Growth Factors and Stem Cells in Failed Rotator Cuff Repairs

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The following relationships exist and have been fully disclosed and approved by the

**STATE OF CONNECTICUT ETHICS BOARD**

1. Royalties and stock options - **NONE**
2. Consulting income - **ARTHREX INC**
3. Research and educational support - **ARTHREX INC**
4. Other support - Educational and Research grants from **OREF, Donaghue Foundation**

**COMITTEES LEADERSHIP**

Program Chairman for the 2014 AOSSM International Sports Medicine Meeting

Research Committee of the Arthroscopy Association of North America (AANA) 2010 to Present

Continuing Education Committee of the American Shoulder and Elbow Surgeons (ASES) 2009 to Present

Founder and Executive Committee Member of the New England Shoulder and Elbow Society (NESES) 2003 to Present
Thanks to

This Research has been presented at the
2009 and 2011 ISAKOS Meeting
2009 and 2011 European Shoulder and Elbow Society,
Beitzel et al Arthroscopy 2011 and AJSM 2012
Introduction

Review of Literature for failed rotator cuff repairs-
This is where clinically “biologics” may provide a real benefit

Biologic analysis of non healing tissue

Operative solutions for failed rotator cuff repairs
  Arthroscopic RCR
  Reverse Ball and Socket
  Latissimus Dorsi Transfer
  Mini open with PRP and Bone Marrow Concentration

Algorithm for revision RCR
Rotator Cuff Tears-Major Clinical Problem

1998-2004: Physician Visits
- Over 5,000,000 visits for rotator cuff related problems

2005: Shoulder/Rotator Cuff related injury
- More time out of work than any other body site
- ~37.5% required greater than 31 days before return
Clinical Data - Rotator Cuff Repairs have a 30-94% failure rate

Boileau JBJS 2005
65 ARCR (tension band technique) assessed with MRI or CT arthrogram
71% (46/65) Watertight
4% (3/65) Partial Repair
25% (16/65) Recurrent Tear

Galatz JBJS 2004
18 RCR >2cm evaluated at 1 and 2 yrs with Ultrasound
17/18 Recurrent Tear

Gerber JBJS 2000
29 Massive RCR evaluated with MRI
17/29 (58%) Repair Intact
12/29 (42%) retear

Galatz JBJS 2004
18 RCR >2cm evaluated at 1 and 2 yrs with Ultrasound
17/18 Recurrent Tear

Ozbaydar Acta Ortho 2005
16 RCR mini open nonretracted
3/16 (19%) Re Tear
13/16 (81%) intact

Large or Massive generally do not heal or retear
While smaller tears have a lower retear rate
Miller et al AJSM 2011-41% of all RCR failed and 80% of these failed within 3 Months-Never healed!!!!

Gamradt et al JSES 2010-At 3 months following repair, the majority of blood flow to the repair is derived from the peribursal soft tissues and the anchor site. The tendon, particularly those with a defect at 3 months, is relatively avascular.
ROTATOR CUFF TEARS

• Common Problem

• Costly Treatment

• Variable Results
Failed Rotator Cuff Repair (RCR) Confirmed on MRI Arthrogram

**SYMPTOMATIC**
- Pain with activity
- Night Pain & Awakening
- Weakness
- Loss of ROM

**ASYMPTOMATIC**
- Small Tear or gap
- No Pain
- Full ROM
- Happy/low demand/older

**Offer Operative Intervention**

**Observe at 1 year intervals with repeat MRI Arthrograms**
OPERATIVE INTERVENTION
Symptomatic Failed RCR

- Non-retracted tears
- No x-ray changes
- Good muscle/tendon quality

Arthroscopic Revision RCR

- Proximal migration humeral head x-ray
- > 70 years of age
- Horrible pain at rest
- Massive RC tear

Reverse Ball and Socket Prosthesis

- > 1 primary RCR
- Young patient
- Retracted tear
- Changes on x-ray

Latissimus Dorsi Transfer

OR

Arthroscopic RCR with concentrated bone marrow and allograft patch
Limited Reports on Revision RCR


Less pain

Unpredictable functional improvement
Revision RCR-Clinical Improvement usually not structural
Arthroscopic Revision RCR
Clinically three categories but unpredictable “healing rate”

1. Overall failures with no improvement in pain

2. Success with no increase in motion but improvement in pain

3. Success with Increase in motion and pain
Why do we still have failures after maximizing the biomechanics of the repair and the post operative rehab?
Multiple Cell Types Present at Tendon/Bone Interface

Supraspinatus Tendon Insertion: Bone-Calcified Fibrocartilage-Fibrocartilage-Tendon

- Calcified Fibrocartilage
- Uncalcified Fibrocartilage
- Bone
- Articular Cartilage
- Tide Mark
- Tendon-Dense fibrous connective Tissue

