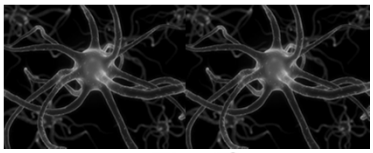
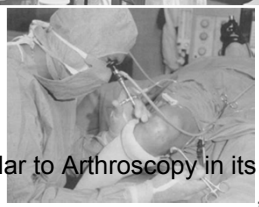
UPDATES IN REGENERATIVE MEDICINE



The NON-SURGICAL CENTER OF TEXAS
SPORTS • SPINE • ORTHOPEDICS
Annette M. Zaharoff, M.D.

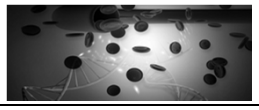
Regenerative Medicine 2016



Similar to Arthroscopy in its infancy

Objectives

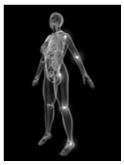
1. Biology of Platelet Rich Plasma (PRP)/Mesenchymal Stem Cells (MSC) and rationale for their use
2. Inherent variables in PRP/MSC use
3. Clinical applications of PRP/MSC in musculoskeletal medicine
4. Review relevant PRP/MSC research



Sports Medicine

Musculoskeletal Medicine

- MSK injuries leading cause of disability and pain
- Increasing prevalence
- Increase healthcare costs
- Decrease productivity and quality of life



Platelet Rich Plasma

- Google hits
  - 2011=461,000
  - 2015=~2,000,000
- > 7800 references for PRP
- > 500 new pub med references in 2015 alone

Regenerative Medicine

New Musculoskeletal Regeneration Program- pipeline from biomedical discovery to clinical implementation

MUSC Medical University of South Carolina — Changing What's Possible

MAYO CLINIC

Barshop Institute UT Health Science Center San Antonio

Penn IRM — Institute for Regenerative Medicine

HARVARD STEM CELL INSTITUTE — 1000 SCIENTISTS. ONE GOAL. REINVENTING HEALTH.

South Texas Blood & Tissue Center

UTHealth — The University of Texas Health Science Center at Houston

BioBridge Global

Regenerative medicine has been called the "next evolution of medical treatments," by the U.S. Department of Health and Human Services. With its potential to heal, this new field of science is expected to revolutionize health care.

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Mesenchymal Stem Cells

- BMAC~5-6x increase vs Adipose
- Trophic Effects (Drugstore?)
  - Cytokines, chemokines, GF
  - Angiogenesis, mitosis, anti-scarring, anti-apoptotic
- Local Modulation
  - Anti-inflammatory
  - Immunomodulatory
  - Anti-microbial

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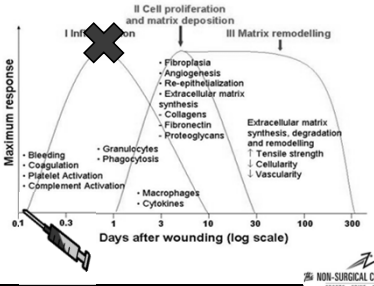
No Quick Fix for Connective Tissue Tendon Healing Insufficiency



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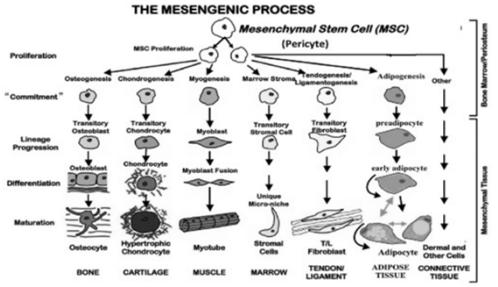
Stem Cell Differentiation



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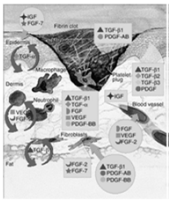
Platelet Rich Plasma

- >4x baseline concentrations
- Growth factors
  - Cell proliferation, tissue growth
- Cytokines
  - Intercellular interactions
- Chemokines
  - Attract stem cells and macrophages



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Why Is the Literature Confusing?

