Platelet rich plasma and bone marrow aspirate concentrate: How, when and where for tendon and joint injuries.

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Some fundamental definitions

- **Regenerative therapy** - a broad definition for innovative medical therapies that will enable the body to repair, replace, restore and regenerate damaged or diseased cells, tissues and organs.

- **Biological products** are made from living material--human, plant, animal, or microorganism, and they're used for the treatment, prevention or cure of disease.
Optimal Biologic

- Tissue engineering prerequisites
  - Cells, scaffold, growth factors
- Patient-side, Point-of-care
- Autogenous
- Trans-endoscopic, percutaneous
Biologics

- PRP – platelet rich plasma
- BMAC – bone marrow aspirate concentrate
- MSC – mesenchymal stem cells (cultured)
- ADSC – adipose derived stromal cells
- UCMC - umbilical cord matrix cells
Questions and controversies

- Which biologic
- When to start treatment
- Dose / volume of treatment
- Treatment protocol – number of doses, exercise
PRP history

- Oral maxillofacial surgery
- Prevailing rationale for use of PRP arises from the growth factors released from platelet alpha-granules (TGF, PDGF, VEGF, BMPs, \textit{IGF})
- Some is good > More is better
  - Changing the paradigm
ligand (GF)

nucleus

transcription: DNA $\rightarrow$ mRNA

translation: mRNA $\rightarrow$ protein

ubiquinated / degraded

recycled
It’s much more than platelets

- Composite of all blood elements

PLASMA - 55% of Total Blood Volume
- 91% Water
- 7% Blood Proteins (fibrinogen, albumin, globulin)
- 2% Nutrients (amino acids, sugars, lipids)
  - Hormones (erythropoietin, insulin, etc.)
  - Electrolytes (sodium, potassium, calcium, etc.)

CELLULAR COMPONENTS - 45% of Total Blood Volume
- Buffy Coat
  - White Blood Cells (7000-9000 per mm³ of blood)
  - Platelets (250,000 per mm³ of blood)
- Red Blood Cells (RBCs)
  - About 5,000,000 per mm³ of blood
Proteomic and Phospho-Proteomic Profile of Human Platelets in Basal, Resting State: Insights into Integrin Signaling

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Abstract

During thrombogenesis and vascular inflammation quiescent platelets are activated to increase the surface expression and ligand affinity of the integrin αIIbβ3 via integrin-out signaling. These signals such as thrombin, ADP and epinephrine transduce signals through their respective GPCRs to activate protein kinases that ultimately lead to the phosphorylation of the cytoplasmic tail of the integrin αIIbβ3 in platelets. The signaling pathways involved in this process are not well defined. In an effort to better understand these pathways, we employed a combination of proteomic profiling and computational analyses of isolated human platelets. We analyzed ten independent human samples and identified a total of 1507 unique proteins in platelets. This is the most comprehensive platelet proteome assembled to date and includes 190 membrane-associated and 325 phosphoproteins, which were identified via independent proteomic and phospho-proteomic profiling. We used this proteomic dataset to create a platelet protein-protein interaction (PPI) network and applied novel contextual information about the phosphorylation step to introduce limited directionality in the PPI graph. This newly developed contextual PPI network computationally recapitulates an integrin signaling pathway. Most importantly, our approach not only provided insights into the mechanism of integrin αIIbβ3 activation in resting platelets but also provides an improved model for analysis and discovery of PPI dynamics and signaling pathways in the future.

