Comparison of the Platelet Concentrate Collection System with the Plasma-Rich-in-Growth-Factors Kit to Produce Platelet-Rich Plasma: A Technical Report

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Purpose: *The aim of this study was to compare a new method for the production of platelet-rich plasma (PRP), the plasma-rich-in-growth-factors kit (PRGF kit; G.A.C. Medicale San Antonio, Vitoria, Spain), with an established method, the Platelet Concentrate Collection System (PCCS; 3i/Implant Innovations, Palm Beach Gardens, FL) with respect to resulting cellular and growth factor contents.* ***Materials and Methods:*** *Whole blood was drawn from 51 healthy donors (20 men, 31 women) aged 19 to 59 years (mean ± SD 35.12 ± 9.65 years), and PRP was prepared by both methods.* ***Results:*** *Platelet counts differed significantly (signed rank test, P < .001 for all) between the donor blood (274,200 ± 54,050/μL), the PCCS PRP preparation (1,641,800 ± 426,820/μL), and the PRGF kit PRP preparation (513,630 ± 139,470/μL). The PCCS concentrated leukocytes (whole blood, 6,992 ± 2,011/μL; PCCS PRP, 14,153 ± 7,577/μL), while the PRGF kit produced a leukocyte-poor PRP (65 ± 108/μL). Higher concentrations of transforming growth factor β1 (TGF-β1) and platelet-derived growth factor AB (PDGF-AB) were found in the PCCS PRP (TGF-β1, 290 ± 95 ng/mL; PDGF-AB, 157 ± 62 ng/mL) than in the Anitua PRGF kit PRP (TGF-β1, 73 ± 26 ng/mL; PDGF-AB, 47 ± 21 ng/mL). Statistical analysis showed significant differences (P < .001 for TGF-β1 and P < .01 for PDGF-AB).* ***Discussion:*** *The results of this study and some data in the literature indicate that the content of growth factors in PRP can vary tremendously, depending on the system used for the preparation of PRP.* ***Conclusion:*** *PCCS collects more platelets and leukocytes than the PRGF kit. This results in significantly higher growth factor levels. Further in vivo studies are needed to determine whether this results in a clinically different biologic effect.* Int J Oral Maxillofac Implants 2005;20:118–123

Key words: *platelet concentrate, platelet-rich plasma*

Platelets are a source of many growth factors, such as platelet-derived growth factor (PDGF), transforming growth factor-β1 (TGF-β1), transforming growth factor-β2 (TGF-β2), insulin-like growth factor (IGF), epidermal growth factor, epithelial cell growth factor, and a growth factor for hepatocytes.[1] These growth factors are recommended for accelerating and improving bone regeneration. Marx and colleagues demonstrated increased bone formation and bone density in 44 patients 6 months after autologous bone grafting using platelet concentrates (platelet-rich plasma, PRP) as a source of autologous growth factors.[2] The latest animal studies have demonstrated different results without respect to the PRP production method.[3–6]

Several methods are currently available for point-of-care production of PRP by the surgeon. Some different production methods result in different compositions of the resulting PRP.[7] A new method proposed by Anitua,[8] the Platelet-Rich-in-Growth-Factors kit (PRGF kit; G.A.C. Medicale San Antonio, Vitoria, Spain), has been available since August 2001, but there are little data available on the resulting PRP. Therefore, this study compared the suitability of

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this new method with that of an established method of producing PRP, the Platelet Concentrate Collection System (PCCS; 3i/Implant Innovations, Palm Beach Gardens, FL) with respect to the resulting platelet concentration, collection efficiency, and the growth factor content of the PRP.

MATERIALS AND METHODS

Between July 24 and August 9, 2001, blood samples were collected from 51 healthy donors (20 men, 31 women) aged 19 to 59 years (35 ± 10 years). All donors included in this study had platelet counts > 130,000/μL.

In this study, the PCCS and the Anitua PRGF kit were compared with respect to the cellular and growth factor levels in the end product (the PRP).

