Dosing - PRP Requirements

Cell Prolif. 2009 Apr;42(2):162-70. doi: 10.1111/j.1365-2184.2009.00583.x. Epub 2009 Feb 24.

Fibroblastic response to treatment with different preparations rich in growth factors.

Anitua E[1], Sánchez M, Zalduendo MM, de la Fuente M, Prado R, Orive G, Andía I.

Author information

Abstract

OBJECTIVES: Preparations rich in growth factors (PRGF) release them plus bioactive proteins at localized sites, with the aim of triggering healing and regenerative processes. The prevailing paradigm suggests that their influence on proliferation, angiogenesis and the extracellular matrix synthesis is minimal. However, variations in their composition and impact on different cell phenotypes have not been examined.

MATERIALS AND METHODS: Sixteen fibroblast cultures obtained from three different anatomical sites (skin, synovium and tendon) of 16 donors were exposed to the molecular pool released from PRGF scaffolds, with increasing amounts of platelets. We evaluated cell proliferation, secretion of angiogenic growth factors (VEGF and HGF), synthesis of type I collagen and hyaluronic acid (HA), considering platelet dose and anatomical origin of the cells. Activity of transforming growth factor-beta (TGF-beta) in type I procollagen and HA synthesis was examined by adding exogenous TGF-beta to plasma preparations.

RESULTS: All plasma preparations induced a significant proliferative response compared to non-stimulated cells ($P < 0.05$). Maximum proliferation rate was obtained with PRGF with 2-fold or 4-fold platelet concentration. Exposure to PRGF stimulated VEGF synthesis exclusively in tendon cells ($P < 0.05$), which also exhibited a different pattern of HGF production ($P < 0.05$). PRGF enhanced HA synthesis ($P < 0.05$), but did not alter collagen I production. Platelet-secreted TGF-beta may be involved in HA, but not in type I procollagen synthesis.

CONCLUSIONS: Optimizing composition and use of platelet-rich products is crucial to enhancing the therapeutic potential of this technology. Our data show that the biological effects of PRGF may depend on concentration of platelets and on the anatomical source of the cells.

PMID: 19250293 DOI: 10.1111/j.1365-2184.2009.00583.x

Dosing - PRP Requirements

Clin Oral Implants Res. 2006 Apr;17(2):212-9.

The in vitro effect of different PRP concentrations on osteoblasts and fibroblasts.

Graziani F[1], Ivanovski S, Cei S, Ducci F, Tonetti M, Gabriele M.

Author information

Abstract

OBJECTIVES: The aim of this study was to assess the biological rationale for the use of platelet-rich plasma (PRP) by evaluating the effect of different concentrations of PRP on osteoblasts (OB) and fibroblasts (FB) function in vitro.

MATERIAL AND METHODS: PRP was obtained from volunteer donors using standard protocols. Primary human cultures of oral FBs and OBs were exposed to both activated and non-activated plasma as well as various concentrations of PRP (2.5 x, 3.5 x and max (4.2-5.5 x)). Cell proliferation was evaluated after 24 and 72 h using an MTT proliferation assay. Production of osteocalcin (OCN), osteoprotegerin (OPG) and transforming growth factor beta1 (TGF-beta1) was evaluated in OB after 24 and 72 h. Statistical analysis was performed using one-way ANOVA.

RESULTS: PRP-stimulated cell proliferation in both OBs and FBs. The effect of different PRP concentrations on cell proliferation was most notable at 72 h. The maximum effect was achieved with a concentration of 2.5 x, with higher concentrations resulting in a reduction of cell proliferation. Upregulation of OCN levels and downregulation of OPG levels were noted with increasing PRP concentrations at both 24 and 72 h. TGF-beta1 levels were stimulated by increasing concentrations of PRP, with the increased levels being maintained at 72 h.

CONCLUSIONS: PRP preparations exert a dose-specific effect on oral FBs and OBs. Optimal results were observed at a platelet concentration of 2.5 x, which was approximately half of the maximal concentrate that could be obtained. Increased concentrations resulted in a reduction in proliferation and a suboptimal effect on OB function. Hence, different PRP concentrations may have an impact on the results that can be obtained in vivo.

