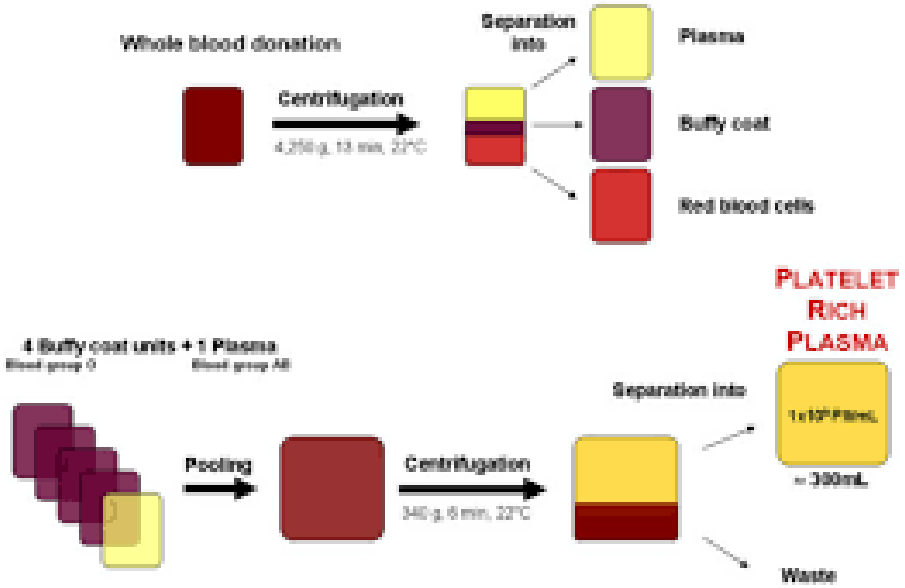
Elde Edilme Yöntemleri

PRP OTURUMU

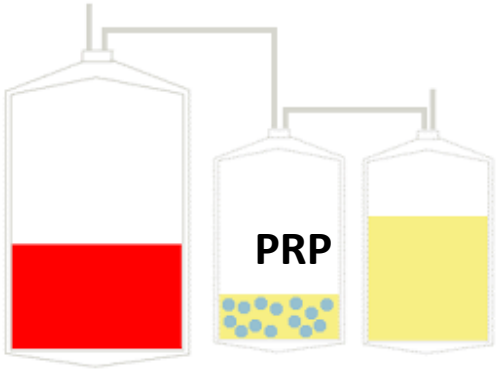
Uz.Dr Nil Banu PELİT

Venedik, 1995

Figure 1. Preparation of buffy coat-derived platelet rich plasma

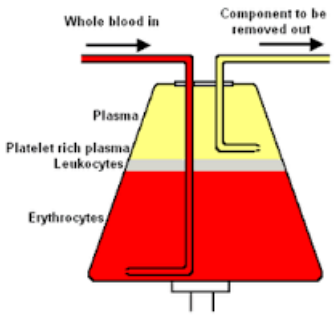


- Trombositlerin bir miktar plazma ile tam kandan konsantre edildikleri süspansiyondur



Kuzey Amerika'da T&T
Avrupa'da T&B sistem tercih edilir
Üstünlük tartışmalıdır

- Aferez cihazları kullanılarak trombositlerin bir miktar plazma ile tam kandan konsantre edildikleri süspansiyondur



Kullanım Alanları

- Diş hekimliği
- Maksillofasiyal cerrahi
- Ortopedi & travmatoloji
- Oftalmoloji
- Kalp-damar cerrahisi
- Dermatoloji
- Estetik & kozmetik cerrahi

Yeni değildir

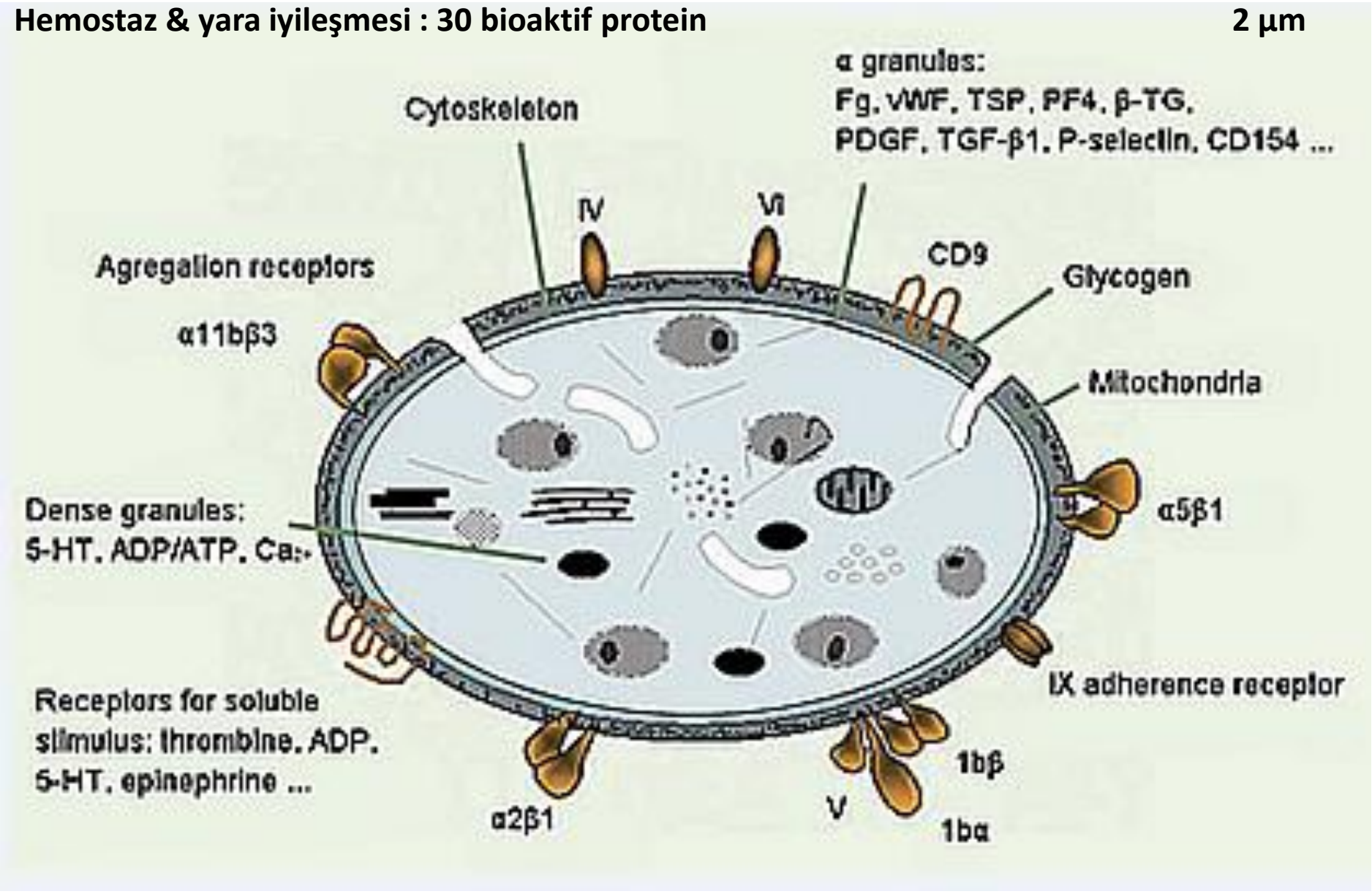
- 2009’da 45 M $’lık ürün pazara sürülmüşken 2016’da 126 M $ olacağı tahmin edilmiştir

- 2009’da protokolleri farklı 16 marka pazarda yer almış

- 2010’da +6 marka protokollerini onaya sunmuştur

The Journal of Arthroscopic and Related Surgery, Vol 30, No 5 (May), 2014: pp 629-638

