Amniotic Stem Cells and What Was Before!
Is There Any Value?

Introduction

- 48% of Common Shoulder and Knee Repairs Fail

- 20-94% Recurrence Rate After Rotator Cuff Repair

- Due to Inadequate Healing at Injury Site

- Suture Pulling Through Repaired Tendon

- Failure to Heal at the Greater Tuberosity

Disclosures

February 2016

- Royalties from Publishers
  - Saunders/Mosby-Elsevier; Thieme
- Medical/Orthopaedic Publications Editorial/Governing Board
- Board Member/Committee Appointments for a Society
  - AAOS; ASES; AANA; ISAKOS; EOA; NY Chapter Arthritis Foundation; NY County Medical Society – Government Affairs
- Principal Investigator - Clinical Trials
  - Anika Therapeutics; Oxford Immunotec; Pfizer; Seikagaku
- Funding, Fellowship
  - Arthrex; ConMed Linvatec; Zimmer
Amniotic Stem Cells and What Was Before
Is There Any Value?

Solution

Optimization of the Healing Environment

- Orthobiologics
  - Made from substances naturally found in the body
  - Delivered in higher concentrations
  - Speed up the healing process

Types
- Recombinant Growth Factors
- Enzyme Inhibitors
- Collagen Substrates
- Marrow Stimulation Techniques
- Platelet Rich Plasma (PRP)
- Mesenchymal Stem Cells (MSCs)
- Autologous
  - Bone Marrow Aspirate
  - Allogenic Amniotic Embryonic

Above Baseline Concentrations

- Platelets
- Growth Factors
- Cytokines

Autologous Blood

Activated Platelets

Release Growth Factors
  - Healing Cascade
    - TGF-b
    - PDGF
    - VEGF
    - EGF
  - Reduce Tendon Inflammation
    - HGF

Sterile Technique

Ultrasound-Guidance

- Accurate Localization of Pathology
- Evaluation of Pathology/Diagnostic

Pre-Injection Guidelines

- No Corticosteroids for 2-3 Weeks
- No NSAIDS 1 Week Prior
- No Anticoagulation 5 Days Prior
- Increase Fluid Intake
- Anti-Anxiety Meds for Specific Patients
Amniotic Stem Cells and What Was Before
Is There Any Value?
Platelet Rich Plasma - Rotator Cuff

Rotator Cuff Surgery
- Prospective, Double-Blind, Randomized Study
  Platelet Rich Fibrin Matrix (PRFM)
  Surgery Vs. Surgery Alone
  N=30 Per Group
  PRFM Group:
  - Significantly Younger
  - Significantly Longer Surgery Time
  No Significant Differences Between Groups
  - VAS Scores
  - Narcotic Use
  - Recovery of Motion
  - Functional Scores

Addition of PRP Did Not Improve Clinical Outcomes

Amniotic Stem Cells and What Was Before
Is There Any Value?
Platelet Rich Plasma - Rotator Cuff

Arthroscopic Rotator Cuff Repair
- Supraspinatus
- 2 Randomized Controlled Trials (N=85)
  Arthroscopic Rotator Cuff Repair ± PRP Injections
  No Improvement with PRP
  - Early Functional Recovery
  - Range of Motion
  - Strength
  - Pain
  No Difference in Structural Integrity of Supraspinatus Repair
  MRI
- 16 Weeks Post Op

Weak Evidence

Amniotic Stem Cells and What Was Before
Is There Any Value?
Platelet Rich Plasma - Rotator Cuff

Arthroscopic RCT Repair ± PRP
- Meta-Analysis¹
  8 Studies Included
  All Tear Sizes
  - No Statistical Differences
  - Overall Outcome Scores
  - Re-Tear Rates
  RCT>3cm
  - Lower Re-Tear Rate with PRP

Systematic Review²
  8 RCTs Included
  No Statistical Differences
  - Functional Outcome Scores
  - Re-Tear Rates