- PRP-Stem Cell variables
- Biology of healing
- Micro-environment of injury site
- What's the real diagnosis?
  - Effects of biotensegrity and biomechanical disruptions

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The PRP-Stem Cell Variables

- Patient
- Equipment / Processing
- Cell counts
  - PRP, MSC
  - RBC, WBC's +/-
  - Dose, Frequency
- Activation +/-
- Other medications

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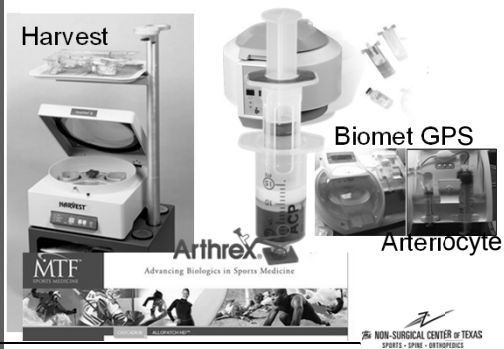
PRP Variables



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The Variables

The

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Comparison of Growth Factor and Platelet Concentration From Commercial Platelet-Rich Plasma Separation Systems

Tiffany N. Castillo,* Michael A. Pouliot,* MD, Hyeon Joo Kim,* PhD, and Jason L. Dragoo,*† MD
Investigation performed at Stanford University, Department of Orthopaedic Surgery, Palo Alto, California

Am J Sports Med. 2011 Feb;39(2):266-71.

Growth Factor and Catabolic Cytokine Concentrations Are Influenced by the Cellular Composition of Platelet-Rich Plasma

Emily A. Sundman,* Brian J. Cole,† MD, MBA, and Lisa A. Fortier,*‡ DVM, PhD
Investigation performed at Cornell University, Ithaca, New York

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PRP Variables

Patient Platelet Count

Patient A 150 x 10^3	Patient B 350 x 10^3
PRP System 2X = 300,000	PRP System 2X = 700,000
PRP System 5X = 750,000	PRP System 5X = 1,750,000

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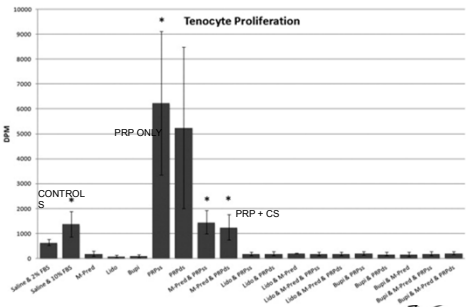
Corticosteroids and Anesthetics



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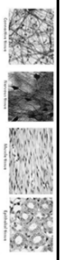
The Biologic Variables

- Microenvironment / healing response
  - Tendinopathy: degenerative, ineffective healing cascade
  - Osteoarthritis: increased catabolic activity, subchondral bone and cartilage injury
  - Mechanism: Conductor vs Orchestra
- Do all tissues respond the same to treatment?



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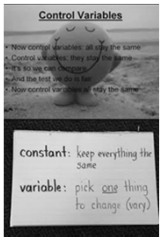
Other Variables

- Patient
  - Immune system
  - Lifestyle/Nutrition issues
- Rehabilitation methods
- Biotensegrity
  - Soft tissue integrity
  - Bone/joint integrity
  - Biomechanical integrity



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Customize Formulations for Specific Indications

- Indications
  - Pro- or anti-inflammatory
  - Target: tendinopathy, OA joint, Subchondral bone?
  - Acute injuries
- Cells and Bioactive Factors
- Activation
- Dose, frequency

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Mechanical Treatment Goals

- Muscular/Myofascial
- Neural
  - Hydrodissection
- Tendon
  - Peri- or Intra-tendinous
  - Enthesis
- Joint
  - Intra-articular
  - Intra-osseous
  - Peri-articular

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PRP Classification

	WBC's	Activation
Type 1	[Increased]	No
Type 2	[Increased]	Yes
Type 3	Minimal to none	No
Type 4	Minimal to none	Yes

Type A: >5x [Platelets]
Type B: <5x [Platelets]

PRP type is likely to affect tissue healing differently.