Volunteers gave written informed consent, as required by the Ethics Board. Before venipuncture, 6 mL of adenosin-citrate-dextrose-acid (ACD-A) solution was drawn into a 60-mL syringe. Venipuncture was then performed using an 18-gauge apheresis needle from the PCCS. Blood was drawn into several different containers in the following order: *(1)* the 60-mL syringe containing 6 mL ACD-A solution (filled with 54 mL of whole blood), *(2)* three 5.0-mL sodium citrate Venoject glass vacuum tubes (Terumo Europe, Leuven, Belgium), and *(3)* a 2.7-mL EDTA monovette (Sarstedt, Nurnbrecht, Germany). The blood-filled syringe and ACD-A vacuum tubes were inverted 5 or 6 times to ensure that the anticoagulant was evenly dispersed. PRP was then prepared from the whole blood in the 60-mL syringe as recommended by the manufacturer of the PCCS kit (see the following section for the method used). The blood in the 5.0-mL glass tubes was used to prepare plasma rich in growth factors (PRGF) by the method of Anitua (see below for method). The platelet counts of the whole blood and the platelet preparations were immediately determined (Cell Dyn 3500; Abbott, Wiesbaden, Germany).

Preparation of PRP Using the PCCS

The PCCS kit contained the following materials: a modified IEC Centra CL-2 centrifuge (IEC Model 7427, International Equipment Company, Needham Heights, MA) with a 4-place swinging bucket rotor for specially designed inserts, a 6-mL ACD-A ampoule, and a PCCS set. The latter is delivered in a sterile box that contains the following: *(1)* a plastic device consisting of 2 flexible plastic bags that are bonded to the underside of a clear plastic cap; *(2)* a 20-gauge needle for adding ACD-A to the collected blood; *(3)* two 60-mL syringes, 1 for collecting whole blood and 1 for transferring the supernatant between the bags; *(4)* an 18-gauge apheresis needle set for collecting whole blood; and *(5)* a 10-mL syringe for collecting the platelet concentrate.

To produce PRP using the PCCS, 60 mL of anticoagulated whole blood were transferred into the polyvinylchloride collection bag via valve no. 1 after closing the clamp on the transfer line. The loaded container was weighed, and the second balancing container was filled with an equivalent amount of water. The blood was then centrifuged for 3.75 minutes at 3,000 rpm in the IEC Centra CL-2 centrifuge. To transfer the platelet-containing plasma into the opposite section of the collection bag, the clamp was opened and air was blown through valve no. 2 until 1 mm^3 of red blood cells followed the plasma through the transfer line. This ensured that the precursor platelet cells were also captured. To form a single platelet pellet, a second centrifugation step was performed for 13 minutes at 3,000 rpm. By pumping 35 mm^3 of air into valve no. 3 after reopening the clamp, platelet-poor plasma refilled section 1 of the collection bag, and only about 5 to 8 mL of platelet-poor plasma remained in section 2 with the platelet pellet. The platelets were resuspended in the residual plasma by carefully massaging the cell mass between the thumb and forefinger for approximately 3 minutes. Finally, the entire contents of section 2 of the bag were transferred to a 10-mL syringe via valve no. 4. After 15 minutes, the PRP was added to Eppendorf tubes for later platelet count analysis.

Preparation of PRP using the Anitua PRGF Kit

The PRGF kit is delivered with a laboratory centrifuge that is specially modified for use with the Anitua system. The PRGF kit consisted of the following components from Terumo Europe: a "winged blood sampling set" (catalog no. MIN-SV21), 5.0-mL citrated vacuum glass tubes that contained 0.5 mL sodium citrate and accommodated 4.5 mL blood (Venoject tubes), and 5.0-mL neutral vacuum glass tubes. The following products from Brand (Wertheim, Germany) are also included in the kit: one 100-μL plunger-operated pipetter, one 500-μL plunger-operated pipetter, 5- to 100-μL filter tips, 50- to 800-μL filter tips, and a Plasticbrand microcentrifuge tube rack.

To produce PRGF-PRP, 3 glass vacuum tubes were each filled with 5.0 mL of citrated blood and centrifuged in the modified laboratory centrifuge for 8 minutes at 1,800 rpm. This resulted in the production of 3 separate layers (from top to bottom): *(1)* plasma, which contains most of the platelets and is defined as PRP by the American Association of Blood Banks[9]; *(2)* the buffy coat layer, which is predominantly composed of leukocytes and about one quarter of the

platelets; and *(3)* the erythrocyte layer, which also contains very young platelets at the top. The rubber closure was then removed from the sterile glass vacuum tube that contained the citrated and centrifuged blood. From the yellow plasma, the top 1 mL was then drawn by careful and accurate pipetting to avoid creating turbulence The remaining plasma is called PRGF. As recommended, the collection of this most important fraction was performed with a 100-µL pipette, and care was taken to avoid creating any turbulence and to avoid the aspiration of red blood cells. For this reason, the buffy coat layer and a small part of the plasma were left in the vacuum tube. Between 5 and 20 pipetting steps were necessary to transfer the PRGF from each glass vacuum tube, depending on the total plasma volume and the individual hematocrit of the donor.