PMID: 16584418 DOI: 10.1111/j.1600-0501.2005.01203.x

Dosing - PRP Requirements

- Low concentration of **LEUKOCYTES**, above all neutrophils
  - Neutrophils potentially have a detrimental effect as they activate/release unspecific proteases and toxic substances[1,2]

- Low concentration of **ERYTHROCYTES**
  - Their degradation releases reactive oxygen species that can induce inflammation and cell death[3]

[1] Diegelmann et al. Wound healing: an overview of acute, fibrotic and delayed healing. Front Biosci 2004; 9: 283-9.
[2] Martin et al. Inflammatory cells during wound repair: the good, the bad and the ugly. Trends Cell Biol 2005; 15(11): 599-607.
[3] Belcher et al. Heme Degradation and Vascular Injury. Antioxidants & Redox Signaling 2010; 12 (2): 233-48

Dosing - PRP Requirements

J Periodontol. 2003 Jun;74(6):858-64.

Platelet-rich plasma-derived fibrin clot formation stimulates collagen synthesis in periodontal ligament and osteoblastic cells in vitro.

Kawase T[1], Okuda K, Wolff LF, Yoshie H.

Author information

Abstract

BACKGROUND: Platelet-rich plasma (PRP) contains several growth factors, including platelet-derived growth factor (PDGF) and transforming growth factor-beta (TGF-beta), at high levels. We have demonstrated the PRP functions like TGF-beta to modulate cell proliferation in a cell-type specific manner. In addition, PRP forms gel-like materials in several, but not all, cell cultures tested. This study was designed to investigate PRP's action on extracellular matrix production in periodontal ligament (PDL) and osteoblastic MG63 cell cultures.

METHODS: PRP was prepared from the plasma obtained from autologous blood of healthy volunteers and stored at -20 degrees C until used. Cells treated with PRP (0.5% to 2%) were immunocytochemically stained for type I collagen and fibrin and the viscosity of the culture media was visually evaluated. Fibrinogen in PRP was detected by immunodot-blotting while endogenous thrombin expression in cells was detected by a modified enzyme-linked immunosorbent assay.

RESULTS: Gel-like material rapidly (< 30 minutes) formed in cultures of either PDL or osteoblastic MG63 cell cultures after addition of PRP (> or = 0.5%). PRP changed cell shape and up-regulated type I collagen at 24 hours. Fibrinogen was detected in the PRP preparations and insoluble fibrin networks were found in the newly formed gel-like material. PRP's action on collagen synthesis was mimicked by purified fibrinogen and blocked by thrombin inhibitor. Thrombin was expressed both in PDL and MG63 cells.

CONCLUSIONS: These findings demonstrated that the gel-like material formed in cell cultures of either PDL or MG63 cells is fibrin clot that is capable of up-regulating collagen synthesis in the extracellular matrix. Our data suggest the possibility that fibrinogen, converted to fibrin, in combination with growth factors present in PRP might effectively promote wound healing at sites of injury in periodontal tissue.

PRP Systems Comparison - IMCAS

	Arthrex ACP	Regenlab BCT	Regenlab THT	Plymouth Medical	Proteal	Glotech
Increase factor in platelets	3.01	1.1** 0.76*	1.16** 0.77*	2.75	2.48	Centrifuge problem; below baseline

** 1 hour after centrifugation

* Immediately after preparation

Magalon J. Product analysis: PRP. Session 037. IMCAS World Congress 2017. 01/26/2017 Paris, France.