Hemostaz & yara iyileşmesi : 30 bioaktif protein **2 µm**



Yara iyileşmesi : 7 temel protein –GFs-

Hücre adhezyon molekülü: fibrin, fibronektin, vitronektin

Aktivasyon → Degranülasyon

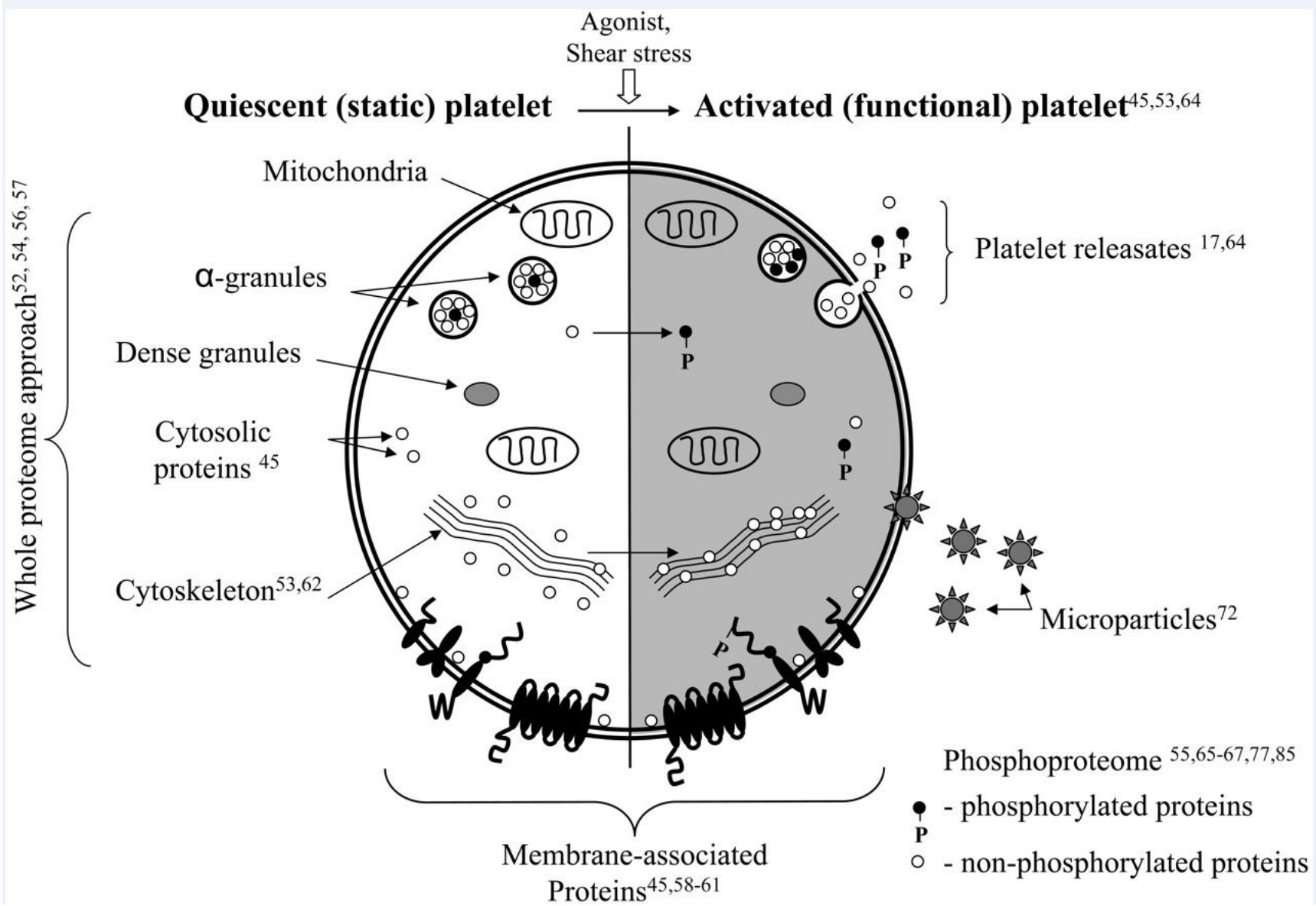
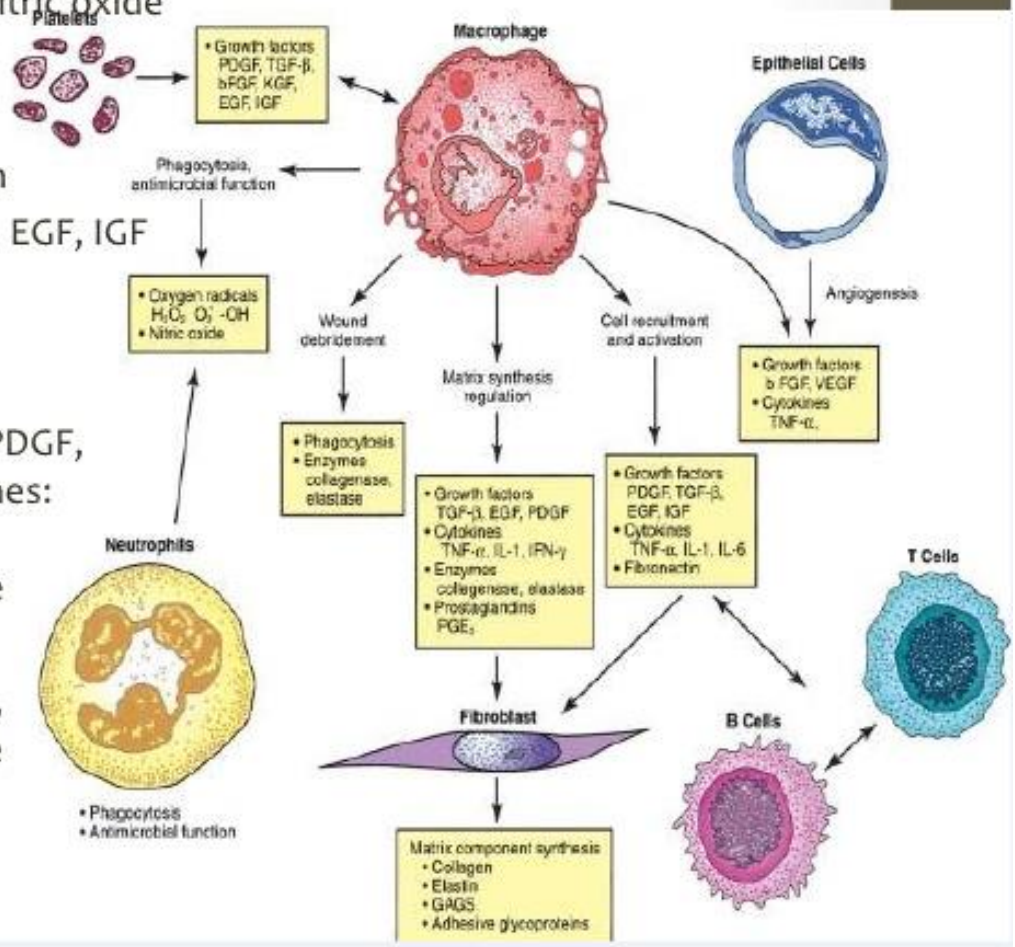


Table 1: Platelet growth factors and their specific characteristics[4]

Platelet Growth Factor Type	Growth factor Source	Biological Actions
Platelet derived growth factor (a-b)	Platelets, osteoblasts, endothelial cells, macrophages, monocytes, smooth muscle cells	Mitogenic for mesenchymal celss and osteoblasts, stimulates chemotaxis and mitogenesis in fibroblast/glial/smooth muscle cells, regulates collagenase secretion and collagen synthesis, stimulate macrophage and neutrophil chemotaxis
Transforming growth factor TGF(alpha –beta)	Platelets, extracellular matrix of bone, cartilage matrix, activated TH1 cells and natural killer cells, macrophages/monocytes and neutrophils	Stimulates undifferented mesenchymal cell proliferation ; regulates endothelial, fibroblastic and osteoblastic mitogenesis ; regulates collagen synthesis and collagenase secretion, regulates mitogenic effects of growth factors, stimulate endothelial chemotaxix and angiogenesis, inhibits macrophage and lymphocyte proliferation
Vascular endothelial growth factor, VEGF	Platelets, endothelial cells	Increases angiogenesis and vessel permeability, stimulates mitogenesis for endothelial cells
Epidermal growth factor, EGF	Platelets, macrophages, monocytes	Stimulates endothelial chemotaxis / angiogenesis, regulates collagenase secretion, stimulates epithelial /mesenchymal mitogenesis
Fibroblast growth factor, FGF	Platelets, macrophages, mesenchymal cells, chondrocytes, osteoblasts	Promotes growth and differentiation of chondrocytes and osteoblasts, mitogenic for mesenchymal cells, chondrocytes and osteoblasts
Connective tissue growth factor CTGF	Platelets through endocytosis from extracellular environment in bone marrow	Promotes angiogenesis, cartilage regeneration, fibrosis and platelet adhesion
Insulin like growth factor – 1 IGF -1	Plasma, epithelial cells, endothelial cells, fibroblasts, smooth muscle cells, osteoblasts, bone matrix	Chemotaxis for fibroblasts and stimulates protein synthesis. enchances bone formation by proliferation and differentiation of osteoblasts

- Macrophages
  - 48 to 96 hours – complete healing
- Phagocytosis
  - Reactive oxygen species, Nitric oxide
- Debridement
  - Collagenase, elastase
- Cell recruitment and activation
  - Growth factors: PDGF, TGF, EGF, IGF
    Cytokines: TNF-, IL-1, IL-6, Fibronectin
- Matrix synthesis
  - Growth factors: TGF, EGF, PDGF, Cytokines: TNF-, IL-1, Enzymes: arginase, collagenase, Prostaglandins, Nitric oxide
- Angiogenesis
  - Growth factors: FGF, VEGF , Cytokines: TNF, Nitric oxide



Doku Onarımı ve Regenerasyonu

Dohan Ehrenfest DM, Rasmusson L, Albrektsson T. Classification of platelet concentrates: From pure platelet-rich plasma (P-PRP) to leucocyte- and platelet-rich fibrin (L-PRF). Trends Biotechnol 2009;27:158-67.

PRP, hücre ve fibrin içeriğine göre:

- **P-PRP**: Saf veya lökositsiz PRP, aktivasyon sonrası düşük dansiteli fibrin ağı
- **L-PRP**: Lökositi bol PRP, aktivasyon sonrası düşük dansiteli fibrin ağı (ticari ve deneysel systemlerin çoğu bu gruptadır)
- **P-PRF**: Lökosittten fakir, aktivasyon sonrası yüksek dansiteli fibrin ağı (jel formasyonu, enjekte edilmez, fibrin glue gibi kullanılmaz)
- **L-PRF**: Lökosittten zengin, aktivasyon sonrası yüksek dansiteli fibrin ağı (II. Jenerasyon kitler)

Dohan Ehrenfest DM, Bielecki T, Mishra A, Borzini P, Inchingolo F, Sammartino G, *et al*. In search of a consensus terminology in the field of platelet concentrates for surgical use: Platelet-rich plasma (PRP), platelet-rich fibrin (PRF), fibrin gel polymerization and leukocytes. Curr Pharm Biotechnol 2012;13:1131-7.

Table 1. Protocol for Each Preparation System Provided by Either Commercial System Instructions (Selphyl System, RegenPRP, Mini GPS III System, and Arthrex ACP) or Publications (Laboratory Preparation)

	PRP Preparation System				
	Selphyl System	RegenPRP	Mini GPS III System	Arthrex ACP	Laboratory Preparation
Sampling needle (gauge)	21	21	18	18-20	21
Anticoagulant	Sodium citrate, 1 mL	Sodium citrate, 1 mL	ACD-A, 3 mL	ACD-A, 1 mL	ACD-A, 6 mL
Net volume of blood collected (mL)	8	8	27	11	30
No. of centrifugation steps	1	1	1	1	2
Common or specific centrifuge	Common	Common	Specific	Specific	Common
Speed and time	1,100*g* and 6 min	1,500*g* and 9 min	3,200 rpm and 15 min	1,500 rpm and 5 min	130*g* and 15 min, 250*g* and 15 min
Removal of PPP	No	Yes, 2 mL	Yes	No	Yes
Platelet resuspension	Return 7-fold	Gently return	Gently return during 30 s	No	Gently resuspend

ACD-A, anticoagulant citrate dextrose solution A.