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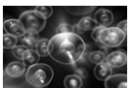
Indications for PRP and MSC's

- Any chronic tendon, ligament or joint injury with pain
  - Accessible to injection therapy
  - failed appropriate conservative management
  - Patient is not interested, poor candidate for surgery
- Subacute / chronic muscle injuries
- Facilitate healing post-op



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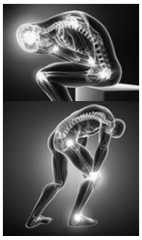
PRP and MSC Treatments

- Common conditions treated
  - Tendons, ligaments, joints
- Cost
- Post-injection care
- Adverse reactions
  - High safety profile



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PRP Studies

Knee OA: PRP vs HA

- Arthroscopy 2011-Kon et al; PRP vs HA: Knee OA
- AJSM 2012-Cerza, F.et al; Comparison between HA and PRP for knee OA
- Am J of PMR 2012-Spakova, T. et al; Autologous PRP vs HA treatment for knee OA
- Arthroscopy 2012-Sanchez, M. et al; RCT evaluating plasma rich in GF's vs HA in knee OA
- AJSM 2013-Patel/Dhillion; PRP vs saline: Knee OA
- Clin Med Insights Arthritis Musculoskelet Disord. 2015-Raeissadat S.A.et al; Knee OA Injection: PRP vs HA, One year RCT

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Summary: PRP / MSC Studies

- Tendinopathies
  - Yes
  - Shoulder, elbow, hip, knee, ankle, foot
- Knee, ankle OA, cartilage damage
  - Getting stronger
- Hip OA
  - Working on it

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PRP and Stem Cell Studies

- Lateral epicondylosis
- Rotator cuff tendinosis
- Patellar tendinosis
- Achilles tendinosis
- Plantar fasciosis
- Knee and Hip OA
- Lumbar disc disease
- Non-union of long bones

MSC Clinical Trials in U.S.
July 2015

Sponsors/Collaborators, by Category	#
Clinical Research Network	4
Government, excluding U.S. Federal	70
Industry	284
National Institutes of Health	18
U.S. Federal Agency, excluding NIH	3
University/Organization	114
TOTAL	493

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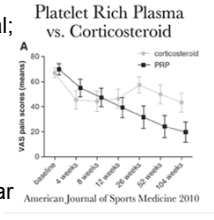
PRP vs Standard of Care

- PRP vs cortisone
  - AJSM 2010-Gosens et al; PRP vs cortisone: Chronic lateral epicondylitis, RCT
  - Foot Ankle Int. 2014-Monto RR. PRP vs cortisone: Chronic plantar fasciitis



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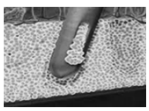
Bone Marrow vs. Adipose Stem Cells

- Pain and donor site morbidities
- Cell number and activity with aging, quantity
- MSC yield (NC/G)
  - Bone marrow: 30,000
  - Adipose: 1,000,000 (500 X)
- FDA regulation



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Stem Cell Research

- Hernigou P, et al: Biologic augmentation of rotator cuff repair with mesenchymal stem cells during arthroscopy improves healing and prevents further tears: A case-controlled study. *Int Orthop* 2014;38(9):1811-1818.
- Vangsness CT Jr,et al: Adult human mesenchymal stem cell intra-articular injection following partial medial meniscectomy: A randomized, double-blind, controlled study. *J Bone Joint Surg Am* 2014;96(2):90-98.

