Biologics in Sports Medicine

Basic Science
Preparation
Applications
Evidence Base

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Biologics

• Natural products that are harvested and used to augment a medical process and/or the biology of healing
3 categories

– Growth factors
  • Harvest and delivery of GFs to site
  • Eg. PRP

– Cell therapies
  • Harvest and delivery of cells to a site
  • Eg. Autologous chondrocyte therapy for cartilage repair

– Tissue therapy
  • Use of tissue to replace damaged structures or augment repair
  • Eg. Meniscal allograft transplantation
3 categories

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Biologics

- PRP
- Stem Cells
Healing

• Inflammation (day 1-5)
  – Hematoma
  – Platelets activate
  – Fibrin-platelet matrix forms
  – Vasodilation
  – Migration of numerous cells

• Proliferative stage (day 3 – wk 6)
  – Fibroblasts produce collagen

• Remodeling (wk 6 – 1 year)
  – Organization of collagen
Biologics and Healing

Provide 3 Elements to the healing process

• Matrix
• Growth Factors
• Stem Cells
Platelet Rich Plasma
PRP

• Defined
  – Autologous blood with concentration of human platelets above baseline values.
    • Normal platelet count is 150,000 – 300,000 per uL
    • PRP by definition has 3-5x the baseline levels.
Platelets

- Blood cells without nucleus, principal role in coagulation
- 3 types of granules (a, d, and l),
- Alpha granules contain growth factors
- Release of this growth factors occur with exposure to collagen, VW-f, thrombin or calcium chloride
<table>
<thead>
<tr>
<th>Factor</th>
<th>Target Cell and Tissue</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>PD-EGF</td>
<td>Blood vessel cells, outer skin cells, fibroblasts and many other cell types</td>
<td>Cell growth and recruitment, cell differentiation, skin closure, cytokine secretion</td>
</tr>
<tr>
<td></td>
<td>Fibroblasts, smooth muscle cells, chondrocytes, osteoblasts, mesenchymal stem cells</td>
<td></td>
</tr>
<tr>
<td>PDGF AB</td>
<td>Fibroblasts, smooth muscle cells, chondrocytes, osteoblasts, mesenchymal stem cells</td>
<td>Potent cell growth, recruitment, blood vessel growth, granulation, growth factor secretion, matrix formation with BMPs (collagen and bone)</td>
</tr>
<tr>
<td>TGF-β1</td>
<td>Blood vessel tissue, outer skin cells, fibroblasts, monocytes, osteoblasts</td>
<td>Blood vessel and collagen synthesis, growth inhibition, apoptosis (cell death), differentiation, activation</td>
</tr>
<tr>
<td>IGF-I, II</td>
<td>Bone, blood vessel, skin, other tissues, fibroblasts</td>
<td>Cell growth, differentiation, recruitment, collagen synthesis with PDGF, cell growth</td>
</tr>
<tr>
<td>bFGF</td>
<td>Blood vessels, smooth muscle, skin, fibroblasts, other cell types</td>
<td>Cell migration, blood vessel growth, cell growth, migration, new blood vessel growth, anti-apoptosis (anti-cell death)</td>
</tr>
<tr>
<td>VEGF, ECGF</td>
<td>Blood vessel cells</td>
<td></td>
</tr>
</tbody>
</table>
• Function
• Augment **healing** response via local secretion of growth factors and recruitment of reparative cells

The phases of cutaneous wound healing
PRP PREPARATION
PRP Preparation

Slight to major difference based on system

• Draw blood
• Mixed with anti-coagulant that binds calcium
• Prevents the conversion of prothrombin to thrombin in the coagulation cascade
  – Prevents platelet activation
PRP Preparation

Centrifugation # 1 ("soft spin")
Separate whole blood into RBC fraction and plasma fraction (plts, wbc, clotting factors)

Centrifugation # 2 ("hard spin")
Platelet poor and PRP

Activation
Addition of coagulant releases growth factors (70% released within first 10 minutes)
PRP Preparation

No universal classification
PAW classification system
- Absolute number of platelets
- Method of activation
- Presence or absence of WBCs
- No evidence base behind this classification
PRP Challenges

• Variability
  – More than 40 available commercial systems
  – Amount of whole blood used
  – Amount of plasma used to suspend concentrated platelets
  – Variability between patients blood samples using same system

• No consensus on appropriate number of platelets
  – More is better?
  – Saturation effect?
  – WBC

• Timing
Stem Cells
Stem Cells

Basic characteristics
• Self Renewal
• Prolonged cell division
• Unspecialized
• Can differentiate into specialized cells when provided appropriate environment

Classified in two ways
• Plasticity
• Source

Stem Cell

Self-Renewal

Differentiation

Mature Cell
Stem Cell Plasticity

Plasticity – ability to become different cell types

Totipotent
Pluripotent
Multipotent
Unipotent
Mesenchymal Stem Cells

- Prime interest in orthopedics
- Source: Bone marrow (also; fat, skin, tendon, periosteum)
- Most common harvest site: Iliac crest
- Multipotent - In appropriate environment can differentiate into bone, tendon, muscle and adipose tissues
Stem Cell in Spots

