Tendinous lesions around the knee
Is there a place for PRP?

H. PEREIRA,
J.M. Oliveira, R.L. Reis, J. ESPREGUEIRA-MENDES

Disclosures: Nothing to declare

MINHO UNIVERSITY
3B's Research Group

European Institute of Excellence on Tissue Engineering and Regenerative Medicine - Headquarters

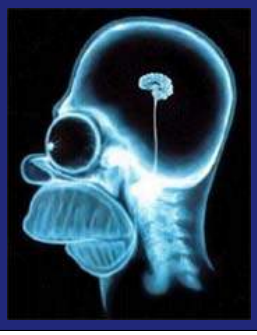
Headquarters and the Home for 3B´s Research

* CEO – Rui L. Reis

3600 m² new state of the art full equipped facility specially designed for TERM research

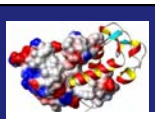
What are Growth Factors?!?
What is PRP?!?

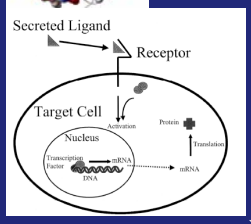
- *Oooops !!?!...*



Local Cells:
- fibroblasts
- Endothelial cells.
- Muscle cells
- stem cell
- (…)

inflammatory cells:
- Macrophages
- platelets
- (…)

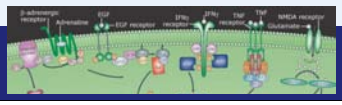




PROLIFERATION | MIGRATION | PROTEIN SYNTHESIS (ex. extra-cellular matrix) | DIFERENTIATION

GROWTH FACTORS -classifications

Growth Factor	Source	Receptor Class	Function
Transforming growth factor beta (TGF-β)	Platelets, bone extracellular matrix, cartilage matrix	Serine threonine sulfate	Pleiotropic growth factor stimulates undifferentiated mesenchymal cell proliferation
Bone morphogenetic protein (BMP)	Osteoprogenitor cells, osteoblasts, bone extra-cellular matrix	Serine threonine sulfate	Promotes differentiation of mesenchymal cells into chon-drocytes and osteoblasts, promotes differentiation of osteoprogenitors into osteo-blasts, influences skeletal pattern formation
Fibroblast growth factors (FGF)	Macrophages, mesenchymal cells, chondrocytes, osteoblasts	Tyrosine kinase	Mitogenic for mesenchymal cells, chondrocytes, and osteoblasts
Insulin-like growth factors (IGF)	Bone matrix, osteoblasts, chondrocytes	Tyrosine kinase	Promotes proliferation and differentiation of osteopro-genitor cells
Platelet-derived growth factor (PDGF)	Platelets, osteoblasts	Tyrosine kinase	Mitogen for mesenchymal cells and osteoblasts; macrophage chemotaxis



GROWTH FACTORS – different roles in different tissues

	Skeletal Muscle	Articular Structures	Meniscus	Ligament or Tendon	Bone
IGF-1	+	+		+	+
bFGF	+	+		+	+
NGF	+				
PDGF	+			+	+/-
EGF		+			-
TGF-β	-	+	+		
BMP-2		+			+
BMP-4					+
BMP-7 (OP-1)					+
VEGF					+/-
Decorin	+				

GROWTH FACTORS:

different roles in different phases of tendon repair

Repair Phase	Activity	Growth Factor
Inflammatory	Stimulates recruitment of fibroblasts and inflammatory cells to the injury site	IGF-1[80–82]
	Regulation of cell migration	TGF-β[42,83–86]
	Expression and attraction of other growth factors (eg, IGF-1)	PDGF[56,87,88]
	Angiogenesis	VEGF, bFGF[61–63,89–92]
Proliferative	Cell proliferation (DNA synthesis)	IGF-1 and PDGF, TGF-β, bFGF, GDF-5, -6, and -7[68–70,72,74]
	Stimulates synthesis of collagen and ECM components	IGF-1 and PDGF, bFGF
	Stimulates cell-matrix interactions	TGF-β, bFGF
	Collagen type III synthesis	TGF-β, GDF-5, -6, and -7
Remodeling	ECM remodeling	IGF-1
	Termination of cell proliferation	TGF-β
	Collagen type I synthesis	TGF-β, GDF-5, -6, and -7

*Tendon repair phases with biological characteristics and ensuing molecular events that are regulated by several cytokines or growth factors.