PRP Systems Comparisson

Patient 1, 37 Y, Male, Healthy

	Baseline 1	**Arthrex ACP**	Baseline 2	**Cellenis PRP (MyCells, Eclipse PRP)**	Baseline 3	**Regenlab BCT**
Platelets	226	**532**	222	**340**	225	**145**
PRP Concentration	x	**2.35**	x	**1.53**	x	**0.64**
WBC	6.8	**0.0**	6.7	**1.0**	6.5	**0.2**
WBC Concentration	x	**0.0**	x	**0,15**	x	**0,03**
RBC	4.9	**0.0**	4.9	**0.0**	4.9	**0.0**

PRP Systems Comparisson

Patient 2, 34 Y, Female, Healthy

	Baseline 1	**Arthrex ACP**	Baseline 2	**Cellenis PRP (MyCells, Eclipse PRP)**	Baseline 3	**Regenlab BCT**
Platelets	286	**623**	288	**424**	290	**191**
PRP Concentration	x	**2.18**	x	**1.47**	x	**0.66**
WBC	7.4	**0.0**	7.3	**0.5**	7.1	**0.2**
WBC Concentration	x	**0.0**	x	**0.07**	x	**0.03**
RBC	4.0	**0.0**	3.9	**0.0**	3.9	**0.0**

PRP Systems Comparisson

Patient 3, 43 Y, Male, Healthy

	Baseline ACP	**Arthrex ACP**	Baseline Cellenis	**Cellenis PRP (MyCells, Eclipse PRP)**	Baseline Regenlab	**Regenlab BCT**
Platelets	219	**465**	211	**202**	211	**153**
PRP Concentration	x	**2.12**	x	**0.96**	x	**0.73**
WBC	5.8	**0.0**	5.5	**0.6**	7.1	**0.4**
WBC Concentration	x	**0.0**	x	**0,11**	x	**0,06**
RBC	5.0	**0.0**	4.8	**0.0**	3.9	**0.0**

PRP Systems Comparisson

	Centrifugation	Closed System	Platelet Concentration	Reduction of WBC / RBC	Anticoagulant	Separation Gel
Arthrex ACP	4 min	Yes	2.22	0.00 0.00	No	No
Cellenis PRP (MyCells, Eclipse PRP)	10 min	No	1.32	0.11 0.00	Yes	Yes
Regenlab BCT	5 min	Yes	0.68	0.04 0.00	Yes	Yes

International Journal of
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MDPI

Article

Comparative Analysis of Different Platelet Lysates and Platelet Rich Preparations to Stimulate Tendon Cell Biology: An In Vitro Study

Franka Klatte-Schulz [1,*,†], Tanja Schmidt [1,†], Melanie Uckert [1], Sven Scheffler [2], Ulrich Kalus [3], Markus Rojewski [4,5], Hubert Schrezenmeier [4,5], Axel Pruss [3] and Britt Wildemann [1]

Int. J. Mol. Sci. **2018**, *19*, 212

2. Results

Blood from 16 donors was taken, and all four different blood products were produced from the blood of each donor to allow the comparison.

2.1. Characterization of Blood Products

The concentration of platelets and leukocytes was quantified in the whole blood and the blood products PRP-ACP, PRP-BCT, and PC and from each individual donor. The strongest enrichment of platelets was found in the PC (3.8 fold higher than blood) followed by PRP-ACP (1.9 fold higher than blood). Surprisingly, PRP-BCT had a reduced platelet count (0.7 fold lower compared to blood). Significant differences between the groups are shown in Figure 1A. Leukocytes were significantly reduced in all blood products compared to the whole blood. PRP-BCT showed a significantly increased leukocyte content compared to PRP-ACP and PC (Figure 1B). PCs used to produce pooled AlloPL