H&E

Benjamin & Ralphs, J. Anat., 1998
However, Tendon and Collagen production have been shown to play a significant role. Genetic Predisposition that may have to be overcome. A Carr SECEC 2011
# Normal and Diseased Tendon vs. Scar Tissue Histology

<table>
<thead>
<tr>
<th>Normal Tendon</th>
<th>Diseased Tendon</th>
<th>Scar Tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relatively avascular</td>
<td>More cells and disorganized collagen</td>
<td>No cells and disorganized collagen</td>
</tr>
<tr>
<td>Thinning of fibrils</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Normal Tendon**
- Relatively avascular
- Thinning of fibrils

**Diseased Tendon**
- More cells and disorganized collagen

**Scar Tissue**
- No cells and disorganized collagen

![Images of tissue samples]
Grade IIC Rotator Cuff Tear with Fatty Infiltration n=8 samples
Human Rotator Cuff Showing Increased Cells and Vasculature within Tendon

- 4x and 10x magnification show increased vascularity (black arrows)
- Tendon appears to have a loose, wavy pattern in some areas
  - Increased cellularity (red arrows)
Not Scar Tissue but Tendon Attempting to Heal

Scar tissue has a distinctly irregular collagen pattern
No Cells

This tissue had cells and a wavy collagen pattern with increased vascularity
Biologic Categories that will enhance the body’s own ability to improve tendon healing?

Cell Based Therapies and Scaffolds-
Mesenchymal stem cells as well as various extracellular patches may improve healing.

Growth Factors and Platelet Rich Plasma—Can modulate various aspects of healing but have a specific dose and time response.

Increasing vascularity during Rotator cuff healing—Poor vascularity exists at the healing cite and agents such as glyceryl trinitrate improved healing (Paoloni et al AJSM 2005).

Matrix Metalloproteinase—Play a role in extracellular matrix production. Tissue inhibitors of Matrix Metalloproteinase’s (TIMMP) such as Doxycycline can improve healing.

We have research and experience on the first two.
Platelet Rich Plasma-Increasing the Growth Factors
Growth Factors Contained in Platelets - protein signals that are ubiquitous to all aspects of cellular growth

- **Platelet derived growth factor (PDGF)**
  - Mitogenic for mesenchymal stem cells and osteoblasts; stimulates chemotaxis and mitogenesis in fibroblast/glial/smooth muscle cells; regulates collagenase secretion and collagen synthesis; stimulates macrophage and neutrophil chemotaxis.

- **Vascular endothelial growth factor (VEGF)**
  - Increases angiogenesis and vessel permeability; stimulates mitogenesis for endothelial cells.

- **Transforming growth factor-beta (TGF-β1)**
  - Stimulates undifferentiated mesenchymal cell proliferation, regulates endothelial, fibroblastic and osteoblastic mitogenesis; regulates collagen synthesis and collagenase secretion; regulates inhibits macrophage and lymphocyte proliferation.

- **Basic fibroblast growth factor (bFGF)**
  - Promotes growth and differentiation of chondrocytes and osteoblasts; mitogenic for mesenchymal stem cells, chondrocytes, and osteoblasts.

- **Insulin-like growth factor 1 (IGF-1)**
  - IGF-1 is one of the most potent natural activators of the AKT signaling pathway, a stimulator of cell growth and multiplication and a potent inhibitor of programmed cell death.

- **Epidermal growth factor (EGF)**
  - Stimulates endothelial chemotaxis/angiogenesis; regulates collagenase secretion; stimulates epithelial/mesenchymal mitogenesis.

- **Hepatocyte growth factor (HGF)**
  - Secreted by mesenchymal cells and targets and acts primarily upon epithelial cells and endothelial cells, but also acts on haemopoietic progenitor cells; has a role in embryonic organ development, in adult organ regeneration, and in wound healing.

Fact-Critical to Understand **DOSE and TIME DEPENDANCE**

Too much, too little, or not at the correct time and you can have a negative effect.
Rotator Cuff Pro PRP-Human Clinical Data

**Randelli et al JSES 2011** - Improved pain in first months and positively affected rotator cuff healing.

**Barber et al Arthroscopy 2011** - The addition of 2 PRPFM constructs sutured into a primary rotator cuff tendon repair resulted in lower retear rates identified on MRI than repairs without the constructs.

**Everts et al Eur Surg Res 2006** - In the PLG-treated group, recovery was faster and patients returned earlier to daily activities and also took less pain medication than control subjects.
Rotator Cuff Anti PRP - Human Clinical Data

Bergeson et al AJSM 2011 - No improvement in retear rates or functional outcomes compared to controls

Hyunchul et al AJSM 2011 - No statistical difference in MRI BUT retear rate of 27% in PRP group and 41% in control

Weber et al AJSM 2012 - PRP Fibrin Matrix did not improve morbidity, structural integrity, or clinical outcome
Definition of PRP
Any level of platelets above “normal” (Normal=150-450 x10³ per microliter)

  - “Platelet-rich plasma (PRP) is an autologous concentration of human platelets in a small volume of plasma.”
  - “PRP is more than just a platelet concentrate; it also contains the 3 proteins (fibrin, fibronectin, and vitronectin) in blood known to act as cell adhesion molecules for osteoconduction and as a matrix for bone, connective tissue, and epithelial migration.”