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TABLE 2
Preparation of PRP Samples[a]

System	Blood Volume, mL	Anticoagulant Volume, mL	Centrifugal Force, g-force	Centrifuge Time, min	Volume Produced, mL
GPS III	52	ACD-A 8	1100	15	6-7
SmartPrep2	52	ACD-A 8	1250/1050	14	6-7
Magellan	52	ACD-A 8	1200	17	6-7
ACP	15	ACD-A 2	1500	5	6-7

[a]ACD-A, anticoagulant citrate dextrose solution A; PRP, platelet-rich plasma.

Trombosit Kazanımı

Table 2: Comparison of various protocols for platelet yield

Study	Volume of WB (mL)	1st Centrifugation		2nd Centrifugation		Platelet count rise
		Force (RCF)	Time (min)	Force (RCF)	Time (min)	
Amable *et al.*	4.5	300	5	700	17	1.4×10^6 to 1.9×10^6 5.4-fold to 7.3-fold
Amanda *et al.*	3.5	100	10	400	10	$1.222\pm166\times10^3$ 5-fold
Khan *et al.*	478	3731	4	—	—	8.3×10^{10}
Slichter and Harker	250-450	1000	9	3000	20	80% Recovery
Landesberg *et al.*	5	200	10	200	10	5.57 to 9.35×10^8
Jo *et al.*	9	900	5	1500	15	$633.2\pm91.6\times10^3$ 4.2 times
Bausset	10	250	15	250	15	3.96 times
Tamimi *et al.*	8.5	160	10	400	10	630.2×10^3
Mazzocca *et al.*	27	1500 rpm	5	6300 rpm	20	472×10^3
Anitua *et al.*	4.5	460	8	Single spin only		2.67 times
Araki *et al.*	7.5	270	10	2300	10	189.6×10^4
Kececi *et al.*	9	250	10	750	10	679.9×10^3

The Effect of Plasma Rich in Growth Factors on Pattern Hair Loss: A Pilot Study

EDUARDO ANITUA, MD, DDS,*† ANDER PINO, MSc,* NAHIKARI MARTINEZ, MSc,*
GORKA ORIVE, PHD,* AND DANIEL BERRIDI, MD*

ISSN: 1076-0512 • Dermatol Surg 2017;0:1–13 • DOI: 10.

Characterization and Comparison of 5 Platelet-Rich Plasma Preparations in a Single-Donor Model

Jeremy Magalon, Pharm.D., Olivier Bausset, Pharm.D., Nicolas Serratrice, Laurent Giraudo, Houssein Aboudou, Julie Veran, Guy Magalon, M.D., Françoise Dignat-Georges, Pharm.D., and Florence Sabatier, Pharm.D.

The Journal of Arthroscopic and Related Surgery, *Vol 30, No 5 (May) 2014: pp 629-638*

Table 3. Mean PRP Characteristics Obtained From Different Preparations and ANOVA Comparis erent Systems

	PRP Preparation System					
	Selphyl System	RegenPRP	Mini GPS III System	Arthrex ACP	y Preparation	*P* Value (ANOVA)
Volume of PRP obtained (mL)	4.10 ± 0.43	3.10 ± 0.61	3.21 ± 0.15	4.03 ± 0.35	3.41 ± 0.92	.0002
Platelet capture efficiency (%)	59.89 ± 15.73	55.28 ± 18.43	46.45 ± 12.66	48.23 ± 7.41	29.63 ± 9.10	<.0001
Relative composition in platelets (%)	73.86 ± 19.72	45.97 ± 24.70	51.84 ± 18.48	80.96 ± 3.10	80.72 ± 3.79	<.0001
Relative composition in WBC (%)	0.18 ± 0.11	1.04 ± 0.47	1.37 ± 0.36	0.08 ± 0.06	0.27 ± 0.24	<.0001
Relative composition in RBC (%)	25.97 ± 19.65	52.99 ± 24.93	46.79 ± 18.51	18.96 ± 3.05	19.01 ± 3.72	<.0001
Relative composition in neutrophils (%)				)	9.63 ± 16.66	.0031
Platelet concentration ($\times 10^9$/L)				7	756.20 ± 195.00	<.0001
Factor increase in platelet concentration					2.64 ± 0.33	—
WBC concentration ($\times 10^9$/L)					2.27 ± 2.01	<.0001
Factor increase in WBC concentration					0.35 ± 0.31	—
% of activated platelets	6.46 ± 2.45	6.10 ± 4.07	5.03 ± 2.95	4.27 ± 3.06	11.08 ± 9.85	.0801
Platelet dose in PRP ($\times 10^9$)	1.36 ± 0.42	1.25 ± 0.49	3.61 ± 0.13	1.49 ± 0.29	2.62 ± 0.12	<.0001

EN SAF

RBC > WBC kontaminasyonu

RBC, reaktif O_2 türlerini artırır

The Journal of Arthroscopic and Related Surgery, Vol 30, No 5 (May) 2014: pp 629-638

Ürün hacmi

Trombosit sayısı ve GF konsantrasyonu en yüksek

Table 4. Mean PRP GF-Releasing Characteristics and ANOVA Comparison Among Different Systems

	PRP Preparation System					
	Selphyl System	RegenPRP	Mini GPS III System	Arthrex ACP	Laboratory Preparation	*P* Value (ANOVA)
VEGF concentration (pg/mL)	394 ± 724.26	366.34 ± 253.14	1,204.58 ± 785.89	153.00 ± 93.78	484.81 ± 438.35	.001
VEGF dose (pg)	1,606.19 ± 2,892.51	1,111.28 ± 746.35	3,831.35 ± 2,459.67	626.16 ± 412.08	1,631.12 ± 72	.0055
PDGF-AB concentration (pg/mL)	9,471.41 ± 5,829.38	22,945.17 ± 16,918.00	29,709.27 ± 24,251.05	13,126.29 ± 9,993.51	30,365.42 ± 29,652.72	.0658
PDGF-AB dose (pg)	38,957.46 ± 24,371.85	71,018.19 ± 51,506.7	95,139.35 ± 75,923.23	52,308.79 ± 39,206.05	107,527.49 ± 103,292.73	.1253
EGF concentration (pg/mL)	898.63 ± 213.66	993.92 ± 411.15	2,617.88 ± 757.95	606.39 ± 423.05	1,170.00 ± 686.20	<.0001
EGF dose (pg)	3,670.08 ± 941.29	3,043.72 ± 1,267.67	8,429.44 ± 2,583.17	2,450.59 ± 1,682.04	3,868.20 ± 2,701.38	<.0001
TGF-β1 concentration (pg/mL)	3,023.88 ± 2,374.69	4,181.20 ± 2,738.81	5,689.75 ± 3,042.30	2,662.08 ± 2,205.91	5,707.46 ± 3,186.69	.0399
TGF-β1 dose (pg)	12,101.84 ± 9,097.80	12,792.28 ± 8,563.38	18,065.21 ± 9,557.29	10,613.60 ± 8,749.41	18,022.96 ± 10,880.65	.2616

PLT dozu ile GF'ler arasında pozitif korelasyon

WBC dozu ile VEGF&EGF arasında pozitif korelasyon

GF ve Sitokin Düzeyleri

Am J Sports Med
2011 39: 2135
August 16, 2011
Emily A. Sundman,

TABLE 1
Platelet, White Blood Cell, and Hematocrit Concentrations in Venous Blood, PRP-1, and PRP-2*

	Platelets × $10^3/\mu L$		White Blood Cells × $10^3/\mu L$		Hematocrit	
	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
Venous blood	183 ± 39.6[a†]	114-232	5.66 ± 1.41[a]	2.8-7.7	35.1 ± 2.51[a]	31-39
PRP-1	361 ± 87.0[a†]	245-493	0.68 ± 0.42[c]	0.2-1.4	0.46 ± 0.52[c]	0-1.0
PRP-2	701 ± 473[b]	114-1520	23.7 ± 5.91[b]	14.3-30.1	4.82 ± 1.83[b]	3.0-8.0

*N = 11. Superscript letters indicate significant difference between groups, but within hematocrit, white blood cell, or platelet-rich plasma (PRP), by analysis of variance with Tukey post hoc test, $P < .05$.
†Indicates significant difference between venous blood and PRP-1 using 2-sample t test, $P < .05$.

Anabolik etki

- **TGF-β1**
- **PDGF AB**

PLT ile pozitif korelasyon

Arthrex ACP; PRP 1
Biomet GPS III; PRP 2

Katabolik etki

- **MMP-9**
- **IL-1β**

NEU ile pozitif korelasyon

↓

MONO ile pozitif korelasyon

Antibakteriyel & İmmunolojik direnç?

Journal of Orthopaedic Science
21 (2016) 683–689
Yohei Kobayashi [a], Y

Table 2
CBC data of the whole blood, LR-PRP, LP-PRP, pure-PRP, and PPP.

	Platelet ($\times 10^3/\mu l$)	Leukocyte ($\times 10^3/\mu l$)	Neutrophil ($\times 10^3/\mu l$)	Lymphocyte ($\times 10^3/\mu l$)	Monocyte ($\times 10^3/\mu l$)	Erythrocyte ($\times 10^6/\mu l$)
Whole blood	206.8 ± 27.1	5.7 ± 0.9	3.7 ± 0.6	1.6 ± 0.4	0.4 ± 0.1	4.7 ± 0.7
LR-PRP	846.5 ± 431.8	14.9 ± 4.5	5.4 ± 3.0	8.1 ± 1.9	1.4 ± 0.6	3.2 ± 1.2
LP-PRP	777.3 ± 253.6	2.4 ± 1.3	0.0 ± 0.0	2.3 ± 0.3	0.1 ± 0.0	0.0 ± 0.0
Pure-PRP	469.2 ± 157.3	0.2 ± 0.2	–	–	–	0.0 ± 0.0
PPP	8.0 ± 5.0	0.1 ± 0.0	–	–	–	0.0 ± 0.0

Data are presented as the mean ± SD.