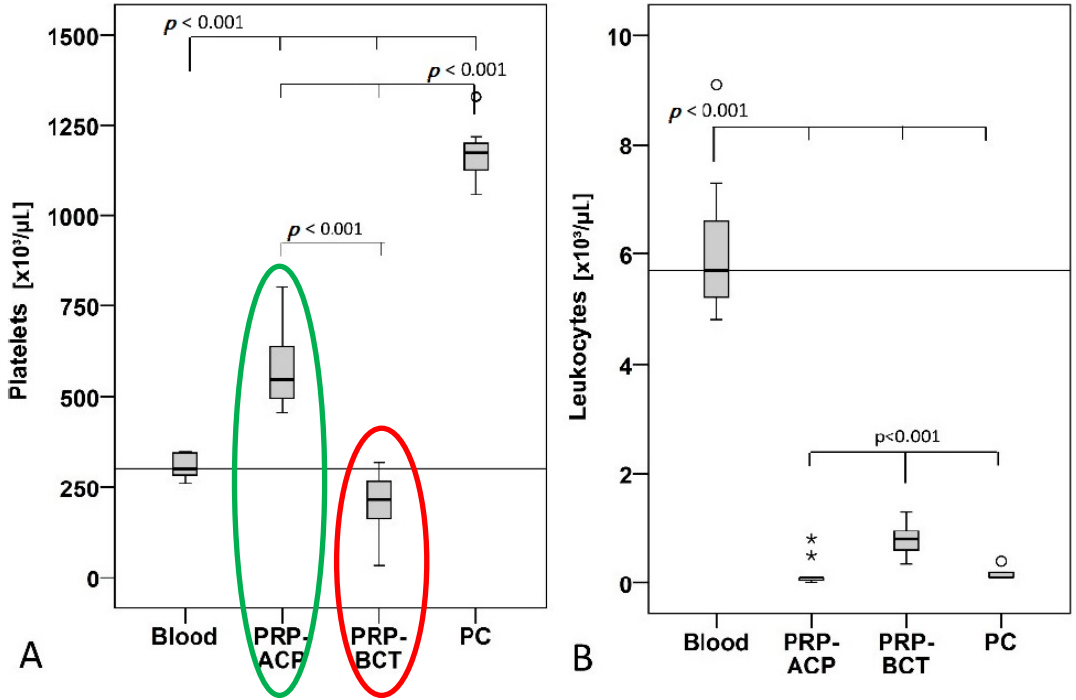


Figure 1. Platelet (**A**) and leukocyte (**B**) concentration in Arthrex, (PRP-ACP), RegenLab (PRP-BCT), and platelet concentrate (PC) compared to whole blood. (**A**) Platelet concentration was significantly higher in PRP-ACP and PC group and lower in the PRP-BCT group. (**B**) Leukocyte concentration was significantly reduced in all groups. °,* indicate outliers, n = 16 individual donors, all blood product were produced from each donor.

Klatte-Schulz F, et al. Comparative Analysis of Different Platelet Lysates and Platelet Rich Preparations to Stimulate Tendon Cell Biology An In Vitro Study. Int J Mol Sci. 2018 Jan 10;19(1). pii: E212.

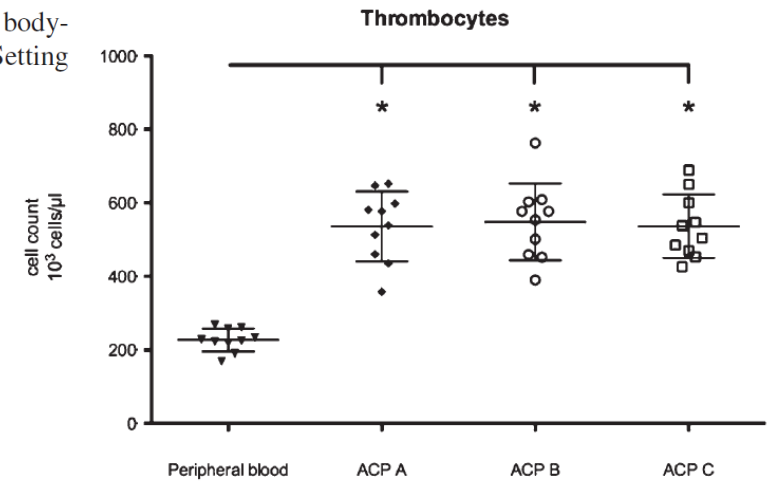
Table 1

Cell counts of thrombocytes, erythrocytes, leukocytes, lymphocytes, monocytes and neutrophils expressed in terms of mean ± standard deviation contained in peripheral blood and the single-spin separation methods ACP-A, ACP-B and ACP-C