  - “Platelet-rich plasma is defined as the volume of the plasma fraction from autologous blood having platelet concentrations above baseline (200000 platelets/ul).”
  - “Studies have shown that clinical efficacy can be expected with a minimum increase of 4-6 fold from this baseline (1 million platelets/ul).”
Density Gradient
Separation of Platelets

Mass density distribution of blood components:
A – Platelets,
B – Monocytes,
C – Lymphocytes,
D – Basophils,
E – Neutrophils,
F – Erythrocytes,
G – Eosinophils

Overlap Platelet and WBC

PRP is obtained by utilizing centrifugation to separate Platelets (A) from other cell types. Due to the overlap and proximity of densities of Platelets and other cell types, specifically white blood cells, the possibility for contamination exists.
Procedure

Drawing blood use 21g butterfly so you do not damage RBCs

If the ACP is cloudy redraw it

**DO NOT** use the anticoagulant its PH is 5.1 & the pt will complain of burning you have 30 mins. to inject
PRP-Two Basic Formats

- **Plasma Based Systems** - isolate plasma and platelets and remove WBC. Slower, shorter spin giving 2-3x baseline platelet levels
  - Arthrex, Cascade

- **Buffy Coat Based Systems** - isolate a platelet poor layer. They obtain all platelets but because of the density also keep a concentration of WBC. Longer, double spin 3-8x baseline levels
  - Biomet, Harvest,
This research is a translational attempt using human cells and clinically accurate concentrations of medicines to answer some common orthopaedic and sports medicine questions about ACP/PRP.
Does ACP/PRP increase Human Tendon, Bone, Muscle and Cartilage Cell Proliferation

YES

Mazzocca et al 2012 AJSM-The positive effects of different platelet rich plasma methods on human muscle, bone, tendon cells
Human Tendon Cell (Tenocyte) Proliferation

Tenocyte Proliferation 5 days after Treatment

DPM x 10^3

2% FBS (Negative Control)
10% FBS (Positive Control)
ACP
Does mixing Steroids, Lidocaine, and Marcaine with PRP/ACP during Injection Help

NO

Mazzocca et al Arthroscopy 2012
Corticosteroids and local anesthetics decrease positive effects of platelet rich plasma: an in vitro study on human tendon cells
Concentrated platelets supplemented with anesthetics (Marcaine and Lidocaine) and/or steroids have a harmful effect on tendon cells in culture.
Does Repeated Dosages of Injections of PRP/ACP Enhance Tendon Growth?

YES

O’Malley et al 2012 - Submitted to Arthroscopy
Repeated dosages of concentrated platelets every 4 days has a beneficial effect on tendon cells in culture.
Does the Platelet Number Matter in Cellular Proliferation
A comparison of ACP, PRP, Biomet, and MTF

It Does Not Seem To!

Mazzocca et al JBBJS 2012-Platelet Rich Plasma differs according to preparation method and human variability
No significant difference in proliferation was seen between any of the devices tested.
Osteoblast Proliferation

N=8 patients

DPM

5% FBS  15% FBS  Blood  ACP  PRP  Biomet

143 x 10^3

361 x 10^3

448 x 10^3

874 x 10^3
Is there variability in PRP within the same patient?

**YES**

Mazzocca et al JBJS 2012-Platelet Rich Plasma differs according to preparation method and human variability
Individual Patient Variability - ACP

ACP Variability

Platelet Concentration (x103)

Day 0
Day 14
Day 35
Individual Patient Variability - Biomet

Biomet Variability

Platelet Concentration (x10^3)

Day 0
Day 14
Day 35

48 yo M, 23 yo M, 23 yo M, 23 yo M, 30 yo M, 33 yo M, 22 yo F, 50 yo F
Can you use ACP to prevent infection with Arthroplasty cases
ACP as an Anti-Bacterial Agent

YES
Is ACP as an Anti-Inflammatory Agent Alone?

YES
Results showing that inflammatory markers VCAM, E-Selectin, and HLA-DR are significantly decreased with application of ACP.

- All groups less inflammation $P<0.05$
- ACP significantly less inflammation

Depomedrol and Depomedrol + ACP also had a significant decrease in fluorescence at 96 hours. Upon examination, and after counting, most of the cells were dead.
Is a Cell Based Approach a Potential Treatment Option?
Mesenchymal Stem Cells are found in Bone, Fat, and Muscle. They can differentiate into tendon and bone for improved healing of Rotator Cuff repairs.
Mesenchymal Stem Cells (MSC)

- Synonyms:
  - Connective Tissue Progenitors (CTP)
  - Mesenchymal Progenitor Cells (MPC)

- Able to differentiate into mesenchymal tissue like bone, tendon, cartilage etc.

