



# PRP – Science, Evidence and Practical Use

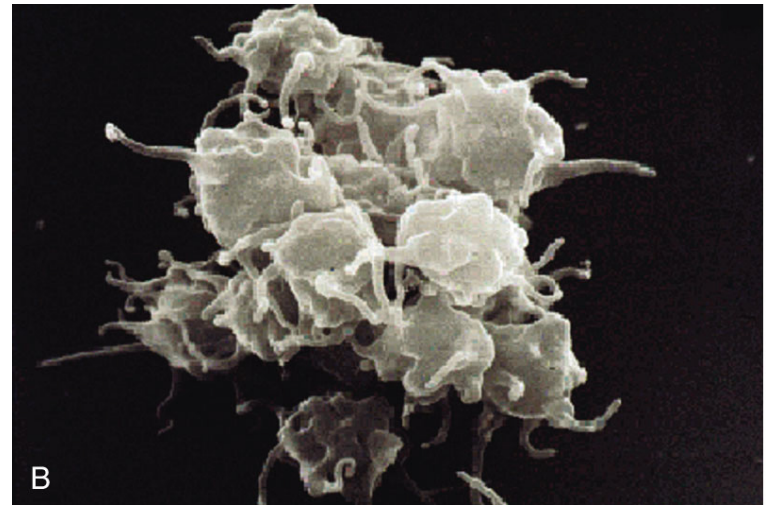
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## But what is it ?

- Autologous blood injection (ABI)
- Platelet rich plasma (PRP)
- Leukocyte rich PRP
- Leukocyte poor PRP
- Platelet rich fibrin (PRF)
- Plasma Rich in Growth factors (PRGF)
- Autologous conditioned serum (ACS)

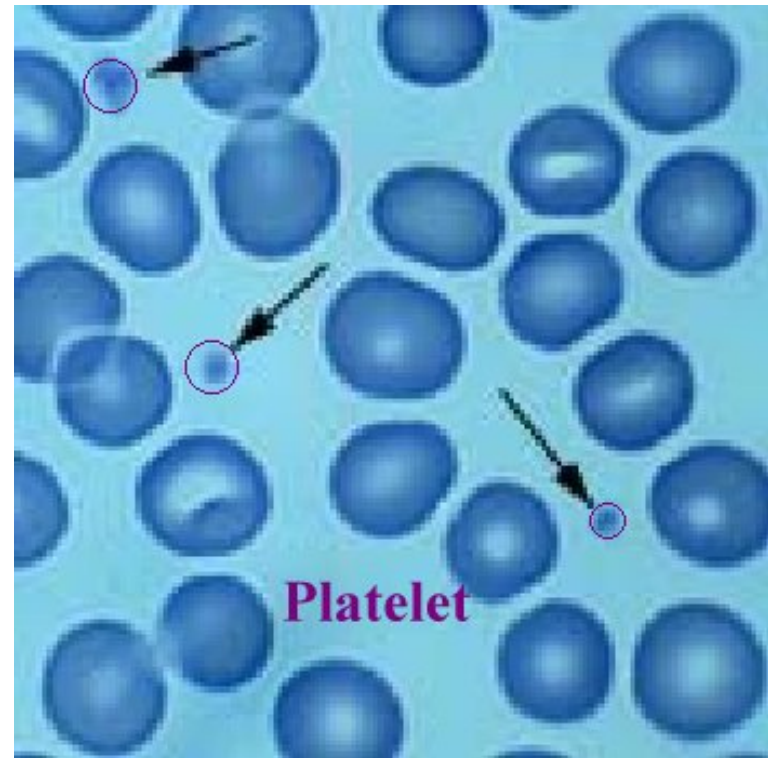
# But what is it ?

- Activated or not activated
  - Thrombin
  - Calcium chloride
  - Photo-activated



# What's in it ?

- RBC's
- WBC's
  - Neutrophils
  - Eosinophils
  - Monocytes
- Platelets
- Fibrinogen
- Water



# Growth Factors

- Growth factors
- Platelet derived growth factor (PDGF)
- Vascular endothelial growth factor (VEGF)
- Insulin-like growth factor 1( IGF-1)
- Hepatocyte growth factor (HGF)
- Transforming growth factor –alpha and beta1(TGF-b1, TGF -a)
- Epidermal growth factor (EGF)
- Fibroblast growth factors (FGF's)
- Keratinocyte growth factor (KGF)
- Connective tissue growth factor (CTGF)
- Granulocyte macrophage colony stimulating factor (GM-CSF)
- Tumour Necrosis Factor alpha (TNF- $\alpha$ )

