone marrow, adipose, synovial tissue and fluid, and most body tissues (likely all)
- Two main tissues being used for orthopedic applications are bone marrow and adipose
- Much, much more research on bone marrow for orthopedic use than adipose
- Urban Myth: adipose has more stem cells than bone marrow-based on a math error (MSCs reported as a % of nucleated cells (NCs), bone marrow NC>>adipose)
Mesenchymal stem cell (MSC)

- An undifferentiated cell that’s held in reserve until replacement or repair is needed.
- It can turn into many cell types of mesodermal origin.
- It can orchestrate a repair response.
Hematopoietic Stem Cells (HSCs)

• Still some controversy over whether they can “transdifferentiate” into MSCs
• A more common physiologic event is cell fusion
• For our purposes, cell fusion is key in muscle repair
• HSCs recruited from the bone marrow to help muscle repair
Endothelial Stem Cells (ESCs)

- Facilitate vascular homoeostasis and neovasculogenesis (new blood vessel formation)
- Many chronically injured MSK tissues have poor blood supply
  - Circulation. 2003 Oct 28;108(17):2041-8
Pericytes

- Recruited from the bone marrow (like EPCs) to form new blood vessels
  - Journal of Leukocyte Biology Volume 80, October 2006
- Art Caplan (the father of MSC research), believes that pericytes create MSCs when injury is detected
Osteochondral reticular (OCR) Cells

- Self-renew and generate osteoblasts, chondrocytes, and reticular marrow stromal cells, but not adipocytes.
- Concentrated within the metaphysis of long bones and not in the perisinusoidal space (where MSCs live) and are needed for bone development, bone remodeling, and fracture repair.
  - Cell. 2015 Jan 15;160(1-2):269-84.
• **MUltilineage-differentiating Stress Enduring cells**
• Can differentiate into all three embryonic layers (endo, ecto, and meso)
• Hard to kill, activated by physical stress
• Involved in regenerative homeostasis and tissue repair
Types of Stem Cell Procedures?
Allogeneic

Cord Blood
Isolated from the blood in an umbilical cord of a fetus.

Amniotic
Cells taken from the fluid or membrane that surrounds a fetus.

Embryonic
Cells taken from the developing embryo.

Synovial
Cells isolated from the synovial fluid or membrane.

Autologous

Bone Marrow Aspirate
Isolated from the liquid part of the bone marrow.

Adipose (Fatty Tissue)
Cells taken from fatty tissue.
Amniotic Stem Cells?
Does Amniotic Fluid and Tissue Have Stem cells?

- Amniotic fluid products being sold under FDA 361 tissue registration contain no viable stem cells
- Sales reps often claim they do have MSCs
- Their FDA registrations state no stem cells
- The Interventional Orthopedics Foundation (501c3 non-profit) tested many products—all had no viable cells
- IOF testing showed they didn’t help MSCs in-vitro
- These products do contain growth factors, cytokines, and extra-cellular matrix—IOF testing showed them to be weaker in biologic effects than PRP
- May have other positive effects—not much data
Can Amniotic Fluid Help Stem Cells?

![Graph showing fluorescence intensity over time with and without amniotic fluid.](image)

**NO Amnio**

**Amniot**

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*Image of Hamburger Helper box with a red 'no' symbol over it.*
Be careful in reviewing the product data...

- As an example, only **a max** of 1.5% of cells in the living/fresh amniotic fluid are stem cells
- Simple live/dead viability rates are low
- In our research the cells are so beat up that they aren’t really viable

<table>
<thead>
<tr>
<th></th>
<th>BioDRestore®</th>
<th>AmnioFix®</th>
<th>SurForce®</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total Cell Concentration</strong></td>
<td>900,000 cells/mL</td>
<td>3,700,000 cells/mL</td>
<td>4,300,000 cells/mL</td>
</tr>
<tr>
<td><strong>Viable Cell Concentration</strong></td>
<td>660,000 cells/mL</td>
<td>1,300,000 cells/mL</td>
<td>2,700,000 cells/mL</td>
</tr>
<tr>
<td><strong>Dead Cell Concentration</strong></td>
<td>240,000 cells/mL</td>
<td>2,300,000 cells/mL</td>
<td>1,600,000 cells/mL</td>
</tr>
<tr>
<td><strong>Viability</strong></td>
<td>73%</td>
<td>36%</td>
<td>62%</td>
</tr>
<tr>
<td><strong>Average Viable Cell Size</strong></td>
<td>12.7 um</td>
<td>9.6 um</td>
<td>13.3 um</td>
</tr>
<tr>
<td><strong>Average Dead Cell Size</strong></td>
<td>7.7 um</td>
<td>10.0 um</td>
<td>6.9 um</td>
</tr>
<tr>
<td><strong>Total Cells Counted</strong></td>
<td>179</td>
<td>725</td>
<td>853</td>
</tr>
<tr>
<td><strong>Viable Cells Counted</strong></td>
<td>131</td>
<td>259</td>
<td>533</td>
</tr>
<tr>
<td><strong>Dead Cells Counted</strong></td>
<td>48</td>
<td>466</td>
<td>320</td>
</tr>
</tbody>
</table>
Amniotic Conclusions?