- Highly Proliferative while retaining their growth and multilineage potential (but not immortal)

- Commonly defined by:
  - Colony Formation
  - Surface Markers (CD105+, CD73+; Negative for CD34, CD45 & CD14)
  - Differentiation into mesenchymal cell lines

Beitzel et al Arthroscopy 2011
Recent Literature showing healing with Stem Cells

- **Hernigou et al., JBJS 2005**
  Autologous bone-marrow grafting efficacy appears to be related to number of progenitors in the graft / aspirated bone marrow should be concentrated (60 Humans)

- **Fortier et al., JBJS 2010**
  Bone marrow concentrate can result in healing of acute full-thickness cartilage defects that is superior to that after microfracture alone (12 adult horses)

- **Okamoto et al., JBJS 2010**
  Transplantation of whole bone marrow cells may be a better treatment for Achilles tendon rupture than cultured mesenchymal stem cells. (BM vs. MSC 87 rats)

- **Ota et al AJSM 2011**
  Stem cell application to muscle improved and accelerated healing
Different Ways to Obtain Bone Marrow Cells

Aspiration is easily achieved during arthroscopic RCR using a bone marrow trocar. Stem cells can be isolated in the OR within 5 minutes.

Cannulated anchors is another way to obtain bone marrow for healing. Vented Anchors and the “Crimson Duvet” technique may assist with healing.
Six Human Studies with Mesenchymal Stem Cell and the Shoulder

Beitzel et al Arthroscopy 2013 in press
Results of a PubMed search in May 2013 using the terms “stem cell OR bone marrow-derived cell” AND “shoulder OR rotator cuff OR proximal humerus”. The search was limited to articles published in English from January 1, 1980 to April 30, 2013.