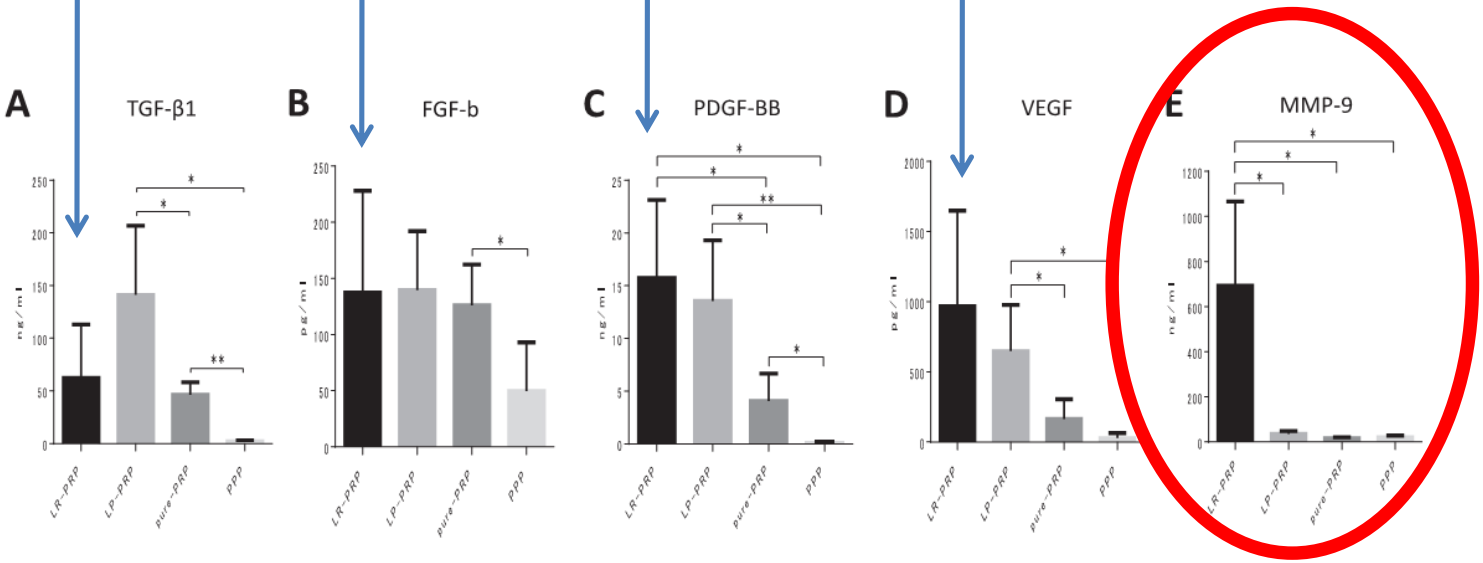


Fig. 3. Growth factor and catabolic cytokine concentrations in LR-PRP, LP-PRP, pure-PRP and PPP. (A) Transforming growth factor-beta 1 (TGF-β1), (B) fibroblast growth factor-b (FGF-b), (C) platelet-derived growth factor-BB (PDGF-BB), (D) vascular endothelial growth factor (VEGF), (E) matrix metalloproteinase-9 (MMP-9). Data are presented as the mean ± SD (*$p < 0.05$, **$p < 0.01$).

Commercial Separation Systems Designed for Preparation of Platelet-Rich Plasma Yield Differences in Cellular Composition

Ryan M. Degen, MD · Johnathan A. Bernard, MD · Kristin S. Oliver, MD · Joshua S. Dines, MD

HSS Journal®
The Musculoskeletal Journal of Hospital for Special Surgery

HSSJ
DOI 10.1007/s11420-016-9519-3

Lökositlerden salınan **sitokinler**:

İnflamatuar reaksiyonu artırır,
Fibrozise zemin hazırlar,
Yapısal olarak daha zayıf bir doku gelişimine neden olur,
Antimikrobial etkisi invivo olarak kanıtlanmamıştır
İntraartiküler kullanımda ağrıyı (asit pH ?) artırır
Hücre ölümü ve sinoviosit aktivasyonuna neden olur

Üründeki **nötrofil** miktarı:

Bakterisidal asit salınımı ile antibakteriyel etkisi vardır
IL-1β miktarı ile pozitif korelasyon vardır
IL-1β inflamatuar sitokindir; yara iyileşmesindeki etkisi tartışmalıdır

AJSM

March 13, 2014

Matthew A. Pifer,* MD,

Matrix Metalloproteinase Content

TABLE 1
Substrates of MMP-2, MMP-3, and MMP-9[a]

Enzyme Name		Extracellular Matrix Substrates
MMP-2	Gelatinase A	Collagens (type I, II, III, IV, V, VII, X, XI), gelatin, elastin, fibronectin, vitronectin, laminin, entactin, tenascin, SPARC, aggrecan, link protein, galectin-3, versican, decorin, myelin basic protein
MMP-3	Collagenase 3	Collagens (type III, IV, V, VII, IX, X, and XI), collagen telopeptides, gelatin, elastin, fibronectin, vitronectin, laminin, entactin, tenascin, SPARC, aggrecan, link protein, decorin, myelin basic protein, perlecan, versican, fibulin
MMP-9	Gelatinase B	Collagens (type IV, V, XI, and XIV), gelatin, elastin, vitronectin, laminin, SPARC, aggrecan, link protein, galectin-3, versican, decorin, myelin basic protein

[a]MMP, matrix metalloproteinase; SPARC, secreted protein acidic and rich in cysteine.

MMP, kas iskelet sisteminin **remodeling ve devamlılığında** önemlidir
Yumuşak doku **remodeling ve iyileşmelerinde** de gereklidir
Yüksek konsantrasyonlarda katabolik etkisi ile iyileşmeyi geciktirir
Ticari kitlerde MMP-9 ve IL-1β gösterilmiştir
Trombosit aktivasyonu sonrası MMP-1 ve 2'nin salındığı saptanmıştır
PRP'nin fibroblastlarda MMP-1 ekspresyonunu artırdığı belirtilmiştir

AJSM

March 13, 2014

Matthew A. Pifer,* MD,

TABLE 2
Hematologic Analysis of Donors Used in MMP Release Experiments[a]

	Platelet Count, $10^3/\mu L$ (-Fold Over Whole Blood)	White Blood Cells, $10^3/\mu L$ (-Fold Over Whole Blood)	Hematocrit
Whole blood			
Donor 1	317	6.71	46.40
Donor 2	217	4.06	41.25
ACP			
Donor 1	418 (1.31×)	0.04 (0.006×)	0.01
GPS			
Donor 2	1268 (5.84×)	31.71(7.81×)	11.77

[a]ACP, Arthrex Double Syringe System; GPS, Biomet GPS III; MMP, matrix metalloproteinase.

?

MMP'ların PLT/WBC veya her ikisinden mi kaynaklı olduğu net değildir.
Enzimatik aktiviteleri tam tanımlanmamıştır.
Kollajen, jelatin, elastin vb proteoglikan matriks maddelerini parçalar
Bu çalışmada hem düşük hem yüksek WBC içeren PRP'de MMP'nin aktif olduğu ve ilk bir kaç saatte en fazla, giderek azalan konsantrasyonlarda 6 gün boyunca salınmaya devam ettiği ve aktif kaldığı gösterilmiştir. Bu durum, fibroblastlardan MMP salınımını düşündürmüş, bu da IL-1β stimulasyonuna bağlanmıştır

Platelet-Rich Plasma Powder

AJSM PreView,
November 30, 2016
Matthias Kieb
Germany

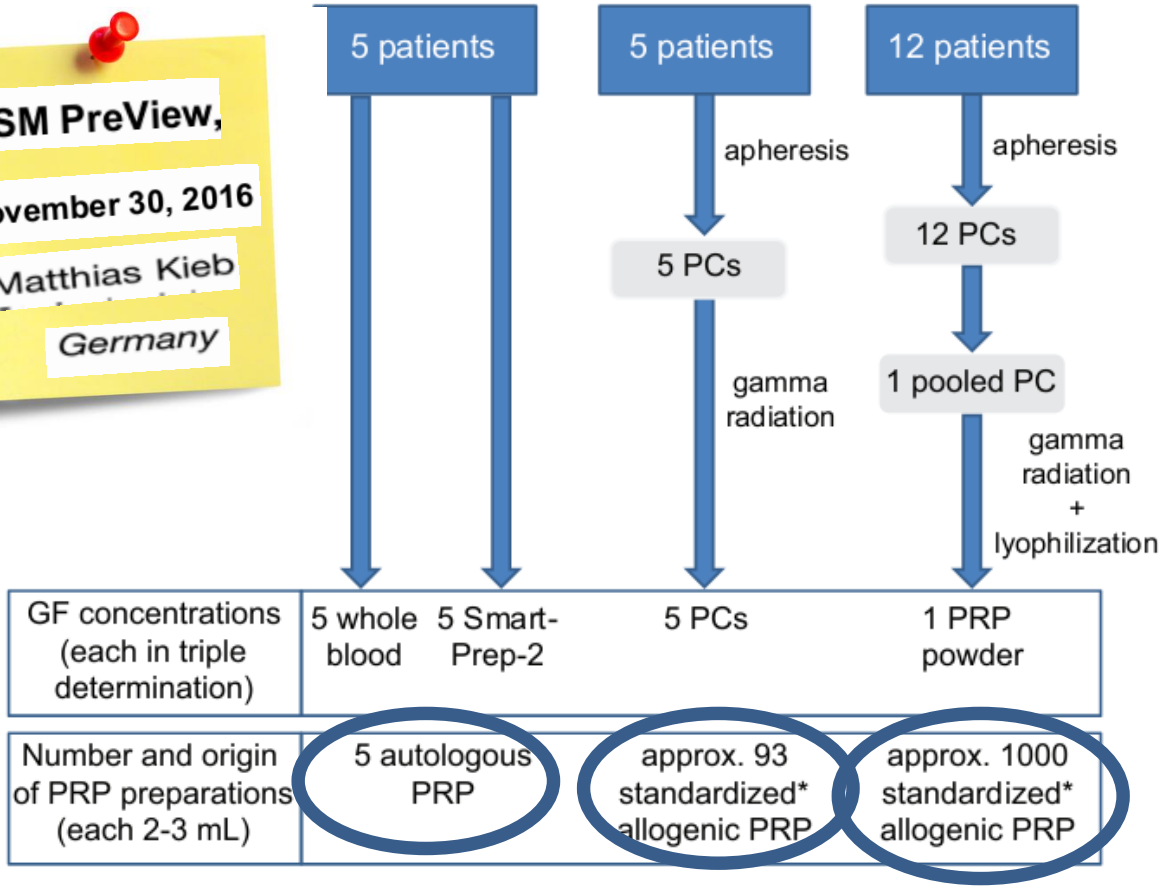


Figure 1. Study protocol. Overview of different preparation methods and number of patients for each study group. *Consecutive potential applications were performed with equal growth factor concentrations. GF, growth factor; PC, platelet concentrate; PRP, platelet-rich plasma.