1. Platelet derived Growth Factors

- Mu
- Au
- Do
- Bio rec
- Pla (m

2. Selectiv

- TC
- Sel
- FD
- Pr

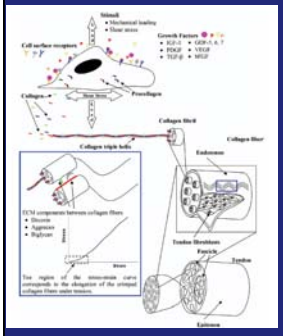
Clinical application considerations

- Short half-life
- Need to repeat effect
- Which Factors? In which proportion? At what time?
- Effective doses
- Limit action to target tissue
- Effect depends on local chemical and cellular environment
- Tissue repair mechanisms are complex:

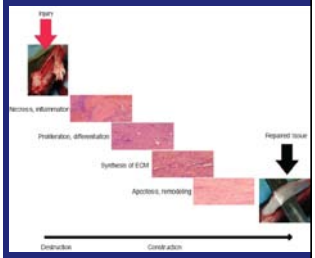
More tissue?... Which final tissue is obtained?...

Tendon: Biology, Biomechanics, Repair, Growth Factors, and Evolving Treatment Options

Roshan James, MS, Girish Kesturu, PhD, Gary Balian, PhD, A. Bobby Chhabra, MD



GF: TISSUE REPAIR MECHANISM



J Hand Surg 2008;33A:102–112

Comparision of Surgically Repaired Tendon Platelet-

- Faster rehablilitation
- Less wound problems
-Tendon thickness

Am J Sports Med 2007 Feb *M Sanchez* et al. 35: 245-251

"...presence of TGF-ß1 could provide some concerns:
1.excessive collagen deposition
2.scar tissue formation,
3.mechanical properties of the repaired tissue.

TGF-ß1 on collagen synthesis was counteracted by the presence of other platelet-secreted molecules
" ... synthesis of VEGF and HGF by tendon cells"

Reciprocal actions of platelet-secreted TGF-b1 on the production of VEGF and HGF by human tendon cells. Plast Reconstr Surg. 2007 Mar;119(3):950-9.
Anitua E, Sanchez M, Nurden AT, et al.

Properties of PRP

"Biological glue"
Coagulation and hemostasis
Wound healing
Provisional scaffold for stem or primary cell migration and differentiation
Intra-articular restoration of hyaluronic acid
Balances joint angiogenesis
Increases glycosaminoglycan chondrocyte synthesis and cartilage matrix
Anti-inflammatory
Antibacterial
Analgesic

NOTE. PRP has multiple properties, including antibacterial and anti-inflammatory effects, coagulation, and hemostasis, as well as analgesic properties. PRP contains platelets that secrete alpha granules. These granules are made up of growth factors (platelet-derived growth factor, transforming growth factor β, VEGF), endostatins, platelet factor 4, angiopoietins, and thrombospondin 1, which are all active in wound healing. Fibrin also contributes to the creation of a scaffold for wound healing and allows PRP to function as a biological glue.

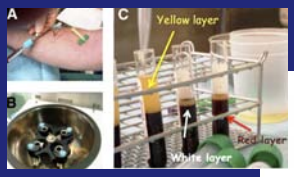
Contraindications to the use of PRP

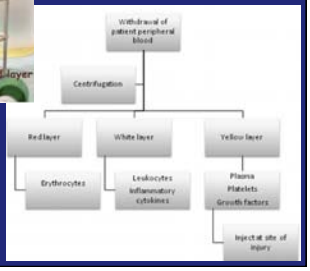
- Contraindications (Absolute)
  - Platelet dysfunction syndrome
  - Critical thrombocytopenia
  - Hypofibrinogenemia
  - Hemodynamic instability
  - Septicemia
  - Sensitivity to bovine thrombin
    - If using bovine thrombin with calcium to make platelet gel
- Contraindications (Relative)
  - Consistent use (anti-inflammatory use) of NSAID's within 48 hours of procedure
  - Corticosteroid injection at treatment site or systemic use of corticosteroids within 2 weeks of graft procedure
  - Recent fever or illness
  - Rash at graft donor site or at receptor site
  - Cancer – especially hematopoetic or of bone
  - Active history or history of *Pseudomonas, Enterococcus* or *Klebsiella* infection, as PRP has been shown in one study to potentially stimulate these patho

How do you get PRP?

The Use of Platelet-Rich Plasma in Arthroscopy and Sports Medicine: Optimizing the Healing Environment

E. LOPEZ-VIDRIERO ET AL.



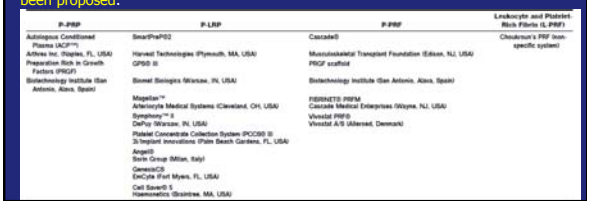


What Is Platelet-Rich Plasma?

