Clinical Update On Stem Cells

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How I Got Here

- B.S. Kinesiology—University of Maryland.
- D.O. degree—PCOM Class of 1999.
- Traditional Internship—PCOM
- Residency in Family Medicine—PCOM
- Sports Medicine Fellowship (CAQ)—Grant Medical Center, Columbus, Ohio.
- Precision Orthopaedic Specialties—Cleveland, Ohio
- Rothman Institute (9 years)—Philadelphia, PA
- Began performing PRP at Rothman in 2008.
- Trained at Bioheart Stem Cell Lab 2014 began performing ADSC procedures.
Regenerative Orthopaedic Medicine

Field of medicine many of “us” have turned to in order to be able to give our patients more interventional options as opposed to surgery steroid injections.

Utilize the bodies own natural healing abilities to augment a failed healing response, injury, degenerative condition.

Hormone Replacement Therapy

Prolotherapy

Platelet Rich Plasma

Autologous Derived Stem Cells

Join different organizations to learn more... agemed.org, aaomed.org, acopms.com, regennex.com, a4m.com, bioheartinc.com
Adult Stem Cells

- Found in almost every organ in the body and tissues.

- Primary function of stem cells is to maintain and repair tissues in which they are found.

- These are multipotent cells which can differentiate into a subset of cell type—as opposed to pluripotent which can differentiate into any cell type.

- They exhibit the ability to become plastic—can take the form of different types of tissue depending on the environment they are surrounded by.
Adipose Derived Stem Cells (Autologous)

Population of cells found in adipose tissue that are different from those found in blood cells.

--Contain subset of multipotent progenitor cells

--Adipogenic, chondrogenic, and osteogenic differentiation potential

--Angiogenic and Vasculogenic
Evidence of Stem Cells In Fat

Disorder called progressive osseous heteroplasia in which bone forms in adipose layer of tissue.

Histology demonstrates presence of osteoblasts, chondrocytes, and adipocytes in tissue.

Defect in gene implies adipose tissue is multipotent.

Evidence of Stem Cells In Fat

- Lipomas and Liposarcomas—soft tissue tumors in subcutaneous or visceral deposits.

- Liposarcoma derived cells can be induced in vitro to differentiate into adipocytes.

- This implies that these tumors derive from a stem cell progenitor.

Circ. Res. 2007; 100;1249-1260
Stem Cells In Fat

- Turnover rate for cells within adipose tissue between 6-15 months.
- Stem Cell Population within adipose tissue is responsible for replacing mature adipocytes.
- Thus adipocytes must be generated from a progenitor or stem cell base.

Circ. Res. 2007;100;1249-1260
How Fat Develops

- Adipose tissue begins to develop during gestation.
- Dense regions of MSCs associated with vascular structures form at sites where adipose tissue develops.
- These stem cells transform into adipoblasts then into adipocytes.
- Adipose tissue expands via hypertrophy of existing fat cells and through the generation of new adipocytes.
- Again stem cell population is responsible for generating mature adipocytes.

High fat feeding promotes production of new adipocytes and expansion of existing adipocytes.

This expansion is supported by mesenchynal and hematopoietic progenitor cells which have been linked to BM origin.

Weight gain promotes the trafficking of BM derived progenitor cells to adipose tissue.
Types of Adipose Tissue

- Bone Marrow
- Brown
- Mammary
- Mechanical
- White—best type of fat
Bone Marrow Adipose Tissue

- Occupies space no longer required for hematopoiesis
- Serves as an energy reservoir and source of cytokines for osteogenic and hematopoietic events.
White Adipose Tissue

- Stores energy
  - Release of insulin from pancreas.
  - Receptors cause a dephosphorylation reaction that releases lipase.
- Stored fat is broken down to fatty acids which are released into blood and taken up by muscle and cardiac muscle as fuel.
- Provides insulation to maintain body temperature.
- 20-25% body weight.
White Fat
Characteristics

Contains large population of hematopoietic cells composed of macrophages and hematopoietic progenitor cells.

The non-hematopoietic population is mainly composed of mesenchymal stem cells.

White fat contains a large proportion of immature cells.

These cells can give rise to osteoblasts, endothelial cells, adipocytes, hematopoietic cells, and cardiomyoblasts.

White fat is most plastic adipose tissue and represents potential and excellent source of stem cells.

Adipose Derived Stem Cell Isolation (ADSC)

- Tumescent Mini-liposuction technique which obtains finely minced fragments of adipose depending on size of cannula.
- Removing hematopoietic cells with washing.
- Enzymatic digestion with collagenase.
- Obtain SVF—which is circulating blood cells, fibroblasts, pericytes, endothelial cells, and adipocyte progenitors.
- Centrifugation to separate SVF.
- Isolating SVF and washing cells with centrifugation.
- Finally cells prepared in final suspension.
Mini-Liposuction
Stromal Vascular Fraction
Collecting ADSCs

The yield can be affected by:
- Surgical technique
- Location of fat
- Enzymatic digestion
Collecting ADSCs

Best collection site for men is abdomen around the umbilicus and for women region did not influence cell yield.