Differential hemogram analysis for peripheral venous blood and each separation method				
	Peripheral blood	ACP-A 1500 rpm, 5 min, brake disabled	ACP-B 1500 rpm, 4 min, brake disabled	ACP-C 3000 rpm, 1 min, brake disabled
Thrombocytes $10^3/\mu l$	226.8 ± 31.20	536.0 ± 95.2	548.5 ± 104.9	536.2 ± 86.66
Erythrocytes $10^6/\mu l$	5.02 ± 0.22	0.04 ± 0.02	0.04 ± 0.02	0.04 ± 0.02
Leukocytes $10^3/\mu l$	6.04 ± 0.78	1.60 ± 0.74	0.05 ± 0.11	0.96 ± 1.32
Lymphocytes $10^3/\mu l$	2.12 ± 0.58	1.30 ± 0.59	0.04 ± 0.11	0.84 ± 1.19
Monocytes $10^3/\mu l$	0.48 ± 0.12	0.20 ± 0.09	0.00 ± 0.00	0.07 ± 0.01
Neutrophils $10^3/\mu l$	3.24 ± 0.50	0.10 ± 0.12	0.002 ± 0.006	0.04 ± 0.05

x2.42

For the remaining 20 subjects (group 1, group 3) and the 4 male patients undergoing elective body-contouring surgeries, ACP was prepared using 1500 rpm for 4 minutes with brake disabled (Setting ACP-B) (Fig. 1 B).



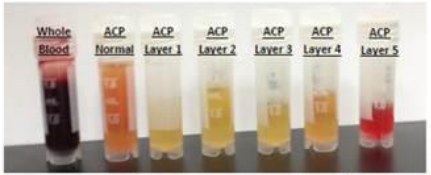
PRP system	Arthrex ACP double syringe (Arthrex Personalized Celltherapy)	Regenkit BCT3	Cellenis PRP (MyCells, Eclipse PRP)
Manufacturer	Arthrex Inc.	Regen Lab SA	Estar Technologies Ltd.
Platelet concentration	x 2.22	x 0.68	x 1.32
Leukocyte concentration	x 0.00	x 0.04	x 0.11
Erythrocyte concentration	x 0.00	x 0.00	x 0.00
Anticoagulant	No	Yes	Yes
Separation gel	No	Yes	Yes
Closed system	Yes	Yes	No
No needles required for preparation	Yes	Yes	No
Preparation time (from the time of blood collection)	8 minutes	8 minutes	15 minutes
Centrifugation time	4 minutes + time needed for centrifuge to come to a halt	5 minutes + time needed to brake the centrifuge	10 minutes + time needed to brake the centrifuge
G force (calculated from revolution rate and radius)	350 G	1500 G	1500 G

Tab 3.6 Summary of the properties of the tested PRP harvesting systems.

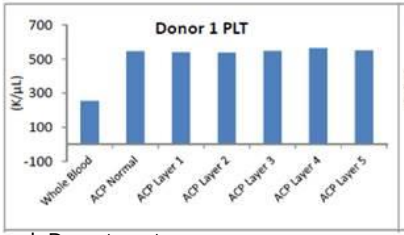
Kolster B. et al. Illustrated Guide to Collagen Induction with Platelet-Rich Plasma (PRP). KVM - der Medizinverlag, Berlin, Germany. 2019.

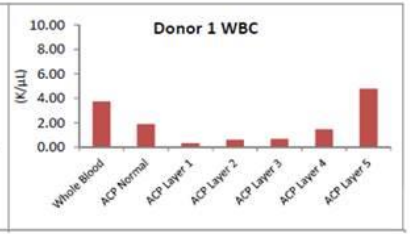
PRP - Arthrex ACP (Leucocyte-poor Fibrin-rich PRP)
Horizontal Slow Speed Centrifugation Technique

Total blood collected: 15ml (no anticoagulant required)
Centrifugation: 350G at 5min.