Figure 2. Small vials of platelet-rich plasma powder. Before use, the powder has to be resuspended in 1 mL of 0.9% saline.

Topikal/iv/im
Tendona Uygulanabilir

TABLE 1
Cell Counts of the Different Samples[a]

	Whole Blood			SmartPrep-2						
	RBC, 10^{12}/L	WBC, 10^{9}/L	Platelet, 10^{9}/L	RBC, 10^{12}/L	WBC, 10^{9}/L	Platelet, 10^{9}/L	PC	RBC, 10^{12}/L	WBC, 10^{9}/L	Platelet, 10^{9}/L
Patient 1	5.27	6.58	209	1.19	16.2	847	PC 1	0	<0.001	1376
Patient 2	4.78	6.64	242	0.55	15.2	915	PC 2	0	<0.001	1288
Patient 3	5.55	6.92	243	0.83	11.3	997	PC 3	0	<0.001	1299
Patient 4	5.34	6.48	221	1.29	14.6	830	PC 4	0	<0.001	1334
Patient 5	4.99	6.19	200	0.68	22.5	915	PC 5	0	<0.001	1256
Mean	5.19	6.56	223	0.91	15.96	900.8	Mean	0	<0.001	1310
Factor[b]				0.18×	2.4×	4.0×	Factor[b]	0×	0×	5.9×

[a]PC, platelet concentrate; RBC, red blood cell in mmol/L; WBC, white blood cell.
[b]Factor indicates the amount of concentration/dilution compared with whole blood.

TABLE 2
Growth Factor Concentrations of Different PRP Preparations[a]

Growth Factor Concentration, pg/mL	Whole Blood (n = 5)	SmartPrep-2 (n = 5)	PC (n = 5)	PRP Powder (n = 1)
VEGF	574 ± 147 (364-723)	528 ± 233 (233-775)	1087 ± 535 (528-1854)	1722 (±2.92%[b])
bFGF	198 ± 164 (59-467)	410 ± 259 (183-843)	151 ± 99 (47-288)	542 (±2.29%[b])
PDGF-AB	2394 ± 451 (1850-2984)	17,846 ± 3087 (13,804-20,870)	18,461 ± 4455 (13,056-21,923)	23,023 (±2.74%[b])
TGF-β1	14,356 ± 4527 (10,242-21,627)	77,533 ± 13,918 (53,092-87,846)	68,582 ± 7388 (56,043-74,280)	87,495 (±0.52%[b])
IGF-1	2317 ± 711 (1531-3365)	1539 ± 348 (1173-2039)	2266 ± 485 (1821-2833)	N/D
IL-1α	111 ± 35 (59-149)	119 ± 44 (55-164)	N/D	N/D
IL-1β	42 ± 16 (26-67)	31 ± 24 (0-60)	N/D	N/D
IL-1RA	767 ± 287 (499-1128)	473 ± 233 (295-833)	N/D	N/D

[a]Data are presented as mean ± SD (range). N/D, not detectable; PC, platelet concentrate; PRP, platelet-rich plasma.
[b]Deviation among triplicate measurements.

İrradiasyon & liyofilizasyon

Vet Clin Pathol
43/4 (2014)
Guillaume L. Hoareau[1]

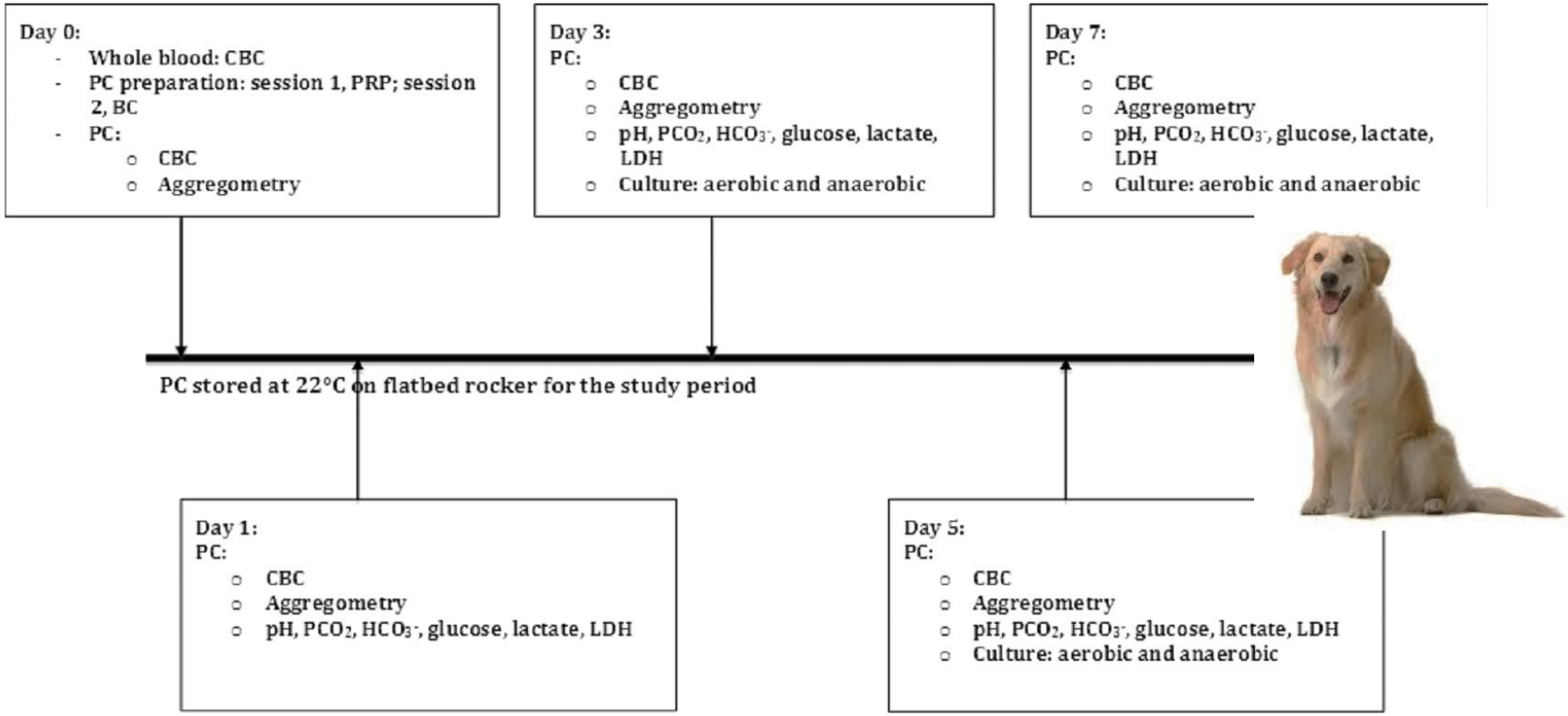


Figure 1. Study design for comparison of platelet-rich plasma (PRP) and buffy coat (BC) protocols for preparation of canine platelet concentrates (PC). PCs were tested up to day 7 after preparation for WBC, RBC, and platelets counts, aggregometry, and variables indicating platelet storage lesion, and for bacterial contamination. pCO_2 indicates partial pressure of CO_2.

Vet Clin Pathol
43/4 (2014)
Guillaume L. Hoareau[1]

Table 1. Platelet (PLT) concentrations and biochemical markers of platelet storage lesion (mean ± standard deviation [SD]) measured in platelet concentrates (PC) prepared by the platelet-rich plasma (PRP) and the buffy coat (BC) protocol, on days 0, 1, 3, 5, and 7 of storage.

	PLT Concentrations		pH		pCO_2* (mmHg)		HCO_3^-*,† (mmol/L)		Lactate*,† (mmol/L)		Glucose*,† (mg/dL)		LDH*,† (unit/mL)	
Day	PRP	BC	PRP	BC	PRP	BC	PRP	BC	PRP	BC	PRP	BC	PRP	BC
0			7.21 ± 0.05	7.17 ± 0.10	39 ± 5	39 ± 8	15 ± 1	13 ± 1	2 ± 0.3	3 ± 0.5	532 ± 29	558 ± 14	42 ± 12	133 ± 76
1	352,250 ± 234,794	323,375 ± 214,043	7.31 ± 0.13	7.05 ± 0.25	28 ± 7	40 ± 16	14 ± 1	10 ± 2	3 ± 1	8 ± 4	526 ± 25	510 ± 46	49 ± 18	221 ± 127
3	334,125 ± 218,241	320,625 ± 237,024	7.36 ± 0.23	7.06 ± 0.36	20 ± 6	21 ± 7	11 ± 3	6 ± 3	6 ± 3	13 ± 6	515 ± 27	461 ± 66	60 ± 18	271 ± 84
5	350,500 ± 223,740	298,250 ± 189,672	7.30 ± 0.3	6.83 ± 0.53	16 ± 4	10 ± 3	8 ± 4	3 ± 4 (n = 7)‡	9 ± 5	18 ± 8	498 ± 41	383 ± 119	92 ± 41	528 ± 497
7	333,000 ± 223,669	322,875 ± 201,878	7.17 ± 0.41	6.62 ± 0.47	12 ± 3	10 ± 4	6 ± 4	4 ± 5 (n = 3)‡	12 ± 6	24 ± 12	466 ± 40	345 ± 140	131 ± 49	1027 ± 812

*Statistically significant change ($P < .001$) over storage time while adjusting for process and dog.
†Statistically significant difference ($P < .05$) between PRP-PC and BC-PC.
‡pH and pCO_2 were out of range; therefore, no HCO_3^- values available for some samples.