No Difference in Outcomes with PRP

Amniotic Stem Cells and What Was Before
Is There Any Value?
Mesenchymal Stem Cells (MSC)

Nucleated Cells
- 3-8x Concentrated in Aspirate

Advantages
- Chondrogenic (Cartilage) Potential
  - Animal Studies
    - Great Potential
  - Human Studies
    - Good Potential
- Osteogenic (Bone) Potential
  - Animal Studies
    - Great Potential
  - Human Studies
    - Good Potential

Disadvantages
- Limited Research
  - No Long Term Studies
  - Need for RCTs
- Cost
  - $1000-5000 Per Patient

Sources
- Bone Marrow (BMAC)
- Adipose Tissue
- Synovial Tissue
- Peripheral Blood
- Periosteum
- Umbilical Cord Blood
- Infrapatellar Fat Pad
- Amniotic Fluid

Progenitor Cells
- Differentiate To:
  - Osteoblasts
  - Chondrocytes
  - Myoblasts
  - Adipocytes

Platelet-Rich Plasma

Mechanism of Action
- Induce Healing Cascade
- Reduce Tendon Inflammation
  - Growth Factor Secretion

Efficacy
- No Improvement
  - Shoulder
Amniotic Stem Cells and What Was Before

Is There Any Value?

Autologous Stem Cell Sources

- Iliac Crest
  - Greatest Concentration
    Bone-Forming MSCs\(^1\)
    - 886.6±1431.5 cells/mL

- Other Sources
  - Proximal Humerus\(^2\)
    - 883.9±577.6 cells/mL
  - Tibial Metaphysis
    - 32.4±135.4 cells/mL
  - Calcaneal
    - 7.1±17.4 cells/mL
  - Distal Femur
    - 551.3±408.1 cells/mL


Amniotic Stem Cells and What Was Before

Is There Any Value?

BMAC - Rotator Cuff

- Arthroscopic RC Repair
  - 45 Patients
    - Symptomatic Ruptures: 1.5-3.5 cm
    - Minimum 10 Year Follow-Up
  - Single Row Rotator Cuff Repair
    - Isolated Repair Vs. Repair + MSCs
  - Faster Healing Rate - 6 Months
    - 100% RCR+MSC Patients
    - 67% RCR Only Patients
  - Prevented Further Tears
    - Intact Rotator Cuff at 10 Years
    - 43% RCR/MSC Patients
    - 44% RCR Only Patients
  - Faster Healing & Lower Re-Tear Rates


Amniotic Stem Cells and What Was Before

Is There Any Value?

BMAC - Rotator Cuff/OA

- Symptomatic Glenohumeral Joint OA and/or Rotator Cuff Tears
  - 102 Patients
    - 115 Shoulders
  - Autologous BMAC Injections
  - Significant Improvement
    - Pre-Op to Final Follow-Up
      - Both Groups
        - DASH Numeric Pain Scale
        - Improvement: 48.6% P<0.001
  - Average Subjective Improvement
    - 48.6% P<0.001
  - No Difference in Outcomes
    - OA vs RCT Shoulders
  - No Treatment-Related Adverse Events
  - Clinical Improvement in Pain and Function

## Amniotic Stem Cells and What Was Before
### Is There Any Value?
#### BMAC Systems - The Problem

<table>
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<th>Biomet BioCue</th>
<th>Arteriocyte</th>
<th>Magellan</th>
<th>Harv est SmartPrep 2</th>
<th>Arthrex Angel</th>
<th>Alliance Spine</th>
<th>Emcyte Donemos CS</th>
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<td>2nd Spin 2800</td>
<td>2nd Spin 3800</td>
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<td>2300/140 x 0.9 mm</td>
<td>3800</td>
<td>2400</td>
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</tbody>
</table>