# Anti-inflammatory and anti-microbial mediators

- Anti-inflammatory mediators
  - IL-1 Receptor antagonist (IL-1ra) – IL-1 blocker
  - Thymosin beta 4 (TB4) – TNF blocker
  
- Anti-microbial factors
  - Microbiocidal peptides
  - Phospholipase A2
  
- Platelet Activation
  - Serotonin
  - Thromboxane A2
  - Calcium
  - Adenosine

## ASC and PA-PRP – Variation on the theme

- **ACS – Autologous conditioned serum**
  - PRP incubated on glass beads for 24 hours
  - Stimulates the release of IL-1 Receptor antagonist from Monocytes
  - Reportedly 30,000 procedures per year in Germany
- **PA-PRP – Photo-activated PRP**
  - Leukocyte Rich PRP placed in polychromatic light for 10 minutes
  - Raises IL-1RA to same level of ACS
- **Photoactivation of blood**
  - Lowers Pro-inflammatory TNF-alpha, IL-6 and IFN
  - Does not raise other pro-inflammatory factors IL-1, IL-4, IL-2

# Cellular Effects

## **Promotion of bone synthesis and inhibition of resorption:**

- Osteoblast proliferation and differentiation
- Inhibiting osteoclastogenesis

(Ogino, 2009, He et al, 2009)

## **Proliferation of fibroblasts:**

- Tendon
- Skin
- Synovium

(Anitua 2007, Anitua 2009).

# Cellular Effects

- Hyaluronic acid and collagen synthesis:
- Increased Hyaluronic acid secretion by synovial fibroblasts
- Increase the synthesis of collagen I by fibroblasts  
(Anitua 2007, Anitua 2009).

# Stem Cell Effects

- Increased proliferation of mesenchymal stem cells
  - Maintain ability to differentiate
    - Chondrocytes
    - Osteocyte
    - Adipocytes
- Stem cell migration and angiogenesis

( Vogel et al 2006, Cenni et al, 2009)

# Growth Factor Effects

- PRP in tendons
  - Increases the synthesis of TGF-beta for 2 weeks post injection (Lyras, et al, 2010)
  - Up regulation of IGF-1 synthesis for 2 weeks post injection (Lyras, et al, 2010 (2))

# Is It Safe?

- PRP in tendons
  - Increases the synthesis of TGF-beta for 2 weeks post injection (Lyras, et al, 2010)
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## Is It Safe?

- Antimicrobial properties
- Decreasing the risk of chest wall infection post cardiac surgery by 93%
- Inhibits growth of
  - MRSA
  - MSSA
  - E. Coli,
  - Enterococcus faecalis

(Khalafi et al. 2008, Beileki et al, 2007).

# Is It Safe?

## **Cancer risk:**

- No study has documented the promotion of hyperplasia, carcinogenesis or tumour growth

(Sampson et al, 2008)

## How many platelets do you need to have a therapeutic effect?

- $1000 \times 10^6$  (Marx 2004) – but fluffy justification
- **Consensus**
  - 2.5-4 times normal platelet concentration  
(Huang 2010, Anitua 2008, Grazini 2006)
  - 10-15% solution in culture of PRP  
(Shan et al, 2009, Qiao 2008)
  - 15-30% causes less cell proliferation  
(Qiao 2008, Hsu, 2010)

# How do you make PRP?

- Multiple different recipes
- 7 commercially available PRP production systems
  - Platelet concentrations from 344-1086 x10<sup>6</sup>.  
(Marx 2004)
- 3 commercially available systems showed
  - Platelet concentration from 569 to 1222 x 10<sup>6</sup>.  
(Everts et al 2006)
- 4 systems with (1 Manual system)
  - Platelet concentration from 430-1358 x 10<sup>6</sup>.  
(Mazzucco et al 2009)

# Mazzucco et al 2009

**Table 2** Volume, platelet and growth factor concentration during the platelet enrichment process, (mean and mean  $\pm$  SD). Table 2a, WB: starting whole blood; PRP: platelet suspension after the first centrifugation step. Table 2b, PRP1: platelet suspension prior gelation

(a)