- These may have growth factor, cytokine, or ECM effects
- The big question for me is whether they’re better than much less expensive PRP
- Some providers have reported success using the ECM quality of these products to bridge gaps...
Two Different Types of Bone Marrow Stem Cell Processes

- **Bone Marrow Nucleated Cell Isolation**
  - The stem cell fraction of bone marrow is isolated via a centrifuge and re-injected the same day.

- **Bone Marrow Mesenchymal Stem Cell Culture**
  - The stem cells themselves are isolated and cultured to greater numbers over a few weeks. This produces a “pure” population of stem cells which is different than the mix of cells produced by same day procedures.

Three Different Types of Fat Stem Cell Processes

- **Simple Adipose Graft**
  - The fat is separated from the oil and liquid and the fat is injected (however the stem cells are still trapped in the fat and are not concentrated).

- **Stromal Vascular Fraction (SVF)**
  - The fat is separated and then chemically digested to release the stem cell fraction, which is then concentrated.

- **Adipose Mesenchymal Stem Cell Culture**
  - The stem cells are isolated and cultured to greater numbers over a few weeks. This produces a “pure” population of stem cells which is different than the mix of cells produced by same day procedures.

### Tissue
- **No FDA Approval**

### Drug
- **Needs FDA Approval**

### Tissue
- **No FDA Approval**

### Drug
- **Needs FDA Approval**

### Drug
- **Needs FDA Approval**
Bone Marrow
Bone Marrow Concentrate (BMC)

- Bone marrow obtained via a bone marrow aspiration
- BMC is made via a bedside unit or in a lab-buffy coat is isolated
- Most of the world literature on orthopedic applications is on bone marrow MSCs
A significant amount of stem cell research has already been published...

(Graphic up to date as of August 2015)
Adipose
Adipose Stem Cells

• AKA ASCs
• Live around blood vessels in adipose tissue
• Mesenchymal stem cells (distant cousins to the bone marrow type)
Adipose tissue obtained through liposuction

- Can be digested via enzymes (collagenase, trypsin, lecithin)
- Centrifuged to obtain a pellet
- This pellet contains many cells, a small percentage of which are MSCs
- This is called “Stromal Vascular Fraction” or SVF
What’s in Adipose tissue obtained through liposuction?
Bone Marrow vs. Adipose
How do bone marrow vs. adipose MSCs compare?

• There are many claims that adipose tissue contains many more stem cells than bone marrow

• Is this true or false or just one big urban myth?
Let’s do the math

• MSC counts in both are reported as a % of the total nucleated cells in the tissue
• Initially, the comparison seems that fat has a huge advantage
• Flow cytometry studies show that MSCs represent 1-5% of the total nucleated cells in fat vs. 0.01-0.5% in bone marrow!
• The problem is that the denominator is quite different for the two tissues!
There is a huge difference in the number of nucleated cells in bone marrow versus fat.
% Comparisons - Example

Bone Marrow

\[
\frac{100,000}{200,000,000} = 0.0005 \times 100 = 0.05\%
\]

Adipose

\[
\frac{100,000}{20,000,000} = 0.05 \times 100 = 5\%
\]
Conclusions

Adipose doesn’t have 500-2,000 times more stem cells.
Bone Marrow >> Adipose for HSCs

- Bone marrow - approx. 1-2% of nucleated cells
  - Blood. 1991 Jun 15;77(12):2591-6
- SVF 0.004% of nucleated cells (which is divided by 1,000 to compare to marrow)=0.000004%
What are the procedural risks of a BMA vs. Liposuction?

UK Registry-Includes both BMA and Trephine Biospies: 
*Haematologica*. 2006 Sep;91(9):1293
- **15 adverse events in 20,323 procedures**

Ultrasound Assisted Lipo: 
- **9 complications in 609 procedures**
When compared head to head, how many research papers show that bone marrow or adipose stem cells are better than the other at making cartilage or its components?