N=141

Abstracts Obtained and Reviewed
N=51

Excluded based on abstract
N=13

Manuscripts Obtained and Reviewed
N=38

Excluded based on manuscript
N=21

Studies meeting inclusion criteria
N=17

In vivo animal studies
N=9

In vitro human studies
N=5

In vitro animal study
N=1

In vitro human and in vivo animal
N=1

In vitro and in vivo human
N=1
<table>
<thead>
<tr>
<th>Authors</th>
<th>Source of Stem Cells</th>
<th>Purpose</th>
<th>Number of Patients/Methods</th>
<th>Results</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kurth et al., 2007</td>
<td>Knee-joint synovial membranes of osteoarthritic human donors</td>
<td>To establish the conditions under which synovial MSCs derived from aging human donors can be induced to undergo chondrogenic differentiation when cultured in alginate.</td>
<td>Six osteoarthritic human patients (aged 59-76 years) undergoing knee-joint surgery. Chondrogenic differentiation capacity was tested by exposure to growth factors, BMP-2, BMP-7, and synthetic glucocorticoid dexamethasone.</td>
<td>Bone morphogenetic protein-2 (BMP-2) induced the chondrogenic differentiation of human synovial MSCs in a dose-dependent manner. The response elicited by BMP-7 was comparable.</td>
<td>Synovial MSCs derived from the knee joints of aging human donors possess chondrogenic potential.</td>
</tr>
<tr>
<td>Mazzocca et al., 2010</td>
<td>Proximal Humerus</td>
<td>1) Arthroscopically obtain BMA from the proximal humerus during RCR, 2) purify and concentrate CTPs in the OR efficiently, and 3) confirm these are stem cells through their ability to differentiate into bone cells.</td>
<td>BMA harvested through anchor tunnel of humeral head during arthroscopic RCR in 23 patients. Twenty-three matched controls selected from a clinical registry to evaluate for increased incidence of complication.</td>
<td>Reverse transcriptase PCR analysis and cellular staining confirmed the osteogenic potential of proximal humerus CTPs. No significant difference in SANE, ROM, and post-operative strength measures compared to control group.</td>
<td>CTPs can be safely aspirated from proximal humerus using the anchor tunnel created during arthroscopic RC surgery without increasing morbidity.</td>
</tr>
<tr>
<td>Mazzocca et al., 2011</td>
<td>Proximal Humerus</td>
<td>To determine if a one-time physiological dose of insulin is capable of differentiating bone marrow-derived Mesenchymal Stem Cells into tendon.</td>
<td>Eleven patients undergoing arthroscopic RCR consented to undergo aspiration of bone marrow. After purification of bone marrow in OR, MSCs were exposed to insulin or tendon-inducing growth factor or left untreated to serve as control.</td>
<td>MSCs treated with insulin showed increased gene expression of tendon-specific markers, increased content on tendon-specific proteins, and increased receptors on the cell surface compared with the control cells.</td>
<td>Bone marrow-derived MSCs treated with a single physiological dose of insulin differentiated into cells with characteristics consistent with tendon.</td>
</tr>
<tr>
<td>Ellera Gomes et al., 2012</td>
<td>Iliac Crest</td>
<td>To investigate the behavior of rotator cuff tears treated with conventional repair technique with the aid of autologous bone marrow mononuclear cells (BMMC).</td>
<td>Fourteen consecutive patients (9 women, 5 men, mean age 59.2 years) with complete rotator cuff tears fixed by transosseous stitches with subsequent injection of BMMC into tendon borders.</td>
<td>After minimum of 12-month follow-up period, UCLA score increased from 12 ± 3.0 to 31 ± 3.2. MRI analysis after minimum of 12-month follow-up demonstrated tendon integrity in all cases.</td>
<td>Implantation of BMMC in rotator cuff sutures appears to be a safe and promising alternative used to enhance tissue quality in affected tendons.</td>
</tr>
<tr>
<td>Tsai et al., 2013</td>
<td>Cells harvested from the rotator cuff</td>
<td>To determine if MSCs could be isolate from human rotator cuff and compare myogenic differentiation potential between bone marrow-derived MSCs (BM-MSCs) and RC-MSCs.</td>
<td>Ten patients (bone marrow samples from 5, RC samples from 5) undergoing surgery to isolate BM-MSCs and RC-MSCs to undergo in vitro evaluation.</td>
<td>Cell surface markers indicated RC-MSCs had the same surface protein profile as BM-MSCs while histo- and immunohistochemistry data suggest that RC-MSCs have the same differentiation potential that are well known for MSCs. Real time PCR and Western blot analysis suggest that RC-MSCs have greater myogenic differentiation potential than BM-MSCs.</td>
<td>Human RC-MSCs could be another cell source for cell therapy in patients with tendon, muscle, or dystrophic muscle disorders.</td>
</tr>
<tr>
<td>Beitzel et al., 2013</td>
<td>Proximal Humerus or Distal Femur</td>
<td>To examine the relations between age, gender, and number of viable mesenchymal stem cells (MSCs) in concentrated bone marrow (BM) obtained from the proximal humerus and distal femur during arthroscopic surgery.</td>
<td>BM aspirated from either the proximal humerus (n=55) or distal femur (n=29) during arthroscopic surgery in 84 patients. MSCs were obtained to undergo in vitro evaluation.</td>
<td>Mean quantity of BM aspirations was 22.6 ± 12.3 mL. The proximal humerus provided 38.7 ± 52.6 x 10⁶ nucleated cells/mL and the distal femur provided 25.9 ± 14.3 x 10⁷ for an overall 766.3 ± 545.3 MSCs/mL of concentrated BM. Values did not significantly differ by age, gender, or donor site.</td>
<td>Arthroscopic aspiration of BM from the proximal humerus and distal femur is a reproducible technique and yields reliable concentrations of MSCs.</td>
</tr>
</tbody>
</table>
Arthroscopic Revision Rotator Cuff Repair with Allograft Patch and Concentrated Bone Marrow
Can we obtain Human Stem Cells without increasing surgical morbidity, time, and number of procedures?

YES

Rapid Isolation of Human Stem Cells (Connective Tissue Progenitor Cells) from the Proximal Humerus During Arthroscopic Rotator Cuff Surgery
Augustus D. Mazzocca, M.S., M.D., Mary Beth McCarthy, B.S., David Chowaniec, B.S., Mark P. Cote, P.T., D.P.T, Robert A. Arciero, M.D, Hicham Drissi, PhD

Rapid Isolation of Human Stem Cells (Connective Tissue Progenitor Cells) From the Distal Femur During Arthroscopic Knee Surgery
Knut Beitzel, M.A., M.D., Mary Beth McCarthy, B.S., Mark P. Cote, P.T., D.P.T., David Chowaniec, B.S., Lauryn M. Falcone, Justine A. Falcone, Evan M. Dugdale, Thomas M. DeBerardino, M.D., Robert A. Arciero, M.D., and Augustus D. Mazzocca, M.S., M.D.
*Arthroscopy, 28 (2012), 74-84*
STEP 1:
Aspiration of Bone Marrow (BM) using the Aspiration Kit and Double-Syringe
Aspiration Technology
No difference in amount of MSC obtained from Fenestrated Cannula vs. Single Hole Cannula

Arthrex® Bone Marrow Aspiration System
Harvest® Bone Marrow Aspiration System

Each system has a trocar with a 14g needle appropriately sized for use in the proximal humerus and to prevent cell damage during harvest.
Insert BMA 14g needle and trocar into area of first anchor.