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PRP + Stem Cells

- ADSC w/ PRP s/p AKS
  - 87% maintained or improved cartilage status w/ 2nd look arthroscopy at 2 years
  - Knee Surg Sport Trauma, 2013 Koh et al

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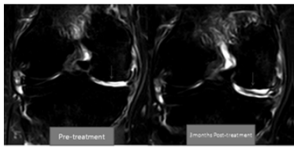
Stem Cell Studies

- MRI comparison of control scaffold, PRP and BMAC treatment for cartilage defects
  - BMAC>PRP>Control
  - AJSM PreView, Nov 16, 2015; Krych, A.



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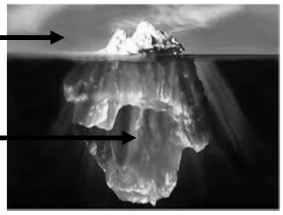
Where are We Headed?

- What we know
- What we don't know



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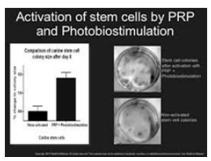
PRP + MSC's

- PRP + MSC Synergy
  - Enhances stem cell and fibroblast proliferation
  - Inflammation
  - Anti-microbial
  - Angiogenic



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Thank you

www.drZmd.com
amzsportsmd@msn.com

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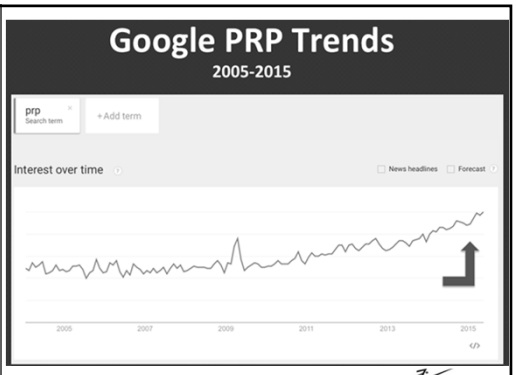
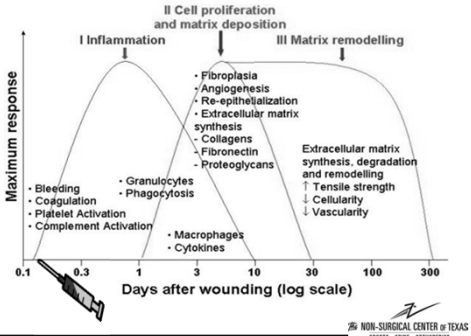
Google PRP Trends
2005-2015



Stimulate Healing Response



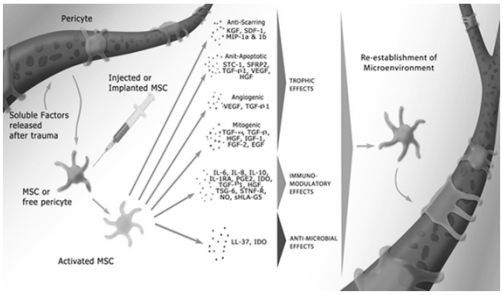
Tendon and Ligament Healing

Rat Model:

- 8-10 days: Inflammatory phase is evident
- 1-12 weeks: Collagen synthesis, cross-linking
- 8 weeks: Collagen begins to align longitudinally
- 3 weeks – 1 year: Collagen remodeling
  (Greenley TK, 1971)

There is no "quick fix" for tendon healing

Stem Cell Differentiation



Use of Corticosteroids and Anesthetics

Corticosteroids and Local Anesthetics Decrease Positive Effects of Platelet-Rich Plasma: An In Vitro Study on Human Tendon Cells

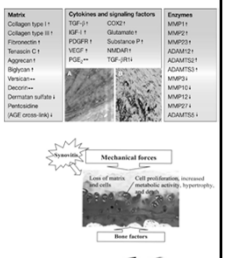
Bradley Carofino, M.D., David M. Chowaniec, B.S., Mary Beth McCarthy, B.S., James P. Bradley, M.D., Steve Delaronde, M.P.H., M.S.W., Knut Beitzel, M.D., Mark P. Cote, P.T., D.P.T., Robert A. Arciero, M.D., and Augustus D. Mazzocca, M.S., M.D.