	WB		PRP							
	Vol ml	plts $10^6$ /ml	Vol ml	plts $10^6$ /ml	PDGF-BB ng/ml	TGF- $\beta$ ng/ml	b-FGF pg/ml	VEGF pg/ml	EGF pg/ml	IGF-I ng/ml
Fibrinet	7.0	263 $\pm$ 70	4.8	346 $\pm$ 105	3.6 $\pm$ 2.2	8.8 $\pm$ 5.0	32 $\pm$ 27	366 $\pm$ 377	1432 $\pm$ 1266	27 $\pm$ 11
Regen	8.0	263 $\pm$ 70	5.0	430 $\pm$ 109	2.3 $\pm$ 1.9	6.2 $\pm$ 4.0	13 $\pm$ 10	133 $\pm$ 121	112 $\pm$ 98	36 $\pm$ 14
Home made	5.0	263 $\pm$ 70	3.0	558 $\pm$ 158	3.0 $\pm$ 1.5	8.1 $\pm$ 2.6	28 $\pm$ 27	172 $\pm$ 135	123 $\pm$ 67	32 $\pm$ 11
Plateltext	6.0	263 $\pm$ 70	3.0	557 $\pm$ 155	4.4 $\pm$ 3.0	12.2 $\pm$ 3.2	1.3 $\pm$ 2.7	225 $\pm$ 153	505 $\pm$ 237	26 $\pm$ 8

(b)

	PRP1									
	Vol ml	plts $10^6$ /ml	Fold increment	PDGF-BB ng/ml	TGF- $\beta$ ng/ml	b-FGF pg/ml	VEGF ng/ml	EGF ng/ml	IGF-I ng/ml	
Fibrinet	1.2	1358 $\pm$ 419	3.9 $\pm$ 0.44	3.6 $\pm$ 2.4	8.8 $\pm$ 5.0	31 $\pm$ 27	0.3 $\pm$ 0.3	1.4 $\pm$ 1.2	27 $\pm$ 11	
Regen	5.0	430 $\pm$ 109	1.65 $\pm$ 0.17	2.3 $\pm$ 1.9	6.2 $\pm$ 4.0	13 $\pm$ 10	0.1 $\pm$ 0.1	0.1 $\pm$ 0.1	36 $\pm$ 14	
Home made	1.9	1196 $\pm$ 188	4.4 $\pm$ 0.48	11.4 $\pm$ 6.7	29.8 $\pm$ 13.5	95 $\pm$ 102	0.6 $\pm$ 0.5	0.4 $\pm$ 0.2	112 $\pm$ 44	
Plateltext	2.0	1160 $\pm$ 164	4.4 $\pm$ 0.36	14.3 $\pm$ 11.3	40.4 $\pm$ 14.9	3.5 $\pm$ 8	0.7 $\pm$ 0.4	1.6 $\pm$ 0.7	88 $\pm$ 34	

# Everts et al 2006

System	WB PLT conc. ( $\times 10^3/\mu\text{L}$ )	PRP PLT	PLT increase Baseline ( $\times 10^3/\mu\text{L}$ )	PLT Yield (%)
CS	219 $\pm$ 81	1051 $\pm$ 276*	4.9 $\pm$ 0.7	48 $\pm$ 12*
GPS	219 $\pm$ 81	569 $\pm$ 247*	2.8 $\pm$ 1.1	36 $\pm$ 15
AGF	1051 $\pm$ 276	1222 $\pm$ 326 <sup>¶</sup>	1.2 $\pm$ 0.1	32 $\pm$ 11

**Table 1.** Characteristics of platelet rich plasma production.

All values represent the mean  $\pm$  SD. \*  $p < 0.001$ ; <sup>¶</sup>  $p < 0.05$ .

