Disclosures

- No financial disclosures.

Outline

- Overview of Orthobiologics
- Classifications/Options
- Biochemical Actions
- Data Review
- Questions
Orthobiologics

- Marketing term from medical device industry.
- Emerging sub-field in Orthopedics and Sports Medicine.
- MSK US – opening door for outpatient use.

Orthobiologics

Bioengineered products + Body’s healing capabilities = Maximized Healing and Regeneration

Orthobiologic Options

- Prolotherapy
- Platelet-Rich Plasma
- Activated PRP
- Mesenchymal Derived Stem Cells
Uses & Investigations
- Orthopedics
- Urology
- Neurology
- Pulmonary
- Cardiology
- Rheumatology
- Dermatology
- Ophthalmology

Orthopedics & SM
- Tendon Injuries
- Ligamentous Injuries
- Muscular Injuries
- Osteoarthritis / Cartilage Damage
- Spinal Pathology – Disk disease, Facet Arthropathies

Orthobiologic Basics
Localized Orthobiologic Therapy
- Enhanced Intrinsic Tissue Regeneration Process
- Local signals direct cells to differentiate into appropriate tissue-specific cell types
- Cells deposit matrix through specific adhesion mechanism
- Created cell-specific molecule(s) target damaged tissue
- Normal tissue cell attrition

Augmented with high GF concentrations and injection fenestration technique.

Wound Healing Process

- Stages:
  1. Inflammatory
  2. Proliferative
  3. Remodeling

Inflammatory

- Initial response to injury.
- Goals are hemostasis and subsequently regeneration.
- Blood escapes damaged vessels:
  - Hematoma formation with platelet recruitment.
  - Growth Factors (GF) and cytokines released.
  - Cell migration, Proliferation, Differentiation and Matrix synthesis.

Proliferative

- Damaged, necrotic tissue removed.
- Replaced with viable tissue, specific to local tissue environment.
- Mesenchymal stem cells produce this viable tissue based on local factors:
  - GFs
  - Cytokine profile
  - Hormones
  - Nutrients
  - pH, O$_2$ tension
Remodeling

- New tissue reshapes/reorganizes to resemble original tissues.
- Reduction of cell density, vascularity, excess matrix.
- Orientation of collagen fibers along lines of stress to strengthen tissue.

Orthobiologic Therapies

Prolotherapy
Prolotherapy

- Prolotherapy has been in use since the 1930s.

- Needling technique with Dextrose solution used as an irritant, to stimulate injured musculoskeletal connective tissue to promote healing.

- Based on the premise that chronic musculoskeletal pain is due to inadequate repair of fibrous connective tissue.


- Stimulates a temporary, low grade inflammation at the site of ligament or tendon injury.

- This inflammatory stimulus activates fibroblasts and native growth factors to stimulate the microenvironment to reinforce local connective tissue.

- Resumes or initiates a new connective tissue healing cascade cycle to complete one which was prematurely aborted or never started.

Platelet-Rich Plasma

History

- Early uses:
  - Wound healing and Plastic Surgery – 1980s
  - Cardiothoracic Surgery – Late 1980s
  - Maxillofacial and Oral/Dental procedures – 1990s
Accelerated Growth

- Mid 2000’s
  - High profile athletes publicized their treatments.
  - $45,000,000,000 Market in 2009
- CME initiatives in Sports Medicine expanded.
- Lucrative, Cash Based Service…

What is PRP?

- Biologic therapy where an autologous blood sample is used to generate a platelet-rich substrate that is reintroduced into the body to promote healing.
- Can be thought of as responding with hematoma in excess of that which would have been physiologically produced.
- Effect of PRP on wound healing likely multifactorial:
  - Platelet concentration.
  - PRP volume delivered.
  - Extent and type of injury.
  - Overall medical condition of the patient.

Why Platelets?