Graduated depth marks ensure the Trocar depth is between 25-30mm.
Aspirate Bone Marrow with Double Syringe

Same Procedure for each Syringe
The first medial anchor is placed inside the tunnel.
Double Syringes (incl. BM) are Centrifuged
(800 rpm / 4 min.)
Double Syringes are Centrifuged to Isolate Cells

Buffy Coat

Fat Cells

Bone Marrow Aspirate

Red Blood Cells

Pre spin

Post Spin

(4 min @ 800 rpm)
Up Draw of Buffy Coat into inner Syringe
Concentrated BMA is Drawn up into the Inner Syringe

2-3 mL is drawn up into each inner syringe of the Double Syringe. Volume can vary between 1-4 mL

Color and Volume varies between tubes
Yield ~ 3mL/DS
Hold Syringe upright to prevent mixing the fat layer
We try not add the fat layer which is easily seen on top

Cells are Transferred to Surgical Field under Sterile Conditions
Clinical Application of BMA

Mix with Collagen

Mix with PRP

Mix with Fibrin Clot
Individually cut small pieces of scaffold to insert arthroscopically
Mini-Open Repair (Margin Convergence) with Human Dermis Allograft... and Stem Cells injected directly
Arthroscopic margin convergence and then mini open implantation of allograft patch
Inject cells and PRP into patch as a scaffold
Final View - Transosseous Equivalent Double Row Repair
Is this safe to the patient?

YES

The University of Connecticut Department of Safety and Quality Control

No increase in risk to the patient of the operative technical aspects of bone marrow aspiration from the shoulder of knee
No increase in complications with the aspirate group in fact less in this group (n=120 aspirations)

No surgical complications (0%)

There were no incidence of RSD, DVT, wound irregularities, hematoma, or septic arthritis (0%)

Incidence of Complications:

<table>
<thead>
<tr>
<th></th>
<th>Wound Infection</th>
<th>Abnormal Pain</th>
<th>Delayed Healing</th>
<th>Stiffness</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ASPIRATE GROUP</strong></td>
<td>0</td>
<td>0</td>
<td>3 (13%)</td>
<td>2 (8%)</td>
</tr>
<tr>
<td><strong>CONTROL GROUP</strong></td>
<td>1 (4%)</td>
<td>3 (13%)</td>
<td>3 (13%)</td>
<td>1 (4%)</td>
</tr>
</tbody>
</table>
How do we know they are Stem Cells?
Bone Marrow–Derived Mesenchymal Stem Cells Obtained During Arthroscopic Rotator Cuff Repair Surgery Show Potential for Tendon Cell Differentiation After Treatment With Insulin

Augustus D. Mazzocca, M.S., M.D., Mary Beth R. McCarthy, B.S., David Chowaniec, B.S., Mark P. Cote, D.P.T., Christopher H. Judson, M.D., John Apostolakos, B.S., Olga Solovyova, B.S., Knut Beitzel, M.A., M.D., and Robert A. Arciero, M.D.

Arthroscopy, 27 (2011), 1459-71

Stem Cells Defined by:

1. Differentiation into bone, fat, tendon, and muscle
2. Colony Forming Units (CFU)
3. Morphology
4. FACS Analysis (CD 73, 90, 45)
How many cells do we get and does it matter?

Comparison of Mesenchymal Stem Cells (Osteoprogenitors) Harvested From Proximal Humerus and Distal Femur During Arthroscopic Surgery

Knut Beitzel, M.A., M.D., Mary Beth R. McCarthy, B.S., Mark P. Cote, D.P.T., Thomas J.S. Durant, P.T., David M. Chowaniec, B.S., Olga Solovyova, B.S., Ryan P. Russell, M.A., Robert A. Arciero, M.D., and Augustus D. Mazzocca, M.S., M.D.

Arthroscopy, 29 (2013), 301-8
Stem cells per syringe per million

![Bar chart showing stem cell counts per syringe for tubes labeled as Tube 1, Tube 2, Tube 3, and Tube 4. Tube 1 has the highest count, followed by Tube 4, then Tube 2, and finally Tube 3.]
How Do We Compare?

Stem Cells

- Mazzocca (No gradient) with n=25
- Mazzocca (Unpublished Data) with n=63
- Mazzocca (AJSM 2010) Shoulder with n=23
- Mazzocca (Arthroscopy) Knee with n=25
- Heringou (JBJS 2005) Iliac Crest with n=60
- Muschler (JBJS 2005) Iliac Crest with n=21
- Muschler (JBJS 2005) Vertebral Body with n=21

CFUs/10^6 Nucleated Cells

4 min @ 800 rpm
5 min @1500 rpm
2-Step Expansion
Now that we have the cells, we need them to stay in place. Will cells stay adhered during arthroscopic surgery?