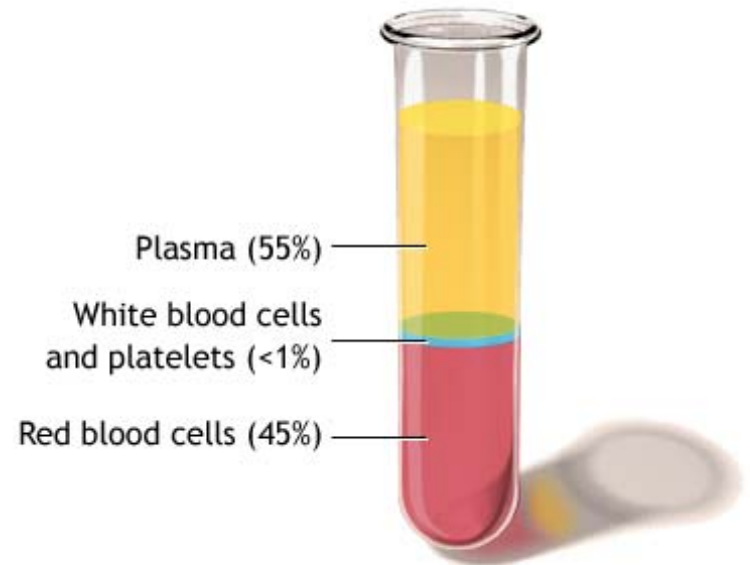
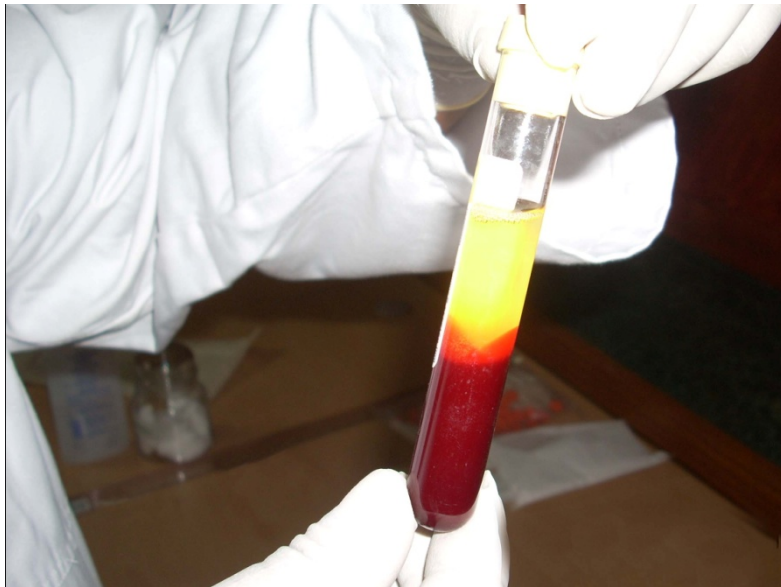
(AGF: autologous growth factor filter; CS: Electa cell-saver; GPS: gravitational platelet sequestration device; Platelet increase Baseline: is a value representing the increase in PRP platelet count compared to the baseline platelet count. PLT: platelet; PRP: platelet-rich plasma; WB: whole blood).

# Weibrich & Kleis 2001

**Table 1.** Descriptive statistics for the platelet counts in the respective blood fractions of the different preparations

	Platelet count in whole blood (1000/ $\mu$ l)	Platelet count in PRP – PCCS (1000/ $\mu$ l)	Platelet count in PRP-Curasan (1000/ $\mu$ l)	Difference in platelet count (PCCS-Curasan) (1000/ $\mu$ l)	Platelet count in erythrocyte sediment Curasan (1000/ $\mu$ l)	Platelet count in PPP-Curasan (1000/ $\mu$ l)
<i>n</i>	47	47	47	47	46	46
Mean	290	2209	1075	1133	75	14
95% Confidence interval	265–316	1944–2473	889–1262	891–1374	64–87	11–17
Median	272	2249	1155	1139	66	12
Standard deviation	86	901	636	845	38	11
Minimum	173	500	64	– 1057	32	2
Maximum	709	4424	2510	3434	182	76
Percentile						
10	211	696	103	52	40	6
25	245	1827	588	583	48	9
75	326	2717	1577	1656	88	16
90	365	3394	1807	2228	146	21

# How do you make PRP?



# Clinical effects – positive uncontrolled trials

- ABI - Lateral epicondylitis (Connell et al, 2006)
- ABI – Lateral Epicondylitis (Suresh, 2006)
- ABI – Patella tendinopathy (James, et al, 2007)
- ABI – Lateral epicondylitis (Gani et al, 2007)
- PRP – OA decrease in pain improved function over 12 months (Kon, 2009)
- PRP - Plantar fasciitis (Barrett, et al, 2004)
- PRP - Decrease in pain in Patella tendinopathy (Kon 2009)
- PRP - Improved myocardial revascularisation post AMI (Wehberg et al, 2009).
- PRP - Decrease post surgical infection (Khalfi et al, 2008)
- PRP - Improved pain control post surgery (Everts et al, 2006, Gardner, et al 2006),
- PRP - Decreased transfusion requirements (Everts et al, 2006, Gardner, et al 2006, Bergoff, et al 2006.)