- In vitro, dose-response relationship seen between platelets and:
  - Proliferation of mesenchymal stem cells.
  - Proliferation of fibroblasts
  - Production of Type I collagen
- Suggests that autologous platelets can enhance wound healing.
Why Platelets?

- In vivo, platelet activation in response to tissue damage:
  - Formation of platelet plug, blood clots.
  - Secretion of biologically activated proteins from \(\alpha\)-Granules:
    - Begin tissue healing
    - Cellular chemotaxis, proliferation and differentiation
    - Removal of tissue debris
    - Angiogenesis
    - Laying extracellular matrix
    - Regeneration of tissues

Mechanism

- After acute injury, platelets migrate to site of injury through inflammatory phase to stop bleeding and signal healing.
- Activation - Process of \(\alpha\)-granule fusion with platelet membrane.
- Process occurs within 10 min of clot formation.
- 95% pre-synthesized GF released in 1st hour.
- Platelets continue to generate and release GFs for the rest of their lifespan, 5-10 days.

Platelet \(\alpha\)-granules:

- Platelet-Derived GF (PDGF) – Promotes chemotaxis of mesenchymal stem cells and endothelial cells to site.
  - Differentiation for fibroblasts and osteoblasts.
  - Up regulate effects of other growth factors.
- Transforming GF - Beta (TGF-\(\beta\)) - Promotes cell mitosis and differentiation for connective tissue and bone.
  - Activates Mesenchymal Stem Cells, pre-osteoblasts and fibroblasts.
  - Inhibits osteoclast formation.

**Platelet α-granules:**

- Insulin-like GF (IGF) – Mitogenic to osteoblast cells and stimulates bone formation from existing osteoblasts.
- Vascular Endothelial GF (VEGF) – Stimulates angiogenesis and chemo-attractive for osteoblasts.
- Epithelial GF (EGF) – Induces epithelial development and promotes angiogenesis.

Platelet Factor 4 (PF4) – promotes coagulation.
- Chemoattractant for neutrophils and fibroblast.
- Interleukin-1 (IL-1) – stimulates fibroblast and keratinocyte growth and chemotaxis.
- Collagen synthesis by fibroblasts.
- Several others…


**Standardized?**

- No uniform PRP collection process.
- No uniform PRP content/ratios.
- No consensus protocols for treatment or rehab.
Collection Methods

- Varying systems: BioMet, Arthrex...
- Differing quantities of blood needed for collection.
- Most require centrifugation to separate out buffy coat, PRP rich layer.
- Platelet concentration ratios from < 2x to > 8.5x.
- Platelet counts average 1 – 1.5 Million / microl.
- < 0.6 M / microl show no better results than whole blood.

PRP Harvest

- Based on harvest system 15 – 60 cc of blood is drawn from patient.
- Platelet filtration performed through centrifugation or filtering system.
- Open vs. Closed systems - Dual syringes vs. Filters
Preparations

Regardless of collection methods:
- PRP preparations yields ~ 3 to 5 cc.

WBCs vs. RBCs

- Presence of RBCs, WBCs, how many?
- There is a large variation in number of RBC in the PRP products across the platforms.
- No clinical data concerning adverse events due to RBC presence in PRP.
- Data has not shown that RBCs activate platelets in PRP.
WBCs vs. RBCs

- WBCs may play antimicrobial role in PRP treatment.
- WBC concentration in PRP accounted for 1/3 to 1/2 variance of growth factor concentration.
- VEGF and PDGF
- No controlled animal studies to discern whether leukocyte-rich PRP increases inflammation, compared with leukocyte-poor PRP.


Deployment

- Topical analgesia recommended.
  - Marcaine – Chondotoxic and toxic to mesenchymal stem cells.
  - Lidocaine – Diluted to 0.25% in sterile saline/water is ideal.
  - Ropivicaine – Diluted to 0.0625%
- Injection with direct visualization.
- Fenestration / Needling technique to promote local inflammation.