PRP-PC > BC-PC

trombositlerin uyarılabilirliği ve saklama lezyonları

SEBEP: BC-PC'deki WBC ve RBC kontaminasyonu
ÖNERİ: Lökosit filtresi ile çalışma
Trombosit fonksiyonları konusunda çalışma
İNVİVO: Trombosit yarı ömrü ve transfüze edilmiş trombosit fonksiyonları konusunda çalışma

TABLE 3
Cellular Data[a]

Kit	Cell Type	Mean, $\times 10^9$/L	SD, $\times 10^9$/L	Median, $\times 10^9$/L	Min, $\times 10^9$/L	Max, $\times 10^9$/L
Control	Platelets	269	106	290	154	362
	WBC	8.73	3.75	8.9	4.9	12.4
	RBC	4.7	0.436			5.2
ACP	Platelets	412	140			546
	WBC	1.3	0.781			1.8
	RBC	0.0333	0.0577			0.1
GPS	Platelets	964	551			1588
	WBC	35.8	10.8			42.3
	RBC	1.03	0.289			1.2
SmartPrep	Platelets	1224	560			1764
	WBC	24.7	8.69			32.6
	RBC	1.43	0.306			1.7
Magellan	Platelets	1266	831			2148
	WBC	31.4	9.4			38.3
	RBC	1.03	0.153			1.2

[a]RBC, red blood cell count; WBC, total white blood cell count.

TABLE 5
pH and Glucose Data

Kit	Mean ± SD, mmol/L	Median (Range), mmol/L
Control		
pH	7.1 ± 0.28	7.12 (7.07-7.12)
Glucose	4.2 ± 0.529	4.4 (3.6-4.6)
ACP		
pH	6.87 ± 0.256	6.99 (6.57-7.04)
Glucose	18.5 ± 2.73	17.7 (16.2-21.5)
GPS		
pH	7.05 ± 0.02	7.05 (7.03-7.07)
Glucose	15.8 ± 1.07	15.2 (15.1-17.0)
Smart Prep		
pH	6.59 ± 0.329	6.61 (6.55-6.61)
Glucose	23.6 ± 1.36	23.8 (22.2-24.9)
Magellan		
pH	6.66 ± 0.102	6.69 (6.54-6.74)
Glucose	22.3 ± 0.907	22.7 (21.3-23.0)

Glukoz konsantrasyonu 4-6 kat artmış
Proloterapide (dekstroz/mannitol) Kullanımı düşünülmüş
Not: Proloterapide dekstroz %12-20'lik konsantrasyonunda kullanılmaktadır

Sıvı veya Jel PRP

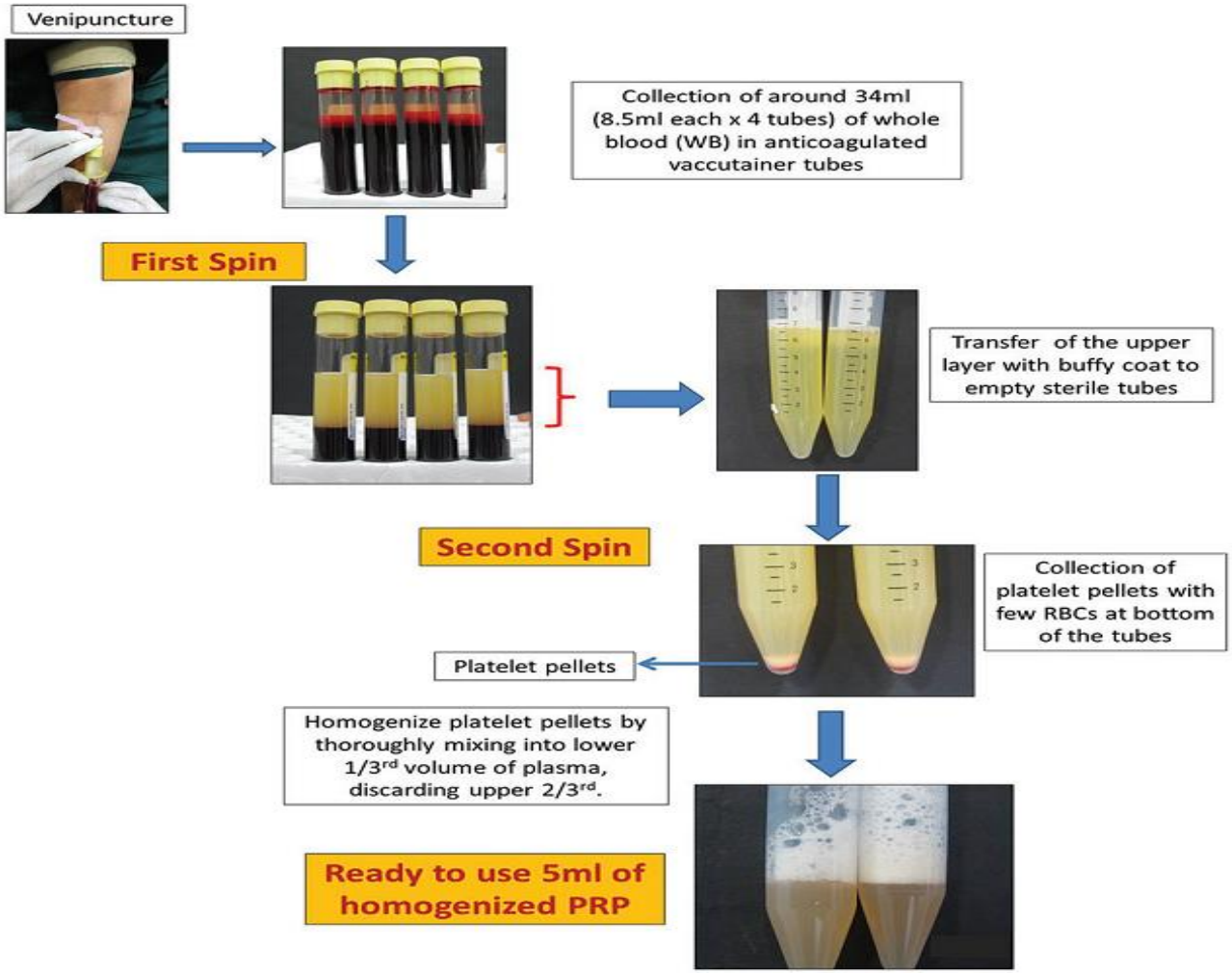


Figure 1: Flowchart describing preparation of PRP

Bireysel Farklılıklar

- Biyolojik farklılıklar

  Yaş ve cinsiyet
- Trombosit boyutu
- Hematokrit düzeyi (dilüsyon faktörü)

- Uygulama dozu (2-6)
- Uygulama aralığı (2-4 hafta)

Journal of Cutaneous and Aesthetic Surgery - Oct-Dec 2014, Volume 7, Issue 4

Kan Alma

- Süre (uzun ise trombosit aktivasyonuna ve trombosit sayısında azalmaya neden olur)
- Karıştırma (uygun olmazsa fibrin ağı gelişir ve trombosit sayısı azmış gibi görünür)

- İğne kalınlığı > 22G

Journal of Cutaneous and Aesthetic Surgery - Oct-Dec 2014, Volume 7, Issue 4

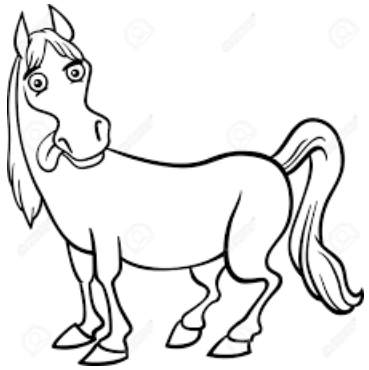
Antikoagülan

- Trombosit fonksiyonları, integritesi ve morfolojisinde önemlidir
- Agregasyon, pH ve ionize Ca üzerine etkilidir
- EDTA (trombosit membranına zarar verir) önerilmez
- Sitrat (trisodyum sitrat) veya dextrozlu sodyum sitrat (ACD-A) en sık kullanılanıdır
- CPDA-1 de az oranda kullanılmaktadır, trombosit viabilitesinde %10 azalma görülmüştür

Journal of Cutaneous and Aesthetic Surgery - Oct-Dec 2014, Volume 7, Issue 4

Effects of sodium citrate and acid citrate dextrose solutions on cell counts and growth factor release from equine pure-platelet rich plasma and pure-platelet rich gel

Carlos E Giraldo*, María E Álvarez and Jorge U Carmona



Giraldo *et al. BMC Veterinary Research* (2015) 11:60
DOI 10.1186/s12917-015-0370-4

SC: Sodyum sitrat + Sitrik asit
ACD-A: Trisodyum sitrat + Sitrik asit + Dekstroz
ACD-B: Trisodyum sitrat + Sitrik asit + Dekstroz

ACD-B, hücre sayısı ve GF içeriği en kötü olan olarak çıkmış; **istatistiksel olarak anlamlı değil**

Table 2 Means (± s.d) of the TGF-$\beta_1$and PDGF-BB concentrations (pg/mg of total protein (TP)) in every blood component obtained with every anticoagulant

Variable	Blood component				
	Plasma	P-PRP lysate	P-PRG	PPP lysate	PPG
SC					
PDGF-BB (pg/mg of TP)	0.9 (0.6)[a,b]	25.2 (14.4)[c]	19.0 (29.4)[d]	8.3 (5.4)[d]	4.5 (5.5)
TGF-β1(pg/mg of TP)*	26.8 (10.4)[a,e]	90.7 (30.7)[c,e]	54.5 (33.1)	45.2 (10.3)[d]	29.2 (17.1)
ACD-A					
PDGF-BB (pg/mg of PT)	1.0 (0.6)[a,b]	28.2 (20.1)[c]	11.3 (30.6)	8.2 (5.0)	5.8 (8.0)
TGF-β1(pg/mg of TP)*	30.0 (8.7)[a]	101.5 (31.2)[c,e]	56.4 (39.1)	47.8 (12.2)	33.3 (17.9)
ACD-B					
PDGF-BB (pg/mg of TP)	1.1 (0.9)[a]	18.4 (13.4)[b]	6.6 (17.3)	7.2 (4.1)	4.8 (6.5)
TGF-β1(pg/mg of TP)*	27.5 (10.0)[c]	87.8 (23.0)[d]	50.8 (31.2)	44.6 (12.9)	31.3 (12.7)

*Data are presented as medians (IR). P-PRG: pure platelet-rich gel; PPG: platelet-poor gel. Lowercase letters represent independent significant differences for every blood component obtained with a specific anticoagulant. SC: blood component different with a: P-PRP and PPP lysates ($P < 0.001$); b: P-PRG and PPG ($P < 0.05$); c: PPP lysate and PPG ($P < 0.001$); d: PPG ($P < 0.05$); and e: P-PRG ($P < 0.05$). ACD-A: blood component different with a: P-PRP and PPP lysates ($P < 0.001$); b: P-PRG and PPG ($P < 0.05$); c: PPP lysate and PPG ($P < 0.001$); d: PPG; and e: P-PRG ($P < 0.05$). ACD-B: blood component different a: all blood components ($P < 0.05$); b: PPP lysate and PPP ($P < 0.05$); c: P-PRP lysate ($P < 0.05$); d: PPP lysate and PPG ($P < 0.05$).