# Clinical effects: Control trials

- **PRGF** - Improved ACL ligamentisation (Sanchez Et al, 2010)
- **PRGF** – Osteoarthritis Improved pain with comparison to synvisc (Sanchez, et al 2008) (retrospective)
- **PRP** – Decreased pain in Patella tendinopathy with 3 injections (Filardo, 2009)

# Clinical Effects: RCT's Positive

- ABI – Lateral Epicondylitis ABI vs methyl pred. ABI better at 4 wks onwards (Kazemi et al, 2010)
- PRP - Lateral epicondylitis greater decrease pain compared to cortisone (Mishra et al, 2006)
- PRP - Lateral Epicondylitis greater decrease in pain at 12 months comparison to Cortisone with multi-stab injection technique (Joost, 2010)
- PRP - Increased healing rate with diabetic ulcers (Driver et al, 2006)
- PRP - Decreased post operative drainage following thyroidectomy (Yoo, 2008)
- PRP - Decreased pain and swelling post subacromial decompression (Everts et al, 2008)
- PRGF - Improved healing of cutaneous ulcers with PRGF (Anitua, 2008)
- ACS – Greater decreased pain in comparison to Synvisc and Normal Saline at 6, 12 and 24 months (Baltzer et al, 2008)
- ACS - Radicular pain decreased pain with comparison to kenokort with CT guided injection (Becker et al, 2007)
- ACS – Muscle tear increased rate of healing, decreased oedema on MRI, with comparison to traumeel and Activegan (Wright-Carpenter, et al, 2008)

## Clinical Effects: RCT' s Equivocal or Negative

- **ABI** – No different to use of cortisone in plantar fasciitis (Kiter, et al, 2006)
- **ABI** – Cortisone better than ABI in treatment of plantar fasciitis (Lee, et al 2007)
- **PRP** - No difference in treatment of Achilles tendinopathy with comparison to normal saline (de Vos 2010)
  - 5 drops of PRP through a 22g needle at 3 sites in the tendon.
  - This is less than 0.3 ml of PRP - ?therapeutic dose.
- **PRP** - No benefit was shown in the post surgical healing of ACL grafts (Silva et al, 2009).
- **PRP** - No benefit with ACL healing (Nin et al, 2009)
- **PRP** - no benefit in the treatment of intra-bony defects (Demir, 2007)

# How can it be used?

## Osteoarthritis

- Exercise program
- Weight loss
- Strength program
- Pain relief
  - NSAIDS
  - CSI
  - Synvisc
  - ACS or PRP

# How can it be used?

## Osteoarthritis

- High level evidence ACS is better than synvisc
- Moderate level evidence for PRP and PRGF
- Dan's Anecdotal experience (not supported by Kon 2009)
  - Less effect in grade 1-2 (use synvisc or cortisone)
  - Good for grade 3-4 OA
  - Effective for chondral lesions and fissures

# How can it be used?

- Tendinopathy/enthesopathy
  - Not clear whether PRP, PRGF, ABI, ACS is better as no comparative studies
  - Personal experience the injection is painful with decreasing as below
    - $ABI > PRP > PRGF > ACS$
    - ACS – nearly no pain

# How can it be used?

## **Tendinopathy / enthesopathy**

- Suggested approach (Based on Cook and Purdam, 2009)
- <3 months or has done no rehab
  - Basic rehab – eccentrics avoiding compressive forces
  - Load modification – No greater than 2/10 pain
  - Review at 6 weeks
    - If improved continue
    - If not improved add in plan B

# How can it be used?

## **Tendinopathy / enthesopathy**

- >3-6mths pain
- Failure to progress with conservative approach
- Impatient patient.
- Ultrasound of tendon
  - No tear (Clarke, 2010)
    - Shockwave
    - GTN patch (pain reliever)
    - More eccentrics (avoid compressive forces)
    - Load modification – No greater than 2/10 pain

# How can it be used?

## Tendinopathy / enthesopathy

### Suggested approach

- >3-6mths pain, or failed conservative approach
- Ultrasound of tendon
  - Tear (Clarke, 2010)
    - **US guided** Injection into tear (blood product)
    - Consider GTN patch
    - More eccentrics – avoid compressive forces
    - Load modification – they must load it, taking 2 weeks off does not work.

# Questions?