• Purpose in Sports Medicine
  – Much of the soft tissue in sports medicine heals poorly (tendons, cartilage etc.) via formation of scar tissue that is biomechanically inferior to the native tissue
  – Delivery of regenerative cells may provide cells for improved healing and replacement of the native tissue
Isolation of Stem Cells
Isolation of Stem Cells

- No consensus regarding optimal number of cells for healing in MSK tissues
- Iliac crest BM aspirate yield a poor number of MSCs
- MSCs decrease with age
- Success of bone marrow aspirate of MSCs may be due to number progenitor cells (Hernigou et al. JBJS, 2005)
Stem Cells Isolation

• Many Sources and Techniques
• Example in Brief
• Bone Marrow Obtained from Iliac crest in Aspiration needle loaded with heparin
Next one of two steps

- Concentration via centrifuge
- Growth on commercially available mediums
Stem Cell Isolation

- MSC Characteristics
  - Adherence to plastic under normal culture conditions
  - Fibroblast-like morphology
  - Presence of surface CD73, CD90, CD105
  - Lack of surface CD11b, CD14, CD19, CD34, CD45, CD79a and HLA-DR

- Play an important role in isolation
- In US centrifugation remains only FDA approved method of concentration
Clinical Applications of Biologics
Tendon Injury
Biologics and Tendon Injury

• Tendons
  – Damaged tendon tissues heals very slowly and rarely attains the structural integrity and mechanical strength or normal, undamaged tendon
    • Poor vascularity
    • Microtrauma
  – Tendon tissue that is torn is often degenerative and prone to failure with surgery
PRP and Tendon Injury

- Complex healing process
- Many of GF and Cytokines involved are present in PRP
- Additionally PRP can aid in angiogenesis
- Anti-Inflammatory properties
- Most studies Elbow, Knee and Ankle
PRP and Tendon Injury

• Patellar Tendinopathy
• Recent literature
  – Comparison of PRP to ESWT and DN
  – Enhanced structural integrity at 3 mos (Charousset et al. AJSM, 2014)
PRP and Tendon Injury

• Lateral Epicondylitis

• Recent Literature
  – PRP vs GC vs Saline
    • (Krogh et al. AJSM 2013)
    • Improvement without significant difference
  – PRP vs Autologous whole blood
    • (Raeissadat et al. BMC Sports 2014)
    • Improvement without significant difference
PRP and Tendon Injury

- **Achilles Tendinopathy**
  - No therapeutic effect
    - Two level I studies comparing PRP vs Saline for Achilles tendinopathy
    - No difference between the control groups

- **Achilles Tendon rupture**
  - Conflicting evidence on PRP for augmentation during Achilles tendon repair (Sanchez et al. AJSM 2007 vs Schepul et al AJSM 2010)
  - Strong support in animal studies
PRP and Tendon Injury

• Limitations and future studies
  – Lack of placebo
  – Lack of non-treatment group
  – Larger studies, better controls

• Considerations
  – Cost
  – Placebo affect
  – Natural history of disease process
Stem Cells and Tendon Injury

- Limited small case series currently showing benefit

- Summary (Ahmad et al. Arthroscopy, 2012)
  - Animal studies
    - Increase collagen fiber density,
    - Enhance tissue architecture
  - Stem cells may regenerate tendon tissue and enhance tendon repair construct
Clinical Applications of Biologics
Rotator Cuff Repair
Biologics and RTC Repair

- Increasing incidence with aging population
- Common cause of shoulder pain and occupational disability
- Improvements in arthroscopic instrumentation and suture anchor technology
- Tendon failure is very high in the literature
- Retear rate ~20%
PRP and Rotator Cuff

• Several level I (8), studies investigated PRP augmentation at time of RCR

• Summary (Chalal et al. Arthroscopy, 2013)
  – Retear rates same for Large to massive tears
  – Potential decrease in retear rate for small to medium tears (7.9 vs 26.8%)
  – No improvement in clinical outcomes when compared to control.
  – Future outcomes stratified by tear size
Stem Cells and Rotator Cuff

Hernigou et al. Int. Orth. 2014

- RCR w/ BMMSC vs RCR w/o
  - 2 groups of 45 pts, tears 1.5-3.5cm, isolated supraspinatus. Treated from 2000-2005, avg size 2.2cm
  - Control from historical data, matched to patient intervention group, previous to 2000

- US/MRI eval of tendon @ 3mo, 6mo, 1y, 2y, 10Y

- Results at 6 mos – 100% of MSC group intact vs 67%
- Results at 10 years – 87% of MSC group were intact, compared to 44% in the non-MSC group