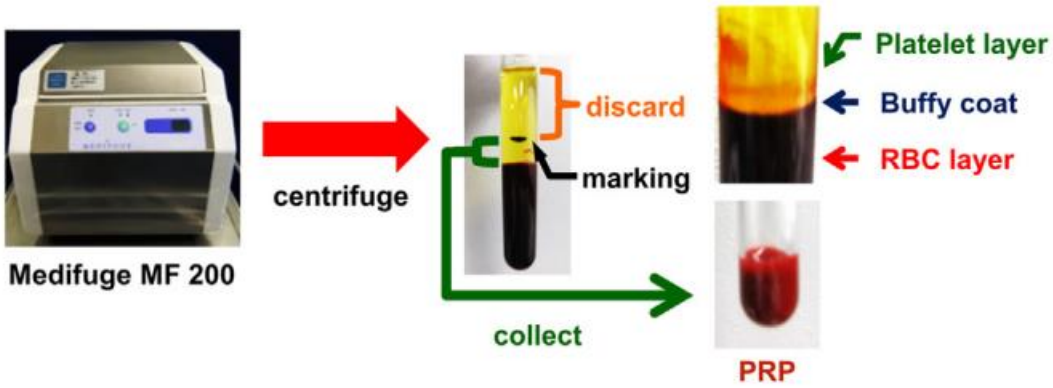
Santrifügasyon

- Hacim
- Devir
- Akselerasyon/deselerasyon
- Süre (uzarsa: PLT artar, WBC azalır)
- Isı (21-24°C-AABB, 12-16°C'de çalışmalar var ama üretim değil tanı amaçlı testler için)
- Sayı (iki kez yapılacaksa 2 devir arasında trombositler hızla ayrılmalıdır, aksi halde PPP tarafına trombositler difüze olur)
- Separasyon süresi (trombositler dipteki rbc yüzeyine adsorbe olabilir, %20)

Journal of Cutaneous and Aesthetic Surgery - Oct-Dec 2014, Volume 7, Issue 4

Dondurarak Saklama

Archives of Oral Biology
73 (2017) 172–178
Yuya Nakatani,



2.3. Lyophilization of PRP

PRP or serum was pre-frozen at −80 °C for 12 h, and was then lyophilized for 12 h using a freeze dryer (EYELA FD-1000; Tokyo Rikakikai, Tokyo, Japan). After freeze-drying, samples were stored at −20 °C for one month in order to prevent contamination and transformation. We produced different concentrations (equal and 3-folds; ×1FD-PRP, × 3FD-PRP) by resolving FD-PRP to equal or 1/3 amount of distilled water at the evaluation of FD-PRP.

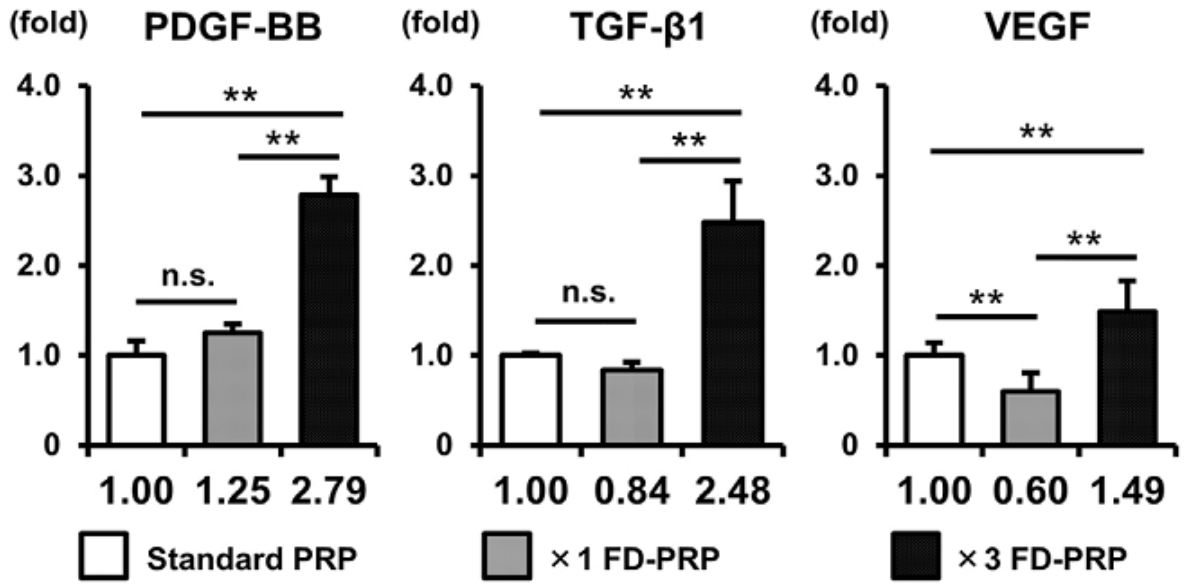


Fig. 3. Comparison of GFs between storage methods.
Concentrations of GFs (PDGF-BB, TGF-β1, and VEGF) were assessed, and the ratio of GF concentration to that in s-PRP among the PRP groups was calculated (×1FD-PRP and ×3FD-PRP). Statistical analysis was performed among s-PRP, ×1FD-PRP, ×3FD-PRP. Data are presented as means ± standard deviation (n = 3). $^{**}p < 0.01$, n.s.: Not significant.

Avantajları: Sınırlı sayıda personel ile kullanılabilirlik,
tek seferde kan alma;
konsantre edilerek volüm azaltılabilir

Dezavantajları: Cihaz gerektirir
İşlem sırasında ürün kontamine olabilir
Oksidasyon sebebiyle bozulma olabilir

Hindawi Publishing Corporation
Journal of Blood Transfusion
Volume 2015, Article ID 706903, 5 pages
http://dx.doi.org/10.1155/2015/706903

Nada Hosny *Egypt*

Table 2: Platelet yield of fresh and one-week and three-week frozen/thawed PRP.

	Platelet count $\times 10^3/\mu$L (mean ± SD)	Platelet yield (mean ± SD)	p value
Whole blood	282 ± 56		
Fresh PRP	991.4 ± 378	3.5 ± 0.9	<0.001*
Frozen PRP (1 week)	453.2 ± 158	1.7 ± 0.6	<0.001*
Frozen PRP (3 weeks)	441.6 ± 154	1.6 ± 0.6	0.001*

* Statistically significant difference ($p < 0.05$).

Table 3: VEGF concentration (pg/mL) in calcium versus calcium and thrombin activated fresh and frozen/thawed PRP samples.

	Ca activated PRP VEGF concentration	Ca and thrombin activated PRP VEGF concentration	p value
Fresh	545.2 ± 349	608.7 ± 494	0.695 (NS)
Frozen/thawed (1 week)	339.5 ± 190	365.2 ± 189	0.743 (NS)
Frozen/thawed (3 weeks)	339.9 ± 189	365.6 ± 188	0.741 (NS)

NS: not significant.

SONUÇ:

Dondurma-çözme VEGF'yi etkilemez

Sık uygulama

PLT sayısı X VEGF konsantrasyonu

VEGF aktivitesini göstermek için PLT sayısı uygun bir indikatör değildir

Hem PLT hem GF ve aktiviteleri birlikte değerlendirilmelidir

Aktive edilmeli mi?

Journal of Cutaneous and Aesthetic Surgery - Oct-Dec 2014, Volume 7, Issue 4

- Kalsiyum klorid/glukonat
- Kalsiyum klorid/glukonat + Trombin
- Mekanik travma (Santrifüj/dondurma-çözme)

- Yumuşak dokuda kullanımında exogen aktivasyon gerekmez

Table 1. Comparison between PAS, autologous serum and PRP

Autologous serum
•Autologous serum conditioned
•Anti-inflammatory
•Inhibition of tissue degeneration
•Competitive blockade of IL-1 receptors
PRP
•Plasma with significantly elevated thrombocyte concentration from venous blood
•Activation of tissue regeneration by growth factors
•Growth factors are continuously released after the activation of thrombocytes
PAS
•Platelet is activated by platelet activation beads
•Including higher concentrated growth factors than autologous serum and PRP
•CELLAID® can be preserved at 4 °C or −80 °C to −20 °C
•Platelet activation beads contained in CELLAID® complete all serum preparation steps within 1 h

PAS contained higher concentrations of cell growth factors (VEGF, TGF-β and PDGF-BB; released by shaking with platelet-activating beads) compared with autologous serum and PRP. PAS could be separated from the blood components using a closed and sterile procedure with CELLAID® kits, and could be cryopreserved at –80 °C for over 1 year without any loss in growth factor concentrations.
IL, interleukin; PAS, platelet-activated serum; PRP, platelet-rich plasma.