Arthroscopy. 2012 May;28(5):711-9.

Human tenocytes cultured in PRP alone, or in combination with corticosteroids and/or anesthetics (lidocaine, bupivacaine)

Biologic Treatment Goals

- Tendinopathy
  - Degenerative tissue with ineffective healing cascade
- Osteoarthritis
  - Increased catabolic activity, subchondral bone injury, cartilage destruction
- Pro- or Anti-inflammatory PRP?



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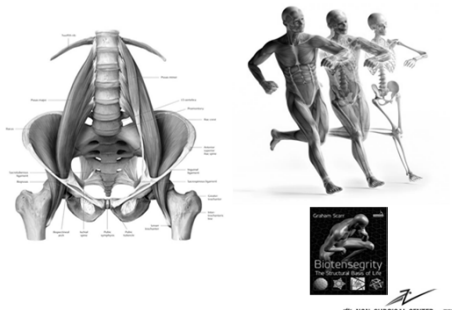
Disruption of Biotensegrity



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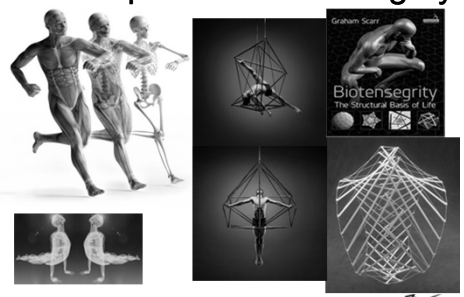
Disruption of Biotensegrity



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Mechanical Treatment Goals

- Tendon
  - Peri- or Intra-tendinous
  - Entheseal
- Joint
  - Intra-articular
  - Intra-osseous
  - Peri-articular
- Muscular/Myofascial
- Neural
  - Hydrodissection

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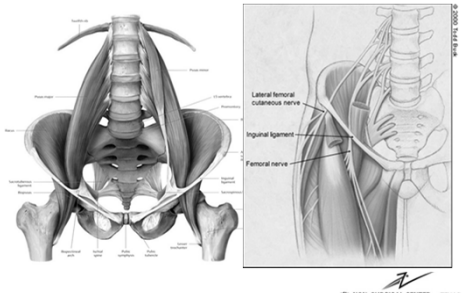
Disruption of Biotensegrity



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Common Conditions Treated

- Tendon, Ligaments, Muscles
  - Tendinosis/partial tears,
    - Rotator cuff, Tennis elbow, Patellar, Achilles, Peroneal, Plantar fasciosis
  - Knee MCL / LCL sprain, Elbow UCL sprain
  - Chronic muscle strain injuries
- OA
  - Hip, Knee, Ankle

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PRP and MSC Treatments

- Common conditions treated
  - Tendons, ligaments, joints
- Cost
- Post-injection care
- Adverse reactions
  - High safety profile

Adverse reactions

- Pain during and after injection
  - Brief immobilization (24-72 hours) helps
- May require short term narcotics
  - AVOID NSAIDs pre-, post-injection
- High safety profile
  - No adverse events reported

PRP Cost

- ~$1000-$2000 with/without U/S guidance
  - Kits cost $250, Facility, Professional charges
- Coding: PRP CPT Code = 0232T
- Not universally covered by insurers
  - Prior authorization process in place
  - Worker's Compensation views favorably in some states

PRP Studies-Summary

- Tendinopathies
  - Yes
  - Shoulder, elbow, hip, knee, ankle, foot
- Knee OA
  - Better
- Hip OA
  - Working on it

PRP Post-injection Care:

- Brief (72 hr) period of immobilization/ protection, early AROM
- Avoid NSAIDs x 2 weeks
  - Ice, Acetaminophen o.k.
- Begin progressive PT program within 2 weeks of injection
- Low intensity tendon loading for first 6-8 weeks, then activity as tolerated