- 39 intact patients had avg # of MSCs of 54,000 injected
- 6 patients with retear had avg # of MSCs of 19,000
Clinical Applications of Biologics
Cartilage Repair
Biologics and Cartilage Repair

- Highly organized tissue
- Complex biomechanical properties
- Poor intrinsic capacity for healing
- Large defects heal via fibrocartilage, with inferior biomechanical properties
- Damage from Trauma or degeneration results in morbidity and functional impairment
Biologics and Cartilage Repair

• PRP and Cartilage defect
• Theory
  – Promote cell survival and chondrocyte proliferation
• Growth Factors
  – IGF-1 – anabolic growth factor for articular cartilage
  – TGF-B – stimulates chondrocyte adhesion
• Application
  – With a collagen gel
  – With a scaffold (eg. Polyglycolic Acid)
  – In conjunction with Microfracture, ACI etc.
Limited Research currently

- **Animal Studies**
  - Rabbit studies
  - Larger animal studies (Goats, Sheep)

- **Human studies**
  - Very limited
  - May form “Hyaline-like” repair tissue w/ microfracture (Sciliari et al.)
  - Small # of patients, with short follow up
  - Results are promising, but the paucity of literature may be due to poor results in unpublished studies.
Stem Cells and Cartilage Repair

• Theory supporting use of Stem Cells is related to micro-fracture

• Penetration of subchondral bone results in influx of MSCs

• Formation of fibrocartilage.
Stem Cells and Cartilage Repair

• Cartilage is secreted by chondrocytes
• Low mitotic activity
• ACI - resection of healthy cartilage and culture of Chondrocytes
• Replantation into patients
• Good clinical results,
  – Donor site morbidity
  – Limited supply of chondrocytes,
Stem Cells and Cartilage Repair

• MSCs versus ACI
  – Multiple donor sites that won’t affect healthy cartilage, obviates need for staged procedure
  – High proliferative capacity
  – Multipotency enabling it to differentiate into chondrocytes
Stem Cells and Cartilage Repair

The Evidence – Clinical studies

• Still very limited, good results in several animal studies
• Nejadnik et al. AJSM, 2010 - ACI versus BMMSC
• Gobbi et al. AJSM 2014
  – 25 patients w/ symptomatic large chondral defects (ICRS grade 4)
  – Treated w/ MSCs and collagen matrix w/ 3 year follow up.
  – Results: MRIs showed complete filling of defect in 80% of patients,
  – Second look arthroscopy in 7 patients – smooth healthy, continuous cartilage

• Overall
  – Promising results based on clinical, MRI, histologic and arthroscopic evaluations
  – If results equivalent to ACI, valid replacement for this treatment
  – Still very limited, further studies to evaluate delivery and surgical methods
  – Patient selection important – OA, Malalignment, ligamentous laxity, BMI
Clinical Applications of Biologics
Meniscus Repair
Biologics and Meniscus

- Meniscus - limited healing
- Vascularity provided by Medial and lateral geniculates via attachment of synovium and joint capsule
- Supplies outer $\frac{1}{3}$
- Peripheral $\frac{1}{3}$ predictably heal after surgical repair
PRP and Meniscus

• “Arthroscopic meniscal repair using an exogenous fibrin clot” CORR 1990
  – Meniscal tears augmented with fibrin clot, showed 8% failure rate compared to 41% without
PRP and Meniscus

• Ishida et al Tissue Engineering 2007
  – 1.5mm full-thickness defect in avascular region of rabbit meniscus
  – Significantly greater scores for number of fibro-chondrocytes and production of ECM in PRP group

• Zellner et al J Biomed Mater Res 2010
  – Similar study to Ishida using rabbits but 2cm punch defects in non-vascular zone
  – No improvement with PRP compared to control

• No Current Human studies
Stem Cells and Meniscus

• Meniscus repairs heal better when associated with concomitant ACL reconstruction
  – Clot formation and release of MSCs from bone tunnels and notchplasty

• Argument for both PRP and MSCs
Stem Cells and Meniscus

Animal studies

• BM, synovial and adipose derived MSCs have been applied to tears in avascular zone of Rabbit and porcine menisci

• Apparent regeneration of meniscus tissue via Macroscopic, histologic and MRI evaluation
Stem Cells and Meniscus

Humans Studies

- Very limited
- Vangsness et al. JBJS 2014
- RDBCT
  - 3 groups of 20, patients were candidates for partial medial meniscectomy based on MRI and surgeon’s evaluation
  - Group A – 50 million hMSCs, Group B 150 million hMSCs, Group C control of suspension w/o MSCs
  - Intraarticular injection deliver 7-10 days after surgery

Results

- No adverse effects
- 24% of patients in Group A had significant increase in meniscal volume vs 0% in control (defined >15% increase in meniscal volume) @ 2 years
Summary

• Safe
• Good pre-clinical and lab background
• Early clinical results for Stem Cells promising
• Future
  – Standardization
  – Delivery and Isolation techniques
  – Quantification
  – Larger studies, Better comparison groups