Measurement of Growth Factor Levels

The PRP samples were stored in Eppendorf tubes at –78°C. The samples were thawed and centrifuged for 10 minutes at 10,000 rpm in a microcentrifuge immediately before the growth factor content was analyzed. Commercially available enzyme-linked immunosorbent assay kits that have been validated for measuring the respective growth factors (Quantikine ELISA kit; R&D Diagnostics, Wiesbaden, Germany) were used according to the manufacturer's instructions to quantify the concentrations of TGF-β1, PDGF-AB, and IGF-I, as previously described.[10] All growth factor measurements were performed in duplicate, and no unexpected scattering of the data (all scattering < 10%) was observed. The lower detection limits of these assays reported by the manufacturer are 7 pg/mL for TGF-β, 8.4 pg/mL for PDGF-AB, and 0.026 ng/mL for IGF-I. Since a large proportion of the TGF-β1 is often present in biologic samples in a latent form,[11] TGF-β1 was converted to its active form as directed by the manufacturer in order to estimate the total TGF-β1 content.

Statistical Methods

The collection efficiency was computed in the following manner:

$$\text{Efficiency} = \frac{\text{Platelet count in PRP} \times \text{volume}_{\text{PRP}}}{\text{Platelet count in whole blood} \times \text{volume}_{\text{whole blood}}}$$

All quantitative measurements have been described using summary statistics (n, mean, standard deviation, median, minimum, maximum, and other quantiles). The 3 platelet counts (donor whole blood, PCCS PRP, and Anitua PRGF) were compared using the signed rank test for nonparametric paired data. Scatter plots and the Spearman correlation coefficient (r_s) were used to demonstrate the relationship between the whole blood and PRP platelet counts and growth factor levels.

To account for multiplicity, the *P* values for the respective signed rank tests of the different platelet count measurements were compared with the Bonferroni-adjusted significance level of .05/3 = .0167. All other *P* values must be considered tentative.

RESULTS

The volumes of whole blood collected for the PCCS and Anitua PRP kits (excluding the anticoagulant) were 54 and 13.5 mL (4.5 mL per glass vacuum tube), respectively. The whole blood samples had a mean hematocrit of 41% ± 4% (mean ± SD). The preparation of PRP required about 30 minutes using the PCCS kit and about 25 minutes using the Anitua method. The resulting volume of PRP was 7 ± 1 mL from the PCCS and 3.7 ± 1 mL from the Anitua PRGF kit (1.2 ± 0.3 mL for each glass vacuum tube). The whole blood platelet count averaged 274,200 ± 54,050/µL (Fig 1; see Table 1 for descriptive statistics). Platelet counts differed significantly (by the signed rank test) between the donor blood, the PCCS PRP (1,641,800 ± 426,820/µL), and the Anitua PRGF (513,630 ± 139,470/µL) (whole blood vs PCCS PRP, *P* = .001; whole blood vs Anitua PRGF, *P* = .001; PCCS PRP vs Anitua PRGF, *P* = .001).

Correlations between the platelet count in the donor whole blood and the platelet concentration in the platelet preparations were similar for the PCCS (r_s = 0.60) and for the Anitua PRGF kit (r_s = 0.63).

The collection efficiency of the PCCS kit (76.6% ± 11.9%) was higher than that of the Anitua PRGF kit (48.4% ± 12%) (Table 1).

Platelet preparation produced by the PCCS concentrated the leukocytes that were present in whole blood (leukocytes in whole blood, 6,992 ± 2,011/µL; leukocytes in PCCS PRP, 14,153 ± 7,577/µL). However, the Anitua kit produced a leukocyte-depleted product (65 ± 108/µL) (Fig 2).