Age and BMI did not affect cell yield.

Enzymatic digestion times and concentrations strongly modify the yield and viability of cells.

Highest CD34 cells in the abdominal fat.

Immunofluorescent CD Markers of ADSC

- CD34/CD31 subset

Displays distinct features from the adult mesenchymal and hematopoietic stem cells and are most important!
Characterization of ADSCs

a. Tonsils
   - Harvest, enzymatic digestion of tissue
   - Centrifuge fractionate

b. Liposuction Aspirates
   - Aspirate and seed cells pellet
   - Centrifuge fractionate
   - Fractionate enzyme digest

 MSCs

- Adipocytes (Fat cells)
- Myocytes (Smooth muscle cells)
- Neurons (Nerve cells)
- Adipocytes (Bone cells)
- Chondrocytes (Cartilage cells)

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High levels of:

-- Hepatocyte growth factor (HGF)—major role in embryonic development and in wound healing.

-- Vascular endothelial growth factor (VEGF)—stimulates growth of new blood vessels.

-- Placental growth factor (PGF)—Angiogenesis and vasculosogenesis.

-- Transforming growth factor –B (TGF)—controls proliferation, cellular differentiation, and other functions in most cells.

Cytokines

Moderate expression of:

--Fibroblast growth factor (FGF-2)—involved in wound healing and angiogenesis.

--Angiopoietin (Ang-1 and Ang-2)—promote angiogenesis.

J Atheroscler Thromb 2006 Apr;13(2):77-81
Bioheart Cell Isolation Technique

- Proprietary cell isolation technique provides safe legal method.

- Using tumescent anesthesia—no general, no pain pills.

- Harvested MSC yield a minimum of 30 million cells/60 cc fat extracted.

- Validated procedure via reproducibility and robustness analysis—process consistently produces pre-determined acceptance criteria.
ADSC Collection

The graph shows the CFU-f frequency (1.43) for Adipose Tissue compared to Bone Marrow. The x-axis represents the processed samples (900/MB) and the y-axis represents the CFU-f frequency.
ADSC Separation
Adipose vs Bone Marrow

- 2000 ml of fat
- 50 million nucleated cells/100 ml of fat

Bar chart showing:
- Stem cells per cc Tissue
- Bone Marrow vs Adipose Tissue
Patient Results from 60 cc of Adipose

Average yield of stem cells from 60 cc adipose tissue is 100 millions cells of which about 85% viable cells.

Many more MSC than bone marrow.

Can use fat grats, hyaluronic acid gel, prp.

I use prp. May use HA in the future.
ADSC Clinical Trials
ADSC Clinical Trials

![Bar chart showing SVF and Adipose SC expanded trials from 2011 to 2014.](chart.png)
Osteoarthritis
A simple google search of the internet-”Stem cell treatment for osteoarthritis” yields 677,000 results.

More and more each year interventional regenerative/orthopaedic physicians training to learn these procedures in order to be able to offer their patients more options as opposed to “traditional” therapies; ie. Cortisone injections, visco, physical therapy then surgery if these fail.

Regenexx doing large data collection and reporting results at different conferences.
Ageless Regenerative Technique
Ageless Regenerative Technique

Proprietary technique utilizing 100 millions ADSC from 60 cc fat and PRP to treat patients with degenerative diseases and musculoskeletal injuries.

Cells are suspended in 5 cc PRP and injected into affected areas using sterile technique.
Ageless IRB Study for OA

- 25 active enrolling sites
- 6 month follow up from 3 clinical sites on 103 patients

Study endpoints include:
- Adverse events
- Short Form Pain Questionnaire (SF-MPQ)
- Visual Analog Score (VAS)
- Present Pain Inventory Score (PPI)
Visual Analog Pain Scale

N = 103 patients

3 clinical sites

Showed statistically significant changes in VAS.

75% average improvement over baseline.
Short Form McGill Pain Questionnaire (SF-MPQ)

- N= 18 patients
- 3 clinical sites
- Statistically significant changes in sf-mpq
- 75% average improvement over baseline
Present Pain Inventory Score (PPI)

- N = 18
- 3 sites
- Statistically significant changes in PPI.
- 60% average improvement in baseline.
Krome Pain Score (KPS)

- N= 14 patients (to date)
- All improved thus far up to 80% in one patient!
- All patients have said they would do it again.
- All have been very happy with short term results (less than 7 months).
- Not a scientific study.
Thank you!