Steven P. Arnoczky, DVM,* Demetris Delos, MD,†‡ and Scott A. Rodeo, MD†‡

Oper Tech Sports Med 19:142-148 © 2011

Products vary markedly not only in the final concentration of platelets they produce but also in the amount of red blood cells and/or white blood cells included
Some techniques for creating PRP actively initiate the clotting cascade as part of the process, creating a fibrin scaffold. The inclusion of these additional blood components may affect the indication(s), potency, and efficacy of the final PRP product.
the generic classification "PRP" does not allow distinction between the different systems and protocols. Therefore, to more precisely delineate these various products based on their leukocyte and fibrin content, categories such as pure PRP, leukocyte rich PRP (L-PRP), pure platelet-rich fibrin, and leukocyte- and platelet-rich fibrin have been proposed.



Platelets: The More the Better?

1. studies shown a poor correlation between platelet concentration and GF concentration in some PRP preparations.
2. natural variations in platelet concentration among individuals as well as daily variation within individuals
3. Doseresponse curves of most GFs are not linear, if cell surface receptors for a specific GF are completely occupied, increasing concentrations of the GF has no additional effect
4. some GFs can actually exert an inhibitory effect on cell functions once a high enough concentration is reached
5. Doseresponse relationship is both GF and cell type dependent, the precise concentration of platelets (and their associated GFs) required to optimize the myriad of cell types involved in connective tissue healing in vivo is not clear

Leukocytes: include or not?

pure- PRP or P-PRP: has had leukocytes intentionally removed from the solution
Leukocyte-rich PRP: whether produced by intentionally preserving the leukocytes or because the processing method is not sensitive enough to distinguish between the platelets and white blood cells in the buffy coat

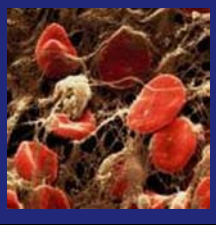
L-PRP preparation has been shown to inhibit the growth of *Staphylococcus aureus* and *Escherichia coli* in vitro although in the same study it had no effect on *Klebsiella pneumoniae* and *Enterococcus faecalis* and *Pseudomonas aeruginosa* activity was actually increased

white blood cells release matrix metalloproteinases and produce reactive oxygen species that can lead to increased muscle damage after injury in the acute inflammatory phase. Similarly, in a recent in vitro study, the concentration of white blood cells in PRP was shown to be positively correlated with expression of the catabolic MMP-3 and MMP-13 genes in equine tendon explants

Plasma differs from serum:

Plasma still contains fibrinogen as well as a number of clotting factors.
When plasma is exposed to thrombin (either by the addition of exogenous thrombin or by coming in contact with tissue thromboplastin (also known as tissue factor), the clotting cascade is initiated and platelets are activated

The resulting formation of a fibrin clot provides a provisional scaffold for cell migration as well as a reservoir of growth factors.



EVIDENCE LEVEL

V

Review TRENDS in Biotechnology Vol.24 No.5 May 2006

New insights into and novel applications for platelet-rich fibrin therapies

Eduardo Anitua[1], Mikel Sánchez[2], Alan T. Nurden[3], Paquita Nurden[3], Gorka Orive[1] and Isabel Andia[1]

Available online at www.sciencedirect.com
ScienceDirect
Biomaterials
ELSEVIER
Biomaterials 28 (2007) 4551–4560
www.elsevier.com/locate/biomaterials

Leading Opinion

The potential impact of the preparation rich in growth factors (PRGF) in different medical fields ☆

Eduardo Anitua[a], Mikel Sánchez[b], Gorka Orive[a,*], Isabel Andia[a]

Can Platelet-Rich Plasma Enhance Tendon Repair?

A Cell Culture Study

Am J Sports Med 2008 36: 1171

Marieke de Mos, Anna E. van der Windt, Holger Jahr, Hans T. M. van Schie, Harrie Weinans, Jan A. N. Verhaar and Gerjo J. V. M. van Osch

Background: Autologous platelet-rich plasma (PRP) application appears to improve tendon healing in traumatic tendon injuries, but basic knowledge of how PRP promotes tendon repair is needed.

Hypothesis: Platelet-rich plasma has a positive effect on cell proliferation and collagen production and induces the production of matrix-degrading enzymes and endogenous growth factors by human tenocytes.

Study Design: Controlled laboratory study.

Methods: Human tenocytes were cultured 14 days in 2% fetal calf serum medium complemented with 0%, 10%, or 20% vol/vol platelet-rich clot releasate (PRCR) the active releasate of PRP) or platelet-poor clot releasate (PPCR). At day 4, 7, and 14, cell amount, total collagen, and gene expression of collagen I⍺1 (COL1) and III⍺1 (COL3), matrix metalloproteinases (MMPs) MMP1, MMP3, and MMP13), vascular endothelial-derived growth factor (VEGF)-A, and transforming growth factor (TGF)-β1 were analyzed.