All 3 of the growth factors that were analyzed were found in high levels in both kinds of PRP preparation, but the relative amounts of the individual growth factors differed in each preparation. TGF-β1 and PDGF-AB were the dominant growth factors in the PCCS PRP (TGF-β1, 289.5 ± 94.6 ng/mL; PDGF-AB, 156.7 ± 61.9 ng/mL). The Anitua PRGF contained only 73.3 ± 25.6 ng/mL TGF-β1 and 47.0 ± 21.5 ng/mL PDGF-AB (Fig 3). The differences in the growth factor contents between PCCS and Anitua PRP preparations were statistically significant for TGF-β1 (signed rank test, *P* < .001) and PDGF-AB

Table 1 Descriptive Statistical Parameters of Donor Age, Collection Efficiency, and Volume of Platelet Preparation

	Mean ± SD	Minimum	Maximum
Age (y)	36.7 ± 13.2	19.0	59.0
PCCS			
Volume (mL)	7.0 ± 0.9	4.6	8.7
Collection efficiency (%)	76.6 ± 11.9	33.4	100.0
PRGF			
Volume (mL)	3.7 ± 0.97	1.5	5.2
Collection efficiency (%)	48.4 ± 12.0	19.7	71.2

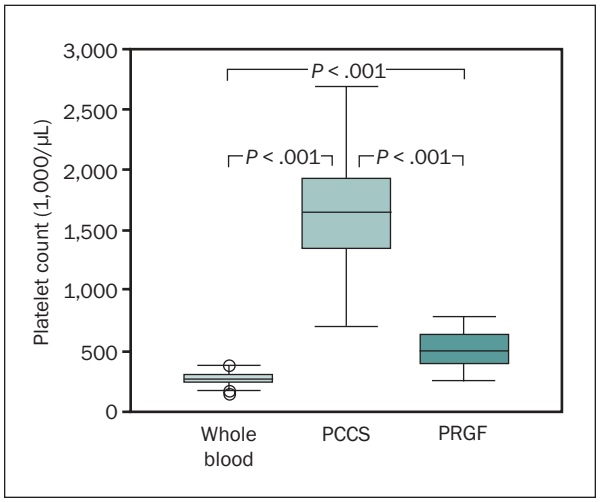

Fig 1 Platelet counts in whole blood, PCCS PRP, and the Anitua PRP preparation. The bars of the box plots indicate the 10th, 25th, 50th, 75th, and 90th percentiles. Circles represent statistical outliers.

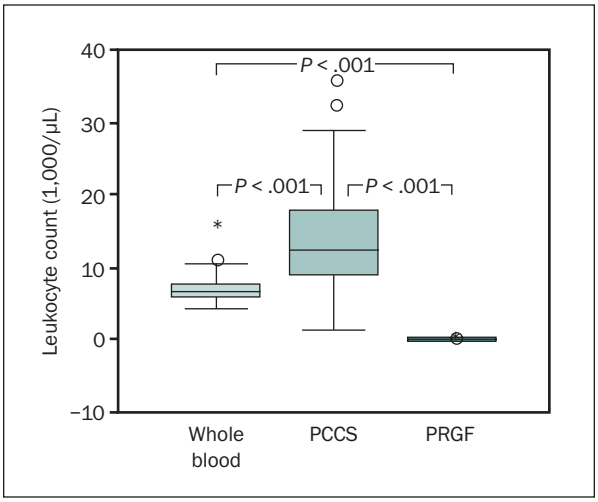

Fig 2 Leukocyte counts in whole blood, PCCS PRP, and Anitua PRGF. The bars of the box plots indicate the 10th, 25th, 50th, 75th, and 90th percentiles. Circles represent statistical outliers; asterisks indicate extreme values.

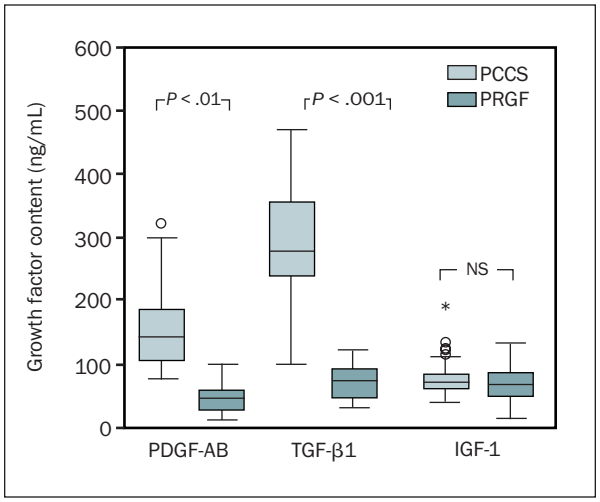

Fig 3 Growth factor content in PCCS PRP and Anitua PRGF. The bars of the box plots indicate the 10th, 25th, 50th, 75th, and 90th percentiles. Circles represent statistical outliers; asterisks indicate extreme values.