Introduction

Platelets are key initiators of hemostatic mechanisms that regulate the vasculature. Platelets also play a central role in cardiovascular disease, cancer, and stroke, which account for the major mortality and morbidity in the United States. In addition, platelets modulate inflammatory pathways in innate, natural and adaptive immunity and a number of inflammatory diseases, such as atherosclerosis. Platelets are multicellular cells that are characterized by a small size and ability to aggregate into large masses. Platelets are also capable of undergoing apoptosis and can be activated to a resting state. When activated, platelets release several molecules, including platelet-activating factor (PAF), fibrinogen, and von Willebrand factor (vWF), which are important in the formation of a blood clot. These molecules bind to specific receptors on the platelet surface and activate a signal transduction pathway known as the integrin αIIbβ3 pathway. The integrin αIIbβ3 pathway is activated by the binding of fibrinogen to the platelet surface, which leads to the recruitment of the alpha IIb beta 3 integrin to the platelet surface and its activation. Once activated, the alpha IIb beta 3 integrin is able to bind to the fibrinogen, which is a key step in the formation of a blood clot. In platelets, such as platelet adhesion, aggregation, and adhesion to vessel walls, are also mediated by the integrin αIIbβ3. A number of proteins, such as alpha IIb beta 3, play a key role in the formation of a blood clot. Alpha IIb beta 3 is a cell adhesion molecule that plays a critical role in the formation of a blood clot. The alpha IIb beta 3 integrin is a key component of the platelet adhesion machinery. When alpha IIb beta 3 is activated, it binds to fibrinogen, which is a key step in the formation of a blood clot. Alpha IIb beta 3 is also important in the formation of a blood clot, as it is a key component of the platelet adhesion machinery.
Vascular response to Injury
Cellular Response to Injury

Normal

1 Week

2 Weeks

4 Weeks

8 Weeks

Courtesy, Linda Dahlgren
Questions and controversies

- Which PRP
- When to treat
- Dose / volume of treatment
- Need to clot / use of thrombin
- Treatment protocol – number of injections, exercise
What is optimal PRP preparation

- **Fold increase in platelets**
  - PRP = $\geq 5.5 \times 10^{10}$ platelets/50mls
  - 2 to 7 fold increase (range 150,000 – 450,000 platelets/µl)

- **WBC, RBC, fibrin**

- **Attempt to classify**
## Product Comparison

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>Platelets (fold change)</th>
<th>WBC (fold change)</th>
<th>RBC reduction (%)</th>
<th>Platelet:WBC</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACP (Arthrex)</td>
<td>2.3</td>
<td>0.09</td>
<td>99</td>
<td>800</td>
</tr>
<tr>
<td>GPS III (Biomet)</td>
<td>8.1</td>
<td>5.4</td>
<td>80</td>
<td>51</td>
</tr>
<tr>
<td>PRP-Human (Harvest Tech/Symphony)</td>
<td>7.6</td>
<td>2.3</td>
<td>52</td>
<td>61</td>
</tr>
<tr>
<td><strong>PRP-Equine (Harvest Tech / Symphony)</strong></td>
<td>4.5</td>
<td>1</td>
<td>99</td>
<td>60</td>
</tr>
<tr>
<td>Magellan (Medtronic)</td>
<td>7.5</td>
<td>3.2</td>
<td>40</td>
<td>84</td>
</tr>
</tbody>
</table>
Experimental methods

- Platelet rich plasma (PRP)
- Bone marrow aspirate (BMA)
- Freeze dried platelets
- Superficial flexor tendon
- Suspensory ligament
Growth factor concentration

TGF-B follows similar trend
McCarrel, et al. JOR, 2009
Anabolic gene expression

COMP follows similar trend

Catabolic gene expression

MMP-3 follows similar trend
## WBC and MMPs

### WBC and matrix synthesis

<table>
<thead>
<tr>
<th>Protein</th>
<th>Platelet Concentration</th>
<th>WBC Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>r²</td>
</tr>
<tr>
<td>COL1A1:COL3A1</td>
<td>0.79</td>
<td>0.62</td>
</tr>
<tr>
<td>COMP</td>
<td>0.73</td>
<td>0.53</td>
</tr>
<tr>
<td>MMP-3</td>
<td>-0.37</td>
<td>0.14</td>
</tr>
<tr>
<td>MMP-13</td>
<td>-0.76</td>
<td>0.58</td>
</tr>
</tbody>
</table>
IL-1B (pg/ml of PRP)