Each knee icon below links to a Pubmed reference. Click any to see abstract.

- Fat Stem Cells Better: 0 papers
- Bone Marrow Stem Cells Better: 13 papers

US National Library of Medicine Search searched 5/4/15 using terms “adipose bone marrow mesenchymal stem cell chondrogenesis”. End date of search was 6/21/07. Only papers showing head to head quantitative chondrogenesis considered. For example, papers that used gene expression differences, ultrastructural comparison of the cartilage repair produced in-vivo, or collagen type comparisons in micro mass.
Is a fat graft a stem cell procedure?
In 2012, we obtained lipo-aspirates and then ran these through a mechanical emulsifier. We got no viable stem cells either through flow cytometry nor through culture.
In 2014, we used a sophisticated vibration assisted lipo-suction machine. We got a few viable stem cells via flow cytometry and culture, but much, much less than one would see from a bone marrow concentrate procedure.
How about the new generation of fat processing systems?

- Read the fine print in the studies
- Research given to physicians uses collagenase to digest the tissue that comes out of these machines
- That’s the only way you’ll get viable stem cells
- If you do that then you’re a drug manufacturer
In the end, there may be something to using a fat graft in a joint


• However, some providers believe this works
Clinical Research
Are these procedures safe?

Main studies:

- N=339-culture expanded, bone marrow MSCs-SAFE-Centeno et al-


- N=2,372-same day BMC/culture expanded MSCs-SAFE-Centeno et al-
  accepted for publication
Stem cells may be able to help RC tear healing

- Hernigou- *Int Orthop*. 2014 Sep;38(9):1811-8. n=45 with BMC and 45 matched controls without BMC. BMC injected at the time of or after RC repair. **Better RC healing** (100% in BMC vs. 67% in control). Re-tear rate halved.
Shoulder RC Tears-US Guided Injection

Our early RCT data on proprietary BMC ultrasound guided injection for partial thickness and full thickness non-retracted RC tears:

**RCT - Preliminary Results at 3 Months (n=20)**

DASH Function (lower value is better)

<table>
<thead>
<tr>
<th>DASH Pre-Treatment</th>
<th>DASH Post-operative Month 3</th>
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<tbody>
<tr>
<td>Exercise Therapy</td>
<td>Adv BMC Inj</td>
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</table>

NPS (0-10)

<table>
<thead>
<tr>
<th>Pain Pre-Treatment</th>
<th>Pain Postoperative Month 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exercise Therapy</td>
<td>Adv BMC Inj</td>
</tr>
</tbody>
</table>
Stem cells likely help knee OA...

<table>
<thead>
<tr>
<th>Author</th>
<th>Study Type</th>
<th>Intervention</th>
<th>Patient n</th>
<th>Stem Cells Used</th>
<th>Functional Improvement</th>
<th>Notes</th>
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</thead>
<tbody>
<tr>
<td>Vagsness</td>
<td>DB RCT</td>
<td>Partial Menisectomy with MSC injection vs. placebo</td>
<td>55</td>
<td>Allo Cultured Bone Marrow MSCs</td>
<td>Yes</td>
<td>1 in 4 patients with increased meniscus Volume</td>
</tr>
<tr>
<td>Centeno</td>
<td>Prospective case series</td>
<td>Image Guided Injection</td>
<td>840</td>
<td>Bone Marrow Concentrate</td>
<td>Yes</td>
<td>2/3rds of patients were TKA candidates</td>
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<tr>
<td>Kim</td>
<td>Prospective case series</td>
<td>Injection</td>
<td>49</td>
<td>Autologous Cultured Bone Marrow MSCs</td>
<td>Yes</td>
<td>Full thickness chondral lesions &lt;6 cm/2 responded best</td>
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<tr>
<td>Vega</td>
<td>RCT</td>
<td>Injection of MSCs vs. HA</td>
<td>30</td>
<td>Allo Cultured Bone Marrow MSCs</td>
<td>Yes</td>
<td>Improved cartilage signal on MRI T2 mapping</td>
</tr>
<tr>
<td>Koh</td>
<td>RCT</td>
<td>Knee MFX + stem cells vs. MFX alone</td>
<td>44</td>
<td>Autologous cultured adipose MSCs</td>
<td>Yes</td>
<td>Better lesion coverage/MRI signal, no diff in 2nd look arthroscopy</td>
</tr>
</tbody>
</table>

Stem cells likely help hip OA?