Yüksek konsantrasyonda GF separasyonu yapan bir kit

Separasyonda ölçüm yok; göz kararı ve buffy coat dahil

JOURNAL OF TISSUE ENGINEERING AND REGENERATIVE MEDICINE
J Tissue Eng Regen Med 2017.
Published online in Wiley Online Library (wileyonlinelibrary.com) **DOI:** 10.1002/term.2238

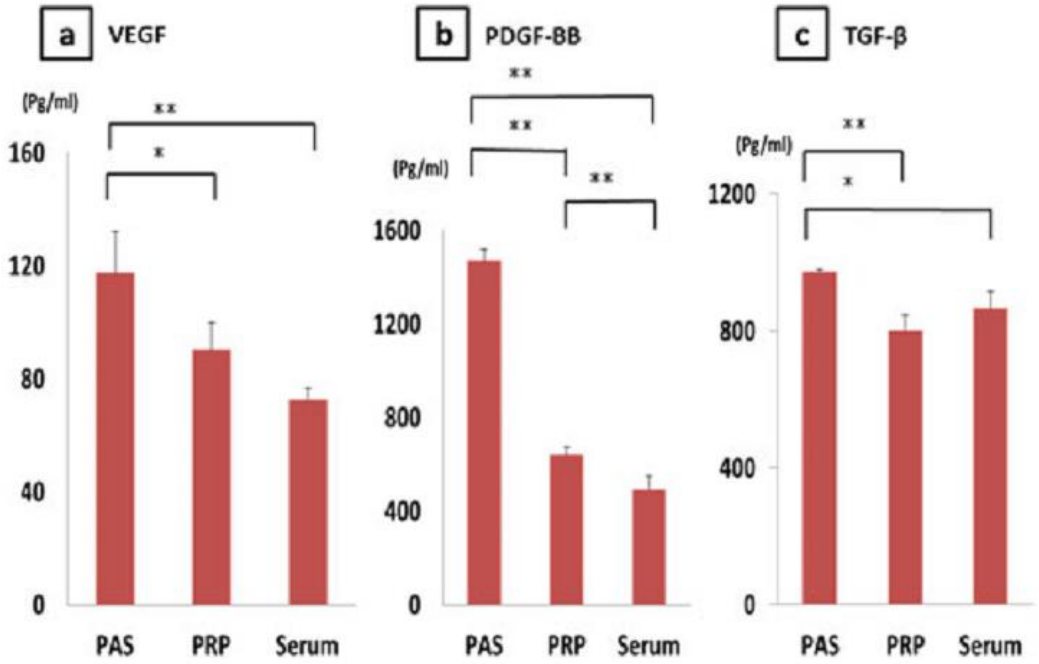
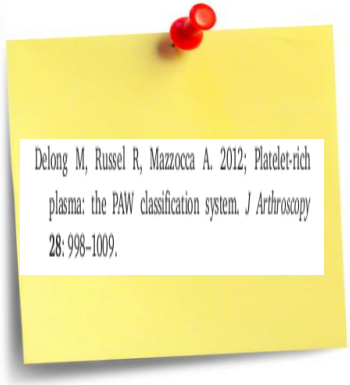


Figure 2. Quantitative analysis of growth factors. Platelet-activated serum (PAS), platelet-rich plasma (PRP) and autologous serum samples were collected from two Japanese White rabbits aged 12 weeks (weight ~2.5 kg). They were analysed for vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF)-BB and transforming growth factor-beta (TGF-β) expression levels using ELISA kits. (a) Significantly higher quantities of VEGF were observed in PAS and PRP than in the autologous serum samples. (b) PDGF-BB expression was significantly higher in PAS than in PRP and in the autologous serum samples, and the PDGF-BB level in PRP was significantly higher than that in autologous serum. (c) A higher TGF-β concentration was observed in PAS than in PRP and autologous serum samples. The vertical axis is in pg/ml; $^{*}P < 0.05$ and $^{**}P < 0.01$ [Colour figure can be viewed at wileyonlinelibrary.com]

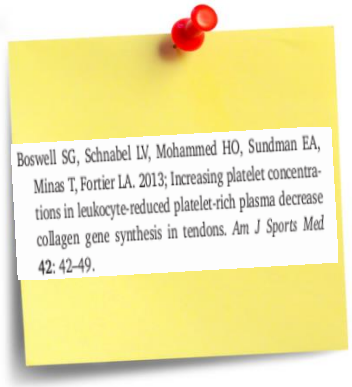
Published online in Wiley Online Library (wileyonlinelibrary.com) **DOI:** 10.1002/term.2238

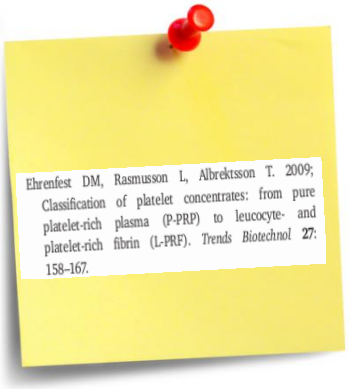
PAW Klasifikasyonu:

1. PLT sayısı
2. PLT aktivasyonu
3. Lökosit sayısı

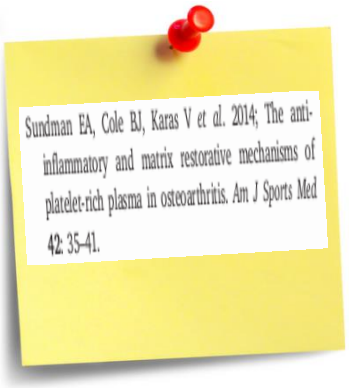

Delong M, Russel R, Mazzocca A. 2012; Platelet-rich plasma: the PAW classification system. *J Arthroscopy* 28: 998–1009.

PRP'deki PLT sayısını artırırken WBC sayısının azaltılması anabolik GF'leri arttırırken proinflamatuar sitokinleri azaltacağı

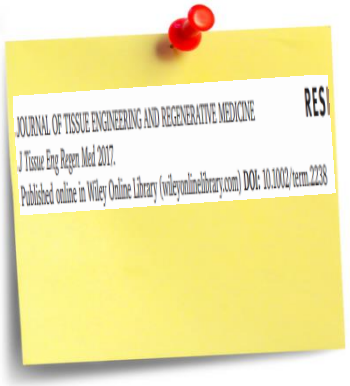

Boswell SG, Schnabel LV, Mohammed HO, Sundman EA, Minas T, Fortier LA. 2013; Increasing platelet concentrations in leukocyte-reduced platelet-rich plasma decrease collagen gene synthesis in tendons. *Am J Sports Med* 42: 42–49.


Ehrenfest DM, Rasmusson L, Albrektsson T. 2009; Classification of platelet concentrates: from pure platelet-rich plasma (P-PRP) to leucocyte- and platelet-rich fibrin (L-PRF). *Trends Biotechnol* 27: 158–167.

Üründeki lökositin etkisi?


Sundman EA, Cole BJ, Karas V *et al.* 2014; The anti-inflammatory and matrix restorative mechanisms of platelet-rich plasma in osteoarthritis. *Am J Sports Med* 42: 35–41.

Konsantre lökositin hasar bölgesine enjeksiyounun doku onarımı ve yara iyileşmesi için uygun olmayabileceği


JOURNAL OF TISSUE ENGINEERING AND REGENERATIVE MEDICINE
J Tissue Eng Regen Med 2017.
Published online in Wiley Online Library (wileyonlinelibrary.com) DOI: 10.1002/term.2238

Üründeki lökositi azaltmak trombositi artırmaktan daha önemlidir

Çalışmalardaki Yetersizlikler

- Örnek sayıları
- İnvivo çalışma
- Sitokin ve GF etkileri
- Mikroçevre
- Kit içerikleri - Klinik sonuçlar
- Farklı ürünlerle uzun dönem karşılaştırma
- Aynı vasıftaki ürünün farklı alanlarda kullanımı
- Akut/kronik olgu
- Etki süresi

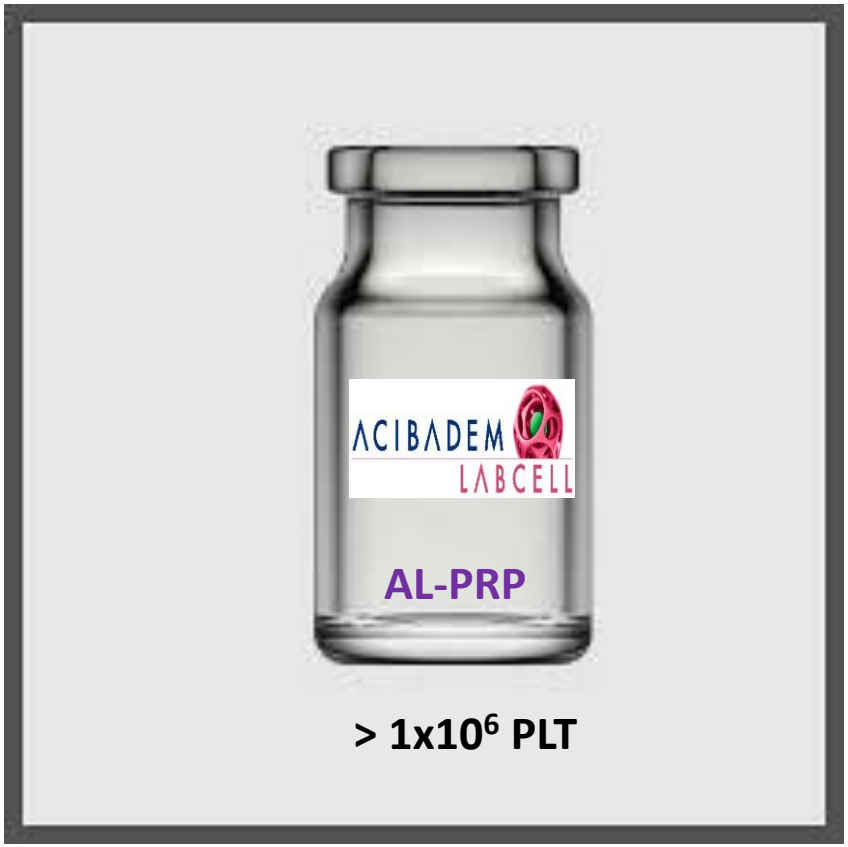
Future prospects and challenges The field of autologous platelet-based therapies is expanding rapidly, however several challenges persist. For example, additional studies involving full proteomic characterization of platelet releasates and concise transcriptional gene response after their application are needed to fully understand the biomolecular mechanism behind the regenerative effect of these therapies. Moreover, due to a number of commercially available kits and homemade protocols, **a more standardized method for preparation is mandatory** to properly correlate clinical results with the composition of the platelet-based therapy. The optimal therapeutic dosage of these biologic therapies is also a current field

International Journal of Dermatology 2017, **56**, 247–251

saklama koşulları

fonksiyonel analiz

ışınlanmış



fizyolojik pH

liyofilize

lökositi azaltılmış

GF/Sitokin içeriği

> $1x10^6$ PLT

teşekkür ederim