- **White blood cell (thou/uL)**
  - $R^2 = 0.91$
  - $P = <0.0001$

- **Neutrophils (thou/uL)**
  - $R^2 = 0.77$
  - $P = <0.0001$

- **Monocytes (thou/uL)**
  - $R^2 = 0.76$
  - $P = <0.0001$

- **Lymphocytes (thou/uL)**
  - $R^2 = 0.73$
  - $P = <0.0001$
## Moving forward

### Aim 1: optimal RATIO

<table>
<thead>
<tr>
<th>PRP “type”</th>
<th>Platelets (*10³/µl)</th>
<th>WBC (*10³/µl)</th>
<th>Platelet:WBC ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>stdPRP</td>
<td>500</td>
<td>8.3</td>
<td>60:1</td>
</tr>
<tr>
<td>lrPRP</td>
<td>500</td>
<td>0.25</td>
<td>2000:1</td>
</tr>
<tr>
<td>hcPRP</td>
<td>1500</td>
<td>25</td>
<td>60:1</td>
</tr>
<tr>
<td>wbcPRP</td>
<td>500</td>
<td>25</td>
<td>20:1</td>
</tr>
<tr>
<td>control</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

### Aim 2: optimal PLATELET #

<table>
<thead>
<tr>
<th>lrPRP “type”</th>
<th>Platelets (*10³/µl)</th>
<th>WBCs (*10³/µl)</th>
<th>Platelet:WBC ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>lrPRP-1</td>
<td>500</td>
<td>0.25</td>
<td>2000:1</td>
</tr>
<tr>
<td>lrPRP-2</td>
<td>1500</td>
<td>7.5</td>
<td>2000:1</td>
</tr>
<tr>
<td>lrPRP-3</td>
<td>200</td>
<td>0.1</td>
<td>2000:1</td>
</tr>
<tr>
<td>control</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
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</table>

### Manufacturer

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</table>
Tendon mRNA

IL-1B

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>PRP 60:1</th>
<th>HC PRP 60:1</th>
<th>LR PRP 2000:1</th>
<th>WBC PRP 20:1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Log2ΔΔCt</td>
<td>a</td>
<td>a</td>
<td>ab</td>
<td>b</td>
<td></td>
</tr>
</tbody>
</table>

TNF-α

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>PRP 60:1</th>
<th>HC PRP 60:1</th>
<th>LR PRP 2000:1</th>
<th>WBC PRP 20:1</th>
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<tr>
<td>Log2ΔΔCt</td>
<td>a</td>
<td>ab</td>
<td>b</td>
<td>a</td>
<td>b</td>
</tr>
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Pre-clinical evidence

Bosch. 6 horses. SDFT. PRP vs PBS. 24 week FU.
- Injected 7 days post mechanical SDF tendonitis
- PRP improved biochemical, histological, mechanical outcome
- PRP improved computed US fiber alignment
- PRP improved neovascularization
Clinical evidence

- Non-randomized clinical trial. 9 STBD.
  - Mid body SL. 3 year follow-up
  - All returned to racing, no re-injury

- Clinical case series: 7 horses
  - SDF, DDF, ICL. 10-13 month follow-up
  - All returned to performance, no re-injury
When should I treat?

- **Acute 0-7 days**
  - Too much endogenous inflammation?
  - Majority of reported “flares”
  - Adhere to conservative treatments

- **Subacute 7-10 days**
  - Inject
Biomechanical forces

- Altered hoof conformation
  - Under-run heels, long toe
How often should I treat?