MSK Ultrasound

- Accurately visualize damaged tissue to ensure appropriate location of deployment.
- Ultrasound vs. anatomical guidance: 95.8% versus 77.8%
- Minimize risk of neurovascular injuries

Post-PRP Care / Rehab

- Post-Injection care guidelines are variable, but may be key to recovery/success.
- Bracing, crutches?
- Analgesia – No NSAIDs for 2 weeks suggested.
  - Tylenol or Vicodin
- Icing?

Post-PRP Care / Rehab

- Progression back to activities?
- Physical Therapy’s Role? Timing?
- Repeat Injections?

What Does the Data Say?
PRP vs. Placebo Knee OA

1. Both groups treated with PRP had better results than did the group injected with saline only.
2. Single dose of WBC-filtered PRP is as effective as 2 injections of PRP 3 weeks apart to alleviate symptoms in early knee OA on WOMAC scale.
3. Results deteriorate after 6 months.

PRP vs. Cortisone

1. Lateral Epicondylitis: PRP vs. cortisone group had reduction of 25% on VAS or DASH scores after 2 yrs.
2. Baseline VAS and DASH scores compared at 2 yr follow-up:
   - Both groups' VAS significantly improved over time.
   - DASH scores of the corticosteroid group returned to baseline, while PRP group significantly improved.
   - No complications related to the use of PRP.

PRP vs. HA in OA

1. Both PRP and HA treatments of OA joints resulted in decreased catabolic gene expression.
2. PRP stimulates endogenous HA production and decrease cartilage catabolism.
3. PRP and HA were similar in suppression of inflammatory mediator concentration and expression of their genes in synoviocytes and cartilage.
4. Clinical Relevance: The anti-nociceptive and anti-inflammatory activities of PRP support its use in OA joints to reduce pain and modulate the disease process.
PRP in Soft Tissue Injury

Metanalysis of 19 trials covered eight types of injury:
- Rotator cuff tears (6)
- Knee ligament reconstruction (6)
- Lateral epicondylitis (3)
- Shoulder impingement syndrome (1)
- Patellar tendinopathy (1)
- Achilles tendinopathy (1)
- Acute rupture of the Achilles tendon (1)

Quality of the evidence is very low.
- Most trials used were randomized, double-blind.
- The trials also used different ways of preparing and applying the platelet-rich plasma.
- Primary outcomes (function, pain, adverse events) for a maximum of 11 studies and 45% of participants.

Very weak (low quality) evidence for a slight benefit of PRP in pain in the short term (< 3 months).

Data does not show that PRP:
- Makes a difference in function in the short, medium or long term.
- There was weak evidence that adverse events (harm) were not significant with PRP vs. other treatments.

In conclusion:
- Evidence is insufficient to support the use of PRP for treating musculoskeletal soft tissue injuries.

What Does This All Mean?

Future Avenues

- Further double blind RCTs.
- Procedural Standardization.
- Long-term outcomes.
- Effects of PRP ratios of WBC, RBC and other growth factors.

Activated PRP
Activated PRP

- Many clinicians have been using activating agents to stimulate growth factor release by PRP to enhance the healing benefits.

- Most commonly performed by adding a solution of 1,000 units of topical bovine thrombin per mL of 10% calcium chloride to PRP.

- Should be used within 10 min of activation.


Why Activation?

- Orthogen (German company that created the procedure) uses a process where autologous conditioned serum (ACS) is generated by taking a sample of the patient’s blood and incubating it in the presence of etched medical-grade glass beads.

- The glass beads are used to grow immuno-factors, (Orthokine) then spun down and re-injected into the affected joint / muscle.
Orthokine vs. PRP

- PRP activation based on exposure of blood leukocytes to pyrogen-free surfaces (e.g., glass spheres) elicits:
  - Accumulation of anti-inflammatory cytokines.
  - Interleukin-1 receptor antagonist
  - Several growth factors: IGF-1, PDGF, TGF-β.