Human Tendon Cell Response to 7 Commercially Available Extracellular Matrix Materials: An In Vitro Study
Kevin P. Shea, M.D., Mary Beth McCarthy, B.S., Felicia Ledgard, B.S., Cristina Arciero, B.A., David Chowaniec, B.S., and Augustus D. Mazzocca, M.S., M.D. Arthroscopy, 26 (2010), 1181-8
Stability of Double-Row Rotator Cuff Repair Is Not Adversely Affected by Scaffold Interposition Between Tendon and Bone

Knut Beitzel, David M. Chowaniec, Mary Beth McCarthy, Mark P. Cote, Ryan P. Russell, Elifho Obopilwe, Andreas B. Imhoff, Robert A. Arciero and Augustus D. Mazzocca

No Difference in Biomechanics of scaffolds soaked in Stem Cells and PRP

Direct Repair with Augmentation – “on top” or as “interposition” between tendon and bone

“Bridging” Showed poor clinical results (Iannotti et al)

Beitzel et al 2012 AJSM
Clinically we compare the allograft patch RCR to the Latissimus Dorsi Tendon Transfer
Latissimus Dorsi Transfer
Indicated for patients that are young with multiple failed RCR.
N=34

Can reduce pain but will not reproducibly increase strength or ROM
Patient Positioning

Bean Bag

Rotator cuff defect

scapular tip
Latissimus Dorsi Transfer
Obtaining Tendon and keeping tendon spread with Locking Suture

Visualization of Lat tendon on humerus

Two rows of Krackow sutures
To keep the tendon spread
Lateral Acromial Osteotomy for Exposure and then tunnel tendon under posterior deltoid

Acromial osteotomy for visualization

Tunnel under posterior deltoid
Pre-insertion of LD tendon at donor site into the rotator cuff defect.
Suture tendon graft to tuberosity and subscapularis tendon anteriorly

Fixation of the Latissimus Dorsi transfer to margin convergence medially, subscapularis anteriorly and tuberosity laterally
Post Operative Course

Admit for 2 nights to hospital
Home in Abduction Immobilizer for 6-8 weeks
Transition to Sling 2-3 with AAROM from 10-16 weeks
Strengthening 16-28
Lat Transfer

ASES

Rowe

CM
Functional UCONN Outcomes N=34
Latissimus Dorsi Transfer

N=5 Failures defined as no improvement in pain. 1 revised to a Reverse Prosthesis

N=20 Success with either no pain or significant improvement in pain with shoulder level or above motion

N=9 Fair-improvement but still having pain and less motion
To avoid more invasive procedures, we have been working with AUTOGRAFT Concentrated Bone Marrow which contains a percentage of Mesenchymal Stem Cells that may assist with ability to heal the RC tendon to bone.

**Arthroscopic Revision Rotator Cuff Repair with Allograft Patch and Concentrated Bone Marrow**

Rapid Isolation of Human Stem Cells (Connective Tissue Progenitor Cells) from the Proximal Humerus During Arthroscopic Rotator Cuff Surgery
Augustus D. Mazzocca, M.S., M.D., Mary Beth McCarthy, B.S., David Chowaniec, B.S., Mark P. Cote, P.T., D.P.T, Robert A. Arciero, M.D, Hicham Drissi, PhD

Rapid Isolation of Human Stem Cells (Connective Tissue Progenitor Cells) From the Distal Femur During Arthroscopic Knee Surgery
Knut Beitzel, M.A., M.D., Mary Beth McCarthy, B.S., Mark P. Cote, P.T., D.P.T.,
David Chowaniec, B.S., Lauryn M. Falcone, Justine A. Falcone, Evan M. Dugdale,
Thomas M. DeBerardino, M.D., Robert A. Arciero, M.D., and Augustus D. Mazzocca, M.S., M.D.
*Arthroscopy, 28 (2012), 74-84*
UCONN Functional outcomes n=21 Allograft Patch and MSC/PRP implantation

N=2 failures with no improvement in pain

N=15 Success with no increase in motion but improvement in pain

N=4 Increase in motion and pain
Biologic Scaffold
Laboratory Cellular Evaluation-Greater cells had a higher chance of clinical success

<table>
<thead>
<tr>
<th></th>
<th>Failure</th>
<th>Success</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nucleated Cells (million/cc BMA)</td>
<td>9.4: Range (1.8-17.6)</td>
<td>14.8: Range (10.75-18.5)</td>
</tr>
<tr>
<td>Colony Forming Units</td>
<td>12.6: Range (0-24.2)</td>
<td>19: Range (13.02-21.5)</td>
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</tbody>
</table>
Cell Data Scattered with some failed having high cell counts
Of the 7 successes, 6 had above average numbers of CFUs/nucleated cells
Pts. that started with lower nucleated cells counts (< 10 million cells/cc BMA), had little or no cellular activity on the LN patch (Panel A) after 14 days in culture. Successful outcomes were associated with pts. that had greater nucleated cell counts and cells appeared to be viable after 14 days in culture (Panel B, arrows).
Radiographs have not shown any increase in HO or excess bone formation.
Pre and Post Outcome data comparing lat transfer to Allograft