Results: Platelet numbers in PRP increased to 2.55 times baseline. Growth-factor concentrations of VEGF and platelet-derived growth factor (PDGF)-BB were higher in PRCR than PPCR. Both PRCR and PPCR increased cell number and total collagen, whereas they decreased gene expression of COL1 and COL3 without affecting the COL3/COL1 ratio. PRCR, but not PPCR, showed upregulation of MMP1 and MMP3 expression. Matrix metalloproteinase 13 expression was not altered by either treatment. PRCR increased VEGF-A expression at all time points and TGF-β1 expression at day 4.

Conclusion: In human tenocyte cultures, PRCR, but also PPCR, stimulates cell proliferation and total collagen production. PRCR, but not PPCR, slightly increases the expression of matrix-degrading enzymes and endogenous growth factors.

Clinical Relevance: In vivo use of PRP, but also of PPP to a certain extent, in tendon injuries might accelerate the catabolic demarcation of traumatically injured tendon matrices and promote angiogenesis and formation of a fibrovascular callus. Whether this will also be beneficial for degenerative tendinopathies remains to be elucidated.

EVIDENCE LEVEL

III-IV

Martha Murray et al

Anterior Cruciate Ligament Healing and Repair
Sports Medicine and Arthroscopy Review: September 2005 – Volume 13 - Issue 3 - pp 151-155

Platelet-rich plasma alone is not sufficient to enhance suture repair of the ACL in skeletally immature animals: an in vivo study J Orthop Res (2008)

Use of a collagen platelet rich plasma scaffold to stimulate healing of a central defect in the canine ACL. J Orthop Res (2006) 24:820–830

Current Status and Potential of Primary ACL Repair
Clinics in Sports Medicine Vol.28, Issue 1, January 2009, Pages 51-61

"despite an active biologic response in the ACL after injury, the two ends of the torn ligament never reconnect. Additional studies have detailed findings after placement of a substitute provisional scaffold in the wound site of the ACL injury to bridge the gap and initiate healing of the ruptured ligament after primary repair."

Knee Surg Sports Traumatol Arthrosc (2009) 17:559–560
DOI 10.1007/s00167-009-0776-2

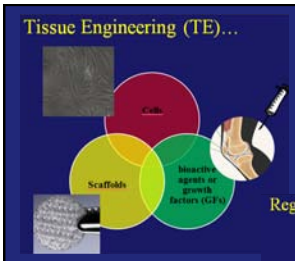
EDITORIAL

Tissue engineering: use of scaffolds for ligament and tendon healing and regeneration

Savio L-Y. Woo

"In the tendon and ligament area, much interest has been given to the use of bioactive molecules including hyaluronic acid (HA), EGF, TGF-beta; and more recently, the ubiquitous platelet rich plasma (PRP) matrices for applications in orthopaedic sports medicine."

Tissue Engineering (TE)…



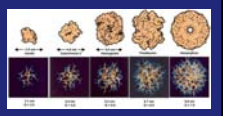
Arthroscopy: The Journal of Arthroscopic and Related Surgery
Vol 27, No 12 (December), 2011: pp 1706-1719

Regenerative Medicine – wider concept…

ICVS/3B's

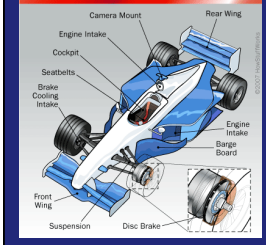
Nanotechnology and Cell engineering

1. Cell 'tune-up'
2. MIMICKING OF PROTEINS



Design and tailor the multi-components/performance

How Formula One Works Car Basics



How cells works Basics

ICVS/3B's

METHACRYLATION OF GELLAN GUM (Gellan Gum-GMA)

Gellan Gum 1% (5-6 mmol) 90°C → Cooling to RT → 1 or 5 days pH 8.5 ← GMA (5-6 mmol) → Gellan Gum -GMA → Precipitation ½ vol acetone → Purification by dialysis (4 days)

ICVS/3B's

GF is not a matter of FAITH….

But there is a great need for studying and learning

Take Home Message

As there are **other interacting factors** in the human body, including inflammatory reactions, circulation, and other cytokines and growth factors, there are **limitations with regard to extrapolating in vitro findings to clinical situations**. The actual effects inside the body need to be further evaluated before the in vivo and clinical effects are known.

J Bone Joint Surg Am Wong et al. 85:1914-1920 (2003)

Take Home Message

"Dynamic comprehension without mind gaps"

Knee Surgery is all about FUN!