Orthokine vs. PRP

- Autologous conditioned serum (ACS) from the incubation of whole blood with glass spheres was developed from these principles and biochemical studies.
- The injection of ACS into affected tissue has shown clinical effectiveness and safety in animal models, as well as in human clinical studies.
- Treatment of osteoarthritis, lumbar stenosis, disc prolapse and muscle injuries.


Data Support Activation?

- 376 patients with knee OA in a prospective, randomized, patient- and observer-blinded, placebo-controlled trial.
- The clinical effects of ACS were compared to HA and saline (placebo) as assessed by patient-administered outcome instruments.

Data Support Activation?

- All treatment groups, intra-articular injections produced reduction in symptoms and improvement in quality of life.
- ACS significantly superior to HA and saline for all outcome measures and time points.
- No differences between the effects of HA and saline.
- Frequency of adverse events was comparable in ACS and saline groups, but higher in the HA group.

Conclusion: The data demonstrates that ACS injection considerably improves clinical signs and symptoms of OA.

Data Support Activation?


Orthokine vs. PRP

- No head to head trials.
- Minimal data supporting Orthokine use over PRP.
- Theoretical advantage with IL-1 Receptor Antagonist.

Dehydrated Amniotic/Chorionic Membrane Injections
Human amniotic membrane have been used for wound healing and various other uses for ~100 years. Only in past decades has it reached sports medicine.

In vivo and in vitro studies demonstrate inflammation reduction and enhanced soft tissue healing. Primary GFs released are EGF, TGF-β, PGF which stimulate epithelial cell migration and proliferation. PDGF A and B which stimulate protein and collagen synthesis, collagenase activity, and chemotaxis of fibroblasts and smooth muscle cells.

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Amnio Injections

- Preservation of tissue required.
- PURRON process - method of sterilizing and drying human amniotic/chorionic membrane obtained from screened and tested donors.
- Allows for allograft material that can be stored at ambient temperature for up to 5 years.
- Micronization process - produces a powder form.
- Powder is dissolved in sterile 0.9% saline solution allowing new uses.
- Injections of dehydrated human amniotic membrane allows treatment of soft tissue injuries.

Refractory Plantar Fasciitis

- Patients with refractory plantar fasciitis receiving single-dose injection of micronized dehydrated human amniotic/chorionic membrane allograft versus Cortisone and Botulinum toxin A experienced:
  - Improved symptoms and function within 1 week.
  - Continued improvement over the 8-week study.
  - 30% over Botulinum and 50% over Cortisone.
Impact During Early Phase Achilles Injuries

- Animal study, Rat Achilles tendons underwent surgical tenotomy and repaired. 72 tendons:
  - Sutured
  - Sutures, injected with Amniotic fluid
  - Sutures, injected with Amniotic fluid + membrane.
- Human amniotic membrane and fluid showed no significant changes on histopathology of healing process of Achilles tendon ruptures in early phase.

Clinical Significance

- Again, limited data…
- Small trial 45 patients
- Funded Trials/Contributions
- Thought to be safe after micronization process.
- Clinical judgment, do no harm.

Mesenchymal Stem Cell Therapies
Mesenchymal Stem Cells

Bone Marrow Aspirate

Adipose Derived

MSCs can be readily extracted from bone marrow of hip and spine, and adipose tissue.

MSCs have potentials to differentiate into bone, cartilage, tendon, muscle and other tissues.
Stem Cell MSK Therapy

- Belief is that there are really only two kinds of stem cells:
  - Embryonic (prenatal) stem cell
  - Adult (postnatal) stem cell.
- Postnatal “adult” stem cells persist after birth:
  - Undifferentiated state
  - Available to maintain tissue homeostasis and allow regeneration.
- Adult stem cells can be found in all tissues in the body in various quantities: major reservoirs in adipose and bone marrow.

Stromal Vascular Fraction (SVF) is the product of lipoaspirate which is obtained from liposuction of excess adipose tissue.