Mesenchymal Stem Cell Studies-Summary

- Tendinopathies
  - Yes
  - Shoulder, elbow, hip, knee, ankle, foot
- Knee OA
  - Getting stronger
- Hip OA
  - Working on it

PRP vs. Steroid for Lateral Epicondylosis

- Randomized Controlled Trial; Level 1
- PRP (n=51) vs. Corticosteroid (n=49)
  - Single injection
  - DASH Scores and VAS scores
- Results
  - Success = >25% ↓ in VAS or DASH, no re-Tx
    - 73% PRP vs 49% Steroid (p<.001)
    - Corticosteroid – better initially then declined
    - PRP – progressive improvement to 1 year

Peerbooms JC, et al. AJSM, 2010 38:255

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PRP – Plantar Fasciosis

Barrett, et al. *Podiatry Today,* 2004
Case Series
9 patients with PF, PRP injection
77.9% complete symptom resolution at 1 year

Martinelli, et al. *Int Orthop, 2012*
Case Series
14 patients with PF, 3 PRP injections, 1 year follow-up
Mean Pain VAS decreased from 7.1 to 1.9

Ragab, et al. *Arch Orthop Trauma Surg, 2012*
Prospective Cohort
25 patients with PF, PRP injection, 10 month follow-up
Mean Pain VAS decreased from 9.1 to 1.6

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PRP Research

- Mishra AK, et al: Efficacy of platelet-rich plasma for chronic tennis elbow: A double-blind, prospective, multicenter, controlled trial of 230 patients. *Am J Sports Med* 2014;42(2):463-471.
- Alsousou J, et al: Effect of platelet-rich plasma on healing tissues in acute ruptured Achilles tendon. *Lancet* 2015;385;S19.

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PRP for Chronic Midportion Achilles Tendinosis

- Randomized Controlled Trial, Level 1
- PRP (n=27) vs. Saline Control (n=27)
  - Both groups performed eccentric exercises
  - 24 week follow-up, VISA-A scores
- Results
  - Both groups improved, PRP not superior
  - VISA-A improvement (12 pts = C.I.D.)
    - PRP 21.7 vs Saline 20.5 (NS)

Unclear if benefits are related to eccentrics or effect of needle trauma / injectant

De Vos, et al. JAMA 2010, 303(2):144

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- Positive outcomes
  - Filardo et al 2012, RCT, level 2
  - Spakova 2012, RCT
  - Patel, Dhillion et al 2013, RCT, level 2
  - Wang-Saegusa, 2011, Case series
  - Jang et al, 2012, case series
  - Filardo et al, 2011, Case series
  - Sanchez et al, 2012, RCT, level 4 (Hip)
  - Multiple Case reports, level 4
- Demonstrate safety of PRP

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No effects of PRP on ultrasonographic tendon structure and neovascularisation in chronic midportion Achilles tendinopathy

R J de Vos,[1] A Weir,[2] J L Tol,[2] J A N Verhaar,[1] H Weinans,[1] H T M van Schie,[1]

Br J Sports Med. 2011 Apr;45(5):387-92.

- Randomized Controlled Trial, Level 1
- PRP (n=27) vs. Saline Control (n=27)
- 6, 12, 24 week follow-up with Ultrasound
  - No difference in ultrasonographic appearance of tendons b/w groups at final follow-up; both improved (p=0.169)
  - No difference in US echo., neovessels at any time point.

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PRP for Patellar Tendinopathy s/p ACL Reconstruction

Participants: 11 patients (9 females, 2 males) following an ACL reconstruction utilizing a patellar tendon autograft

- 9 Females, 2 Males
- Average age = 19 ± 2.19 yrs
- Average Timing of Injection = 34.8 ± 17.1 weeks s/p ACLR
- All failed PT, NSAIDs, rest, iontophoresis

Outcomes: Paired differences of IKDC scores (pre-injection to post-injection) for each patient assessed with the Wilcoxon Signed-Rank Test (p≤0.05) and reported as the median (inter-quartile ranges [IQR]: 25th and 75th).