How often
- +/- q 14 days following re-check examination
- Guideline
  - ≥ 50% improvement in:
    - Palpation and lameness
    - Ultrasound examination x-section and long planes
Volume of PRP

- Enough to fill the lesion
  - Ultrasound guided
Do I need thrombin

- NO
  - Clots when sees exposed basement membrane
  - Most are bovine origin
Rehabilitation Protocols

- Exercise program varies with severity of injury
  - Day 0-14/21: Stall rest, surgery, PRP, BMAC injection
  - > 14 days: depends on exam findings
  - 10% increase in intensity or duration per week
  - keep a spreadsheet

- Ultrasound dictates the transition from each phase

- Reduce work if echolucencies or swelling appear
- No characterization of lesion
- Lack of correlation
  - Lesion size, age, etc.
- No characterization of PRP
  - Platelets, WBC
2 clinical series

- PRP vs HA, cohort of 30 patients; Sanchez et al, *Clin Exp Rheumatol* 2008
  - PRP 33% pain relief success vs HA 10% (p=0.004)
  - Overall WOMAC better in PRP at 5 weeks p=0.010
- 4 IA PRP q21d, 1yr follow-up; Kon et *Knee Surg Sports* 2010
  - Improved IKDC and EQ VAS scores at 6 and 12 months

Clinical observation - pain relief before repair
Pain, OA, and PRP

- Cartilage / synovium from TKA patients → co-culture
- Surrogate markers for pain
  - Substance P, IL-15, IL-1, TNF-α
- Matrix synthesis
  - Synoviocytes: HA, COMP
  - Cartilage: Col2, aggrecan, Col1
Balances

- Advantages
  - Autogenous
  - High concentrations and milieu of growth factors
  - Low cost
  - Ease or preparation, use at time of diagnosis
  - Formation of scaffold

- Disadvantages
  - No stem cells
  - No standard product
Are there “stem cells” in PRP?

Kuwana M et al, Journal of Leukocyte Biology 2004; 74: 833-835 Figure 6.
Stem cell-based biologics

PRP – platelet rich plasma
BMAC – bone marrow aspirate concentrate

- MSC – mesenchymal stem cells (cultured)
- ADSC – adipose derived stromal cells
- UCMC - umbilical cord matrix cells
Stem cells for cartilage repair

- 2 paradigms
  - Acute focal cartilage injury
  - Established osteoarthritis
- The joint as an entire organ system
  - Cartilage
  - Subchondral bone
  - Synovial membrane
  - Menisci, ligaments
Bone marrow MSC for cartilage repair—evidence

- **Microfracture**
  - Horses, non-human primate, humans
    

- Increased fill in defect
- Diminished pain
- Return to function
- Durable?
Bone marrow-derived MSCs

Obtaining sternal bone marrow aspirate from a standing horse.

Lisa A. Fortier
Lauren V. Schnabel
Patient-side bone-marrow derived MSC implant - Proximal P1

+ thrombin

Suspensory desmitis
BMAC
Bone marrow aspirate concentrate

- Model = Equine 2 - 5 years (n=10)
- Lateral trochlear ridge of femur
- 15 mm diameter cartilage defect

- Treatment = BMAC + microfracture
- Control = microfracture

- 16 weeks, second look arthroscopy
- 8 months, euthanasia

Fortier et. al. JBJS, 2010, in press.
60 mls sternal aspirate
15U heparin/ml aspirate

14 minute centrifugation
SmartPrepII
Harvest Technologies
Plymouth MA, USA

BMAC™

TCP + BMAC graft

10:1 BMAC – thrombin
3300 U/ml

supernatant

7 mls BMAC
3T MRI imaging

Left = BMAC

No bony overgrowth
Fill > 75%

Right = control

Fill < 25%

Axial FSE

T2 mapping

T1 rho
Clinical goals to answer?

- PRP
  - Is it the ratio of platelets:WBC
  - Is there a platelet “threshold”
  - Acute vs. chronic disease

- BMAC / stem cells
  - Tissue source
  - Number of cells
  - Delivery mechanism and vehicle
Cate Radcliffe, Lauren Schnabel, Taralyn McCarrel, Julia Flaminio, Doug Antczak, Betina Wagner, Jon Schimenti, Kira Novakovski, Line Grieve