<table>
<thead>
<tr>
<th></th>
<th>Rowe</th>
<th>ASES</th>
<th>CM</th>
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<tbody>
<tr>
<td>Lat Transfer Pre</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patch Pre</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lat Transfer Post</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Patch Post</td>
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</tbody>
</table>

[Bar chart showing comparison of outlet data for Lat Transfer Pre, Patch Pre, Lat Transfer Post, and Patch Post across Rowe, ASES, and CM metrics.]
If Greater than 50% Fatty Infiltration do a Lat Transfer NOT Patch Augmentation

P Value: .003 SIGNIFICANT

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<tr>
<th>Modified Goutiller Classification</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
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<tbody>
<tr>
<td>Total Patients</td>
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<tr>
<td>&lt;50% Fatty Infiltration</td>
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<td>Success</td>
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<tr>
<td>&gt;50% Fatty Infiltration</td>
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<td>Success</td>
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</tbody>
</table>

P Value: .003 SIGNIFICANT
Compare Latissimus Transfer to Stem cell Augmentation to Reverse Arthroplasty
Biologic vs Lat Transfer vs Reverse

• Biologic Patch
  – 15 Patients/ Average Age: 58
  – Average follow-up: 24 Months

• Lat Transfer
  – 22 Patients/ Average Age: 54
  – Average follow-up: 46.3 Months

• Reverse Total Shoulder Arthroplasty
  – 22 Patients/ Average Age: 60
  – Average follow-up: 24 Months
  – Infection converted to Prostalac (abx)
Pre vs Post-Operative ASES

<table>
<thead>
<tr>
<th></th>
<th>Pre-op ASES</th>
<th>Post-op ASES</th>
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<tbody>
<tr>
<td>Biologic</td>
<td>37.9</td>
<td>55.7</td>
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<tr>
<td>Lat Transfer</td>
<td>35.3</td>
<td>47.8</td>
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<tr>
<td>Reverse TSA</td>
<td>11.5</td>
<td>80.5</td>
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</table>
Reverse functional Improvement significantly better for SST 12/12 best score

<table>
<thead>
<tr>
<th></th>
<th>Pre-op SST</th>
<th>Post-op SST</th>
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</thead>
<tbody>
<tr>
<td>Biologic</td>
<td>3.1</td>
<td>6.0</td>
</tr>
<tr>
<td>Lat Transfer</td>
<td>3.3</td>
<td>4.3</td>
</tr>
<tr>
<td>Reverse TSA</td>
<td>1.0</td>
<td>9.1</td>
</tr>
</tbody>
</table>
Biologic patch and Lat Transfer 40-50% failure Compared to Reverse Arthroplasty 5%
Reverse Arthroplasty in the short term clinically does better but the patient has to sacrifice.

My bias is to continue to help the body heal itself NOT replace!
2013 MSC Take Home Facts

1. Mesenchymal Stem Cells exist in the proximal Humerus

2. Can easily withdraw increased volume of Bone Marrow (0-40cc) and spin with standard centrifuge

3. MSC can be found in increased volume aspiration but not as consistent

4. MSC able to adhere to various biologic scaffolds and proliferate

5. Multiple hole vs Single Hole aspiration does not make a difference
2013 MSC Take Home Facts

6. Clinical comparison with Lat Dorsi transfer show that allograft dermal patch plus MSC is as good and inconsistently better than Lat transfer with significantly less morbidity.

7. If humeral proximal migration (acromiohumeral distance > 7) or greater than 50% fatty then would perform Lat Transfer NOT Patch Augmentation.
University of Connecticut Failed Rotator Cuff Repair (RCR) Algorithm Confirmed by MRI Arthrogram

**SYMPTOMATIC**
- Pain with activity
- Night Pain & Awakening
  - Weakness
  - Loss of ROM

**ASYMPTOMATIC**
- No pain
- Full ROM
- Happy

**Offer Operative Intervention**
- Non-retracted tears
- No X-Ray changes
- Good muscle/tendon quality

**Observe at 1 year intervals with repeat MRI Arthrogram**

- Proximal migration on X-ray
- >70 years with massive RC tear
  - Horrible pain at rest

- > 1 primary RCR
  - Young patient with retracted tear
  - Changes on X-Ray

**Arthroscopic Revision RCR**

**Reverse Ball and Socket**

**Lat Transfer VS**

**Allograft & bone marrow**
Biologic Categories that will enhance the body's own ability to improve tendon healing?

Matrix Metalloproteinase - play a role in extracellular matrix production. Tissue inhibitors of Matrix Metalloproteinase’s (TIMMP) such as Doxycycline can improve healing.

Growth Factors and Platelet Rich Plasma - Can modulate various aspects of healing but have a specific does and time response.

Increasing vascularity during Rotator cuff healing - poor vascularity exists at the healing cite and agents such as glycercyl trinitrate improved healing (Paoloni et al AJSM 2005).

Cell Based Therapies and Scaffolds - Mesenchymal stem cells as well as various extracellular patches may improve healing.