Adult derived stem cells (ADSCs) are extracted using collagenases, which are later removed by washing the ADSCs with D5LR solution three times.

After 3 washings, the total amount of collagenase contained in the ADSCs is undetectable.
Procedure

- After each washing, the lipoaspirates are centrifuged, ultimately producing:
  - ~10 mL of ADSCs-containing SVF.

- PRP has been successfully used as a cell culture additive to facilitate growth and differentiation of autologous MSCs.

  Especially when Adipose Derived.

Procedural Differences

- Distinct lack of homogeneity among procedures of MSC collection in animal studies investigating the use of MSCs in the treatment of OA.

- Animal and clinical studies have suggested dose ranges anywhere from $1 \times 10^6$ to $4 \times 10^7$ cells per injection.

- No clear indication of what the initial dose should be.

Embryonic Stem Cell?

- Embryonic stem cells, in theory, are able to transform into any type of tissue.

- They are “totipotent” or “omnipotent” and able to differentiate into any of the three germ layers.

- However there are legal, religious, political and ethical issues which inhibit their use.
Controversy surrounding legality of use of embryonic stem cells nationally.

Avoided with use of autologous adult stem cells, but regulation still exists.

Autologous adult stem cells are considered:
- “Human Cells, Tissues and Cellular-Based Products (HCT/P’s)"
- Regulated by the FDA.

Exemption from regulation exists if the physician:
- "removes HCT/P’s from an individual and implants such HCT/P’s into the same individual during the same surgical procedure."
- At this time, no chemical manipulation of the adipose-derived tissues for isolation and concentration is permitted in the United States by the FDA.

To be considered as occurring “during the same surgical procedure” the cells must be “autologous,” “minimally manipulated,” and “used within a short period time.”

“Minimally manipulated” defined as “processing that does not alter the relevant biological characteristics of cells or tissues.”

“Short period of time” is not exactly defined. Considered to be “a matter of hours (or less), without the need for shipping.”

Harvesting native autologous adipose stem cells does not currently pose any problem as far as FDA regulation is concerned as long as exemption criteria are met.
"More than minimal" manipulation involves: "the use of drugs, biologics, and/or additional devices that warrants regulation of the manufacturing process and the resulting cells as biological products."

This is where the use of enzymes such as collagenase or culture expansion of cells comes into question.

Chemical isolation, concentration, and culture expansion of stem cells, while delivering higher yields, remains problematic in terms of existing FDA requirements.

One of the safety issues that needs to be addressed is the potential of these stem cells becoming neoplasms.

Initially brought on by reports of chromosomal abnormalities in MSCs that have been cultured in vitro. However, when these cells are cultured less than 60 days in vitro, they pose no detectable risk of cell mutation or tumor formation.

Retrospective cohort study demonstrated no evidence of neoplastic complications in any implant sites in 100 injected joints.

Autologous and uncultured ADSCs/PRP therapy in the form of SVF can be considered to be safe when used as percutaneous local injections.

In 2003, Murphy et al. reported:

Improvement in medial meniscus and cartilage regeneration with ASC therapy in animal models.

In 2008, Centeno et al. reported:

Significant knee cartilage growth and symptom improvement in a case report using culture expanded autologous MSC’s from bone marrow.

Intra-articular injections of MSCs improved pain and function scores in knee OA.
The optimal method of MSC administration remains unknown.
The optimal dose, frequency, timing, and number of injections remains unclear.
The optimal source of MSCs (bone marrow, adipose tissue, synovium, umbilical cord blood/tissue) is yet to be identified.
The majority of clinical studies use bone marrow- or adipose-derived MSCs.
There is a need for clinical imaging studies to determine where the MSCs end up after injection.

Many options for Orthobiologic treatments are under investigation.
Know the science behind these technologies.
Don’t just follow the trendy new fad.
Know the Risks vs. Benefits and explain them to your patients.
Collect data and report it!

Thank You!

Questions?
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