### BMAC - Our Series
- **21-70 Year Old**
- **Recreational/ Professional Athletes (N=32)**
  - Knee (N=21)
  - Hip (N=5)
  - Shoulder (N=3)
  - Elbow (N=1)
  - Achilles (N=2)
- **Pain Free Return to Sport**
  - Tennis
  - Skiing
  - Basketball
  - Running
- **No Complications**
- **Great Promise For Restoration Of Anatomic Architecture**

### BMAC - Disadvantages
- **Disadvantage**
- **Limited Ability to Standardize BMAC Content**
  - Heterogeneous Nature of Bone Marrow Cellular Population
  - MSC Population Declines with Age
- **Harvest in the Operating Room**
  - Donor Site Morbidity
- **Excludes International Companies**
- **White Paper Info**

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Amniotic Stem Cells and What Was Before

Is There Any Value?

Amniotic Stem Cells

- Biosynthetic Activity of Isolated Chondrocytes
  - Decreases with Age

Amniotic Derived Factors
- Amniotic Fluid
- Amniotic Stem Cells
- Amniotic Membrane

Recovered During Cesarean Delivery
- Consent, Healthy, Pre-Screened Donors
- No Harm to Newborn or Mother

Chorion Removed
- Eliminate Possible Immune Response
  - If Recipient Not Compatible with Donor Tissue Type

Amniotic Fluid MSCs
- Higher Percentage of MSCs Compared to Adult Bone Marrow
  - Adult BM: 0.001-0.01%
  - Amniotic Fluid: 0.9-1.5%
- Greater Proliferative Capacity
- Higher Expression Levels of Osteogenic Genes
- Lower Immunogenicity
  - Lowest Risk of Graft Vs. Host Disease
- Non-Invasive Cell Isolation
- No Ethical Concerns
  - MSCs Extracted From Fluid Discarded After Amniocentesis

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Amniotic Stem Cells and What Was Before

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Allogeneic Source - Amniotic Stem Cells

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Amniotic Stem Cells and What Was Before
Is There Any Value?
What Are They?

- **Amniotic-Derived Stem Cells**
  - Can Differentiate Into
  - All 3 Germinal Phenotypes
  - Various Cell Types
    - Osteogenic
    - Myogenic
    - Neurogenic
    - Tenogenic
    - Endothelial

- **Types**
  - Human Amnion Epithelial Cells (hAECs)
  - Human Amnion Mesenchymal Stromal Cells (hAMSCs)

Amniotic Stem Cells and What Was Before
Is There Any Value?
Amniotic Membrane

- **Human Amniotic Membrane**
  - Contains
    - Extracellular Matrix Components
      - Collagen Types I, II, III, IV, VII
      - Fibroblasts, Fibronectin, Laminins, Aggrecan, Syndecan-1,4
      - Hyaluronic Acid
    - Growth Factors
      - EGF, FGF, IGF, IGF-I, TGF-α, TGF-β, VEGF
    - Anti-Microbials
      - Defensins, Calprotectin, Lactoferrin, PGRP-S
    - Tissue Modeling/Repair Molecules
      - Angiogenin, Osteoactivin, Osteopontin, Pericostin, MMPs, TIMPs, FAP, FLRG, EMMPRIN

Amniotic Stem Cells and What Was Before
Is There Any Value?
Amniotic Membrane

- **Modulates**
  - Cytokine and Growth Factors Levels in Tissue
  - Unique Properties
    - Pain Reduction
    - Anti-Inflammatory
    - Fibrosis Suppression
    - Antibacterial
    - Improved Wound Healing
    - Capability
    - Suppresses Tumorigenesis

- **Potentially Superior Clinical Outcomes**
  - More Randomized Control Studies Needed
Amniotic Stem Cells and What Was Before
Is There Any Value?
Animal Studies

- Tendon/Ligament Injuries
  - 51 Horses Amniotic Stem Cells vs.
  - 44 Horses Autologous Bone Marrow Derived Stem Cells
    ▪ Amniotic Stem Cells
      - Faster Return to Activity: 4-5 Months vs. 4-12 Months
      - Lower Rate of Re-Injury: 45% vs. 23%
  - Conclusions
    ▪ Amniotic Stem Cells Have
      - Greater Proliferative Capacity
      - Higher Plasticity