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<thead>
<tr>
<th>Author</th>
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<th>Functional Improvement</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Centeno</td>
<td>Prospective case series</td>
<td>Image Guided Injection</td>
<td>196</td>
<td>Bone Marrow Concentrate</td>
<td>Yes</td>
<td>Majority of patients were THA candidates</td>
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<tr>
<td>Emadedin</td>
<td>Prospective case series</td>
<td>Unknown</td>
<td>5</td>
<td>Culture expanded BM MSCs</td>
<td>Yes</td>
<td>Severity unknown</td>
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### Stem cells and Lumbar DDD?

<table>
<thead>
<tr>
<th>Author</th>
<th>Study Type</th>
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<th>Patient n</th>
<th>Stem Cells Used</th>
<th>Functional Improvement</th>
<th>Notes</th>
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</thead>
<tbody>
<tr>
<td>Mochida</td>
<td>Prospective case series</td>
<td>Surgical implant</td>
<td>9</td>
<td>Autologous Nucleus Pulposis Cells</td>
<td>No-minimal MRI changes</td>
<td>Safety study</td>
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<tr>
<td>Pettine</td>
<td>Prospective case series</td>
<td>Injection into IVD</td>
<td>26</td>
<td>Bone Marrow Concentrate</td>
<td>Yes</td>
<td>Possible slight changes in MRI, but within error of DDD grading scale</td>
</tr>
<tr>
<td>Pang</td>
<td>Prospective Case Series</td>
<td>Surgical Implantation</td>
<td>2</td>
<td>Allogeneic Cord Blood MSCs</td>
<td>Yes</td>
<td>No imaging</td>
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<tr>
<td>Orozco</td>
<td>Prospective Case Series</td>
<td>Injection into IVD</td>
<td>10</td>
<td>Autologous culture expanded BM-MSCs</td>
<td>Yes</td>
<td>No improvement in disc height, some increase in T2 signal</td>
</tr>
</tbody>
</table>

Stem Cells for ACL Tears

- Precise guided fluoro injection technique
- Used pre/post MRIs with imaging analysis on n=10
- Improved pain/function and imaging of ACL
- Larger case series of n=40 preparing now
- RCT recruiting
ACL Tear Imaging Analysis
Regulatory
The regulation you need to know is 21 CFR 1271

- If what you produce in your office crosses the “minimal manipulation” line, then it’s a drug
- **There is no protection because you’re a doctor nor because you perform the procedure in a medical setting nor during the same surgical procedure**
The FDA is now telegraphing it’s punches on fat...

- Dec 2014 Draft Guidance on Adipose Tissue Regulation
- Example A-2: Adipose tissue is recovered by tumescent liposuction. Stem cells from the lipoaspirate are then isolated. Cell isolation would typically cause the adipose tissue to no longer be “such HCT/P.” Thus, even if this processed HCT/P from adipose tissue is injected into the same patient from whom it was removed during the same surgical procedure, the establishment would generally not be considered to qualify for the exception under 21 CFR 1271.15(b).
Newest developments

• Warning letter to Goinis
• Same day SVF
• Previously claimed because he didn’t have a “lab” but was working in either an office of surgery center setting—was practicing medicine
• Has now shut down web-sites for 3 clinics
What does being viewed as a drug manufacturer mean?

- You will need to have a BLA/IND in place (cost 2M USD)
- Just getting your protocol IRB approved means nothing (gets you in more trouble not less)
- You will be held to cGMP
cGMP is not what you’ve been told it is...

• Just using a hood and some protocols that claim to be cGMP isn’t the same thing as correctly manufacturing drugs

• For example:
  • The FDA regulated cleaner solution for the hood that is regulated to kill x must be independently tested on coupons of every surface in the lab by an outside lab to kill x
  • Your release testing must include all major pathogens, endotoxins, and viability including functional assays
  • Every deviation of even extra seconds on a processing step must result in the filing of a process deviation form
  • You must have a separate compliance department that the FDA can interact with
The Future
Use of stem cells in orthopedic medicine has already exploded over the last 5 years, that will continue.

By 2020:

- This will be commonplace
- In some “home run” injection applications, we’ll see more precise injections and less surgeries
- Several culture expanded MSC products will be available—insurance coverage for orthopedic applications will be spotty
- PRP will get insurance coverage for a few applications
Conclusions

• Stem cells are likely here to stay
• Early clinical data in knee OA looks promising
• Data coming in many other clinical applications
• Don’t get hammered for the production of an illegal drug
Any Questions?