Donor 1	Platelets (K/µL)	White Blood Cells (K/µL)	Neutrophils (K/µL)
Whole Blood	255	3.76	2.25
ACP Normal	547 x2.15	1.90	0.11
ACP Layer 1	540 x2.12	0.32	0.03
ACP Layer 2	537 x2.11	0.62	0.04
ACP Layer 3	548 x2.15	0.68	0.04
ACP Layer 4	565 x2.22	1.48	0.07
ACP Layer 5	551 x2.16	4.80	0.34





5-6ml of PRP
out of 15ml whole blood
→max. Platelet concentration x3.0

PRP: x2.2-x3.0 Platelets & almost free of leucocytes[1-5]

1. Arthrex. Inc. Research Department
2. Kolster B. et al. Bildatlas Kollageninduktion mit Platelet-Rich Plasma (PRP). KVM - der Medizinverlag, Berlin, Germany. 2018.
3. Loibl M et al. The effect of leukocyte-reduced platelet-rich plasma on the proliferation of autologous adipose-tissue derived mesenchymal stem cells. Clin Hemorheol Microcirc. 2016;61(4):599-614.
4. Magalon J. Product analysis: PRP. Session 037. IMCAS World Congress 2017. 01/26/2017 Paris, France.
5. Magalon J. Product analysis - PRP. Session 279. IMCAS World Congress 2018. 02/03/2018 Paris, France.

The effect of leukocyte-reduced platelet-rich plasma on the proliferation of autologous adipose-tissue derived mesenchymal stem cells[1]

Markus Loibl[a,*], Siegmund Lang[a], Gero Brockhoff[b], Boyko Gueorguiev[c], Franz Hilber[a], Michael Worlicek[a], Florian Baumann[a], Stephan Grechenig[a], Johannes Zellner[a], Michaela Huber[a], Victor Valderrabano[d], Peter Angele[a], Michael Nerlich[a], Lukas Prantl[e] and Sebastian Gehmert[a,d,e]

Table 1

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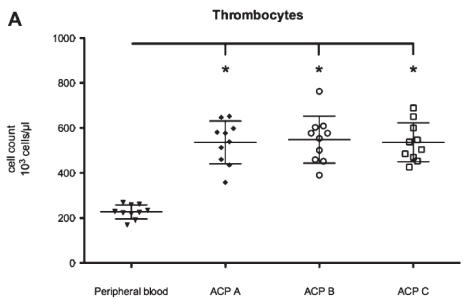

Loibl M, et al. The effect of leukocyte-reduced platelet-rich plasma on the proliferation of autologous adipose-tissue derived mesenchymal stem cells. Clin Hemorheol Microcirc. 2016;61(4):599-614.

Considerations - Preparation of PRP

Evidence	FDA-sanctioned Level I study
Safety	Closed and needle-free systems to prevent infections and needlestick injuries
No Anticoagulants	100 % autologous and a lower injection pain
Ease of use	Intuitive/automated processes to prevent incorrect use
Quality Control	ISO 13485 Sterile & Pyrogen-free
Quality and reliability	Established systems from a market-leading company

The Arthrex ACP® Double Syringe

- The ACP double syringe enables closed and easy production of PRP
- No synthetic separating materials are used as the PRP is separated from other blood components in a purely physical process
- This system enables the production of a PRP with a platelet concentration that is 2 to 3 times greater, coupled with a reduction in white blood cells and erythrocytes
- Using the Arthrex ACP double syringe, PRP can be produced and applied within a few minutes
- System handling is easy and convenient

The Arthrex ACP® Double Syringe

- Optimal composition of PRP for aesthetic application
- Only system **100% Autologous** (no blood contact with anticoagulants, synthetic gels or activators)
- Unique 2 Syringes in 1 Design for PRP production
- Purely physical process
- Lower injection pain
- Closed system
- Needle-free
- Fast (5 min. soft spin centrifugation, 90 degree) for PRP that can be produced and applied within a few minutes
- Application Time - 30 minutes to inject after blood sampling
- Yields PRP for full face application
- Clinically validated (FDA-sanctioned Level 1 Study and Health Canada Approval)