Advantages of Amniotic Stem Cells
- Pluripotent
- Highly Plastic
- Capable of Differentiation into Major Lineages
- Low Immunogenicity: HLA Class II Antigens Not Expressed
- Non-Teratogenic
- Easy Procurement After Parturition

Amniotic Stem Cells and What Was Before
Is There Any Value?
Summary

Products Are Available
- Injectable Mixture
  ▪ Amniotic Fluid
  ▪ Amniotic Stem Cells
  ▪ Chorion-Free Amniotic Membrane
- In-Office Application
- Storage: Small Freezer -80 Degrees F
- Immediate Delivery
  - Thaw 5 Minutes in Hand
  - Inject Within 10 Minutes
  - Longer Than 10 Minutes: Loss of Cell Viability
- One Stop Shopping: Patient Satisfaction
Amniotic Stem Cells and What Was Before
Is There Any Value?
Typical Product On The Market-Even More Molecules

- Found in Typical Product*
  - Growth Factors
    EGF, FGF-4, bFGF, HGF, IGF-I, TGF-α, TGF-β, VEGF
  - Tissue Remodeling/Repair Molecules
    Angiogenin, Osteoactivin, Osteopontin, Periostin, MMPs, TIMPs, FAP, FLSG, ENMIPP
  - Connective Tissue Proteins
    Angiogenin, Osteoactivin, Osteopontin, Periostin, MMPs, TIMPs, FAP, FLSG, ENMIPP
  - Amnion-Derived Cells
    Amniotic Epithelial Mesenchymal Cells
    Capable of Differentiating into Various Cell Types
      Osteogenic, Myogenic, Neuronal, Tenogenic, Endothelial
  - Antimicrobials
    Defensins, Calprotectin, Lactoferrin, PGRP-S

*Specific molecules found in Product through Protein Quantification Array

Amniotic Stem Cells and What Was Before
Is There Any Value?
Safety, Effective and Reproducible

- Plus Standardization
  - For Each Product Lot
    - Current Product Information
      Total Cell Number
      Percent Cell Viability
    - Future Product Information
      Surface Marker Profile
      Cellular Identity
      Cytokine Expression Levels
      Functional Bioassay
      Performance
  - To Assure and Optimize Product Potency

Amniotic Stem Cells and What Was Before
Is There Any Value?
Business Opportunity - Niche Practice

- Cutting Edge
  - New Patient Referrals From Physicians
  - Educate Your Patients & Community
    Safe
    Effective
    In Office
    - No Surgery Required
    - Return to Sport
    - 6-8 Weeks
Amniotic Stem Cells and What Was Before
Is There Any Value?
Business Opportunity - Niche Practice

- **Plancher 2016**
  - Began Christmas 2015
  - 11 Patients
    - Knee & Shoulder
  - Huge Revenue Potential
    - $999-$2500/1cc Injection
    - Know Your Market

- **Insurance Reimbursement**
  - All Insurances
    - Varying Degrees
    - Up to $1800 Reimbursement
    - Excludes Medicare

Amniotic Stem Cells and What Was Before
Is There Any Value?
Overall Conclusions

- **Platelet-Rich Plasma**
  - Mixed Clinical Results
  - No Supporting Evidence
  - Limited Standardization
- **Bone Marrow Aspirate Concentrate**
  - Promising Clinical Evidence
  - Shoulder
  - Limited Standardization
- **Amniotic Stem Cells Are The Future!**
  - Good Preclinical and Clinical Evidence
  - Safety & Effectiveness
  - Shoulder & Elbow
- **More Research Needed**
  - Optimal Concentration
  - Adjuvant Therapies
    - Bioactive Factors, Molecular Signaling

Thank You