- Average follow-up duration: 29.5 ± 17.7 weeks
- 7/11 (64%) demonstrated significant improvement, 4/11 (36%) no significant improvement

	Baseline (median, IQR)	Post-Injection (median, IQR)	Significance
IKDC	48.3 [44.3,60.3]	74.7 [52.9,82.8]	**p=0.02**

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PRP for Acute Muscle Injury

- Design: Controlled Laboratory
- Methods: Rat tibialis anterior strain
  - PRP, PPP (platelet poor = sham), no Tx
  - Single contraction (large strain) vs. Multiple contraction (small strain) injury
  - Outcome: Histology and Contractile force
- Results
  - PRP enhanced recovery from multiple contraction injury
  - No improvement in single contraction injury

Hammond, et al. AJSM 2009

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PRP for Non-Union of Long Bones

60 Patients with > 6 months of non-union

- 42/60 s/p ORIF, all with >90% fracture fragment contact
  - Tibia (n=35), Femur (n=15), Humerus (n=5), Radius (n=5)
  - Injected with 20-30 mL PRP at site of fx non-union
  - Radiographs: 8, 12, 16, 20, 24 weeks

Results

- 55/60 with callus formation at week 8
  - 40/55 with bridging trabeculae at week 12
  - 55/55 with union by week 24
  - All received PRP within 2-4 months of non-union dx
- 5/60 non-union (2-tibia, 2-femur, 1-radius)
  - All received PRP > 12 months since non-union dx

Kumar, et al. AAOS, 2012

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The Biomechanical and Histologic Effects of Platelet-Rich Plasma on Rat Rotator Cuff Repairs

Jennifer Beck, MD*,†, Douglas Evans, MD†, Pietro M. Tonino, MD†, Sherri Yong, MD†, and John J. Callaci, PhD†

†Department of Orthopedic Surgery, Loyola University Medical Center, Maywood, Illinois

Am J Sports Med. 2012 Sept; 40(9) 2037-2044

- Tendon-from-bone supraspinatus tear
- Immediate trans-osseous repair performed
  - PRP augmented vs control repair
  - Histology / Biomechanics 7d, 14d, 21d

Results

- PRP group - increased fibroblastic response and vascular proliferation, @21d more linear collagen aligment
- No difference in strain to failure loads

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PRP for Lumbar Spinal Fusion

Early results have been Mixed

RCT, Level 1

- 40 subjects, Posterior stabilization was achieved with pedicle screws and interbody fusion was attempted with carbon cages filled with autologous bone +/- PRP
- CT Scans at 3, 6, 12, 24m

Results

- No significant difference in patient reported outcomes of ODI, SF-36, VAS
- No significant differences in CT Evidence of healing

Sys J et al. Eur Spine J 2012

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Effect of Platelet-Rich Plasma on the Biologic Activity of the Human Rotator-Cuff Fibroblasts: A Controlled In Vitro Study

Patrick Sadoghi,[1] Birgit Lohberger,[1] Birgit Aigner,[2] Heike Kaltenegger,[1] Jörg Friesenbichler,[1] Matthias Wolf,[1] Tarek Sununu,[3] Andreas Leithner,[1] Patrick Vavken[4,5,6]

J Orthop Res. 2013 Apr 8. doi: 10.1002/jor.22360. [Epub ahead of print]

- Rotator cuff fibroblasts cultured 21d with PRP of 3 different concentrations
  - 1x, 5x, 10x (dose-response relationship) vs controls
  - DNA, GAG measurements @ 1, 7, 14, 21 d
- Results
  - PRP increased (p<0.0001) fibroblast proliferation and elevated GAG and DNA levels.
  - 1x and 5x had most profound effects

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