(signed rank test, $P < 0.01$). IGF-I levels in the 2 kinds of PRP were similar (PCCS PRP, 78 ± 29.9 ng/mL; Anitua PRGF, 78 ± 28.1 ng/mL).

Scatter plots of the PRP platelet counts in the respective growth factor contents showed a definite linear relationship for TGF-β1 and PDGF-AB only, but not for IGF-I (see Table 2 for r_s values).

DISCUSSION

In this study, major differences between the PCCS system and the PRGF kit in their preparation methods and results were found. The PCCS end product has a higher platelet count, which is correlated to higher growth factor levels. The platelet collection efficiency is also higher, so the surgeon can use more of the platelets that are in the whole blood drawn. In contrast to the PRGF kit, the PCCS system is a semi-closed system; whether this results in a reduced risk of bacterial contamination was not investigated in this study.

It is known for some production methods that the growth factor levels in PRP preparations can vary tremendously depending on the resulting platelet and leukocyte concentrations. A comparison[12] of

Table 2 Spearman's Rank Correlation Coefficients Between Platelet Counts, Leukocyte Counts, and Growth Factor Levels

	PCCS-PRP			PRGF		
	PDGF-AB	TGF-β1	IGF-I	PDGF-AB	TGF-β1	IGF-I
Platelet count	0.640	0.770	−0.800	0.827	0.829	0.059
Leukocyte count	0.333	0.346	−0.036	0.026	0.136	−0.264

blood bank PRP with Curasan PRP showed that the higher leukocyte count in leukocyte-rich PRP (blood bank PRP 160 ± 320 leukocytes/μL; Curasan PRP 30,130 ± 12,500 leukocytes/μL, signed rank test $P < .001$) resulted in higher levels of PDGF-AB (PDGF-AB blood bank PRP 133.59 ± 46.26 ng/mL, PDGF-AB Curasan PRP 233.70 ± 111.86 ng/mL, signed rank test $P < .001$), whereas the higher platelet count in the leukocyte-depleted blood bank PRP (Blood bank PRP 1,434,300 ± 351,960 platelets/μL, Curasan PRP 908,500 ± 492,300 platelets/μL, signed rank test $P < .0001$) led to higher levels of TGF-β1 (TGF-β1 Blood bank PRP 268.65 ± 70.77 ng/mL, TGF-β1 Curasan PRP 95.02 ± 60.67 ng/mL, signed rank test $P < .001$).[12] The results of the present study confirm these findings. The very low leukocyte counts in the Anitua PRGF resulted in low PDGF-AB levels, and the low platelet counts of the Anitua PRGF also led to low TGF-β1 levels. The IGF-I levels observed in this study were similar to those obtained from the blood bank PRP and the Curasan PRP (IGF-I blood bank PRP 85.37 ± 25.58 ng/mL, IGF-I Curasan PRP 101.72 ± 47.7 ng/mL, signed rank test $P = .160$),[12] which might be a hint that IGF-I is plasmatic in origin.

The results of this study and the data in the literature on the growth factor levels in PRP demonstrated a tremendous range of concentrations that can be achieved depending on the collection strategy. The amount of TGF-β1 enclosed in PRP has varied from 73.3 ± 25.6 ng/mL (Anitua PRGF) and 95.02 ± 60.67 ng/mL (Curasan PRP) to 268.65 ± 70.77 ng/mL (blood bank PRP) and 289.5 ± 94.6 ng/mL (PCCS PRP). Widely varying concentrations of PDGF-AB contained in PRP have also been found: 47.0 ± 21.5 ng/mL (Anitua PRGF), 133.59 ± 46.26 ng/mL (blood bank PRP), 156.7 ± 61.9 ng/mL (PCCS-PRP), and 233.70 ± 111.86 ng/mL (Curasan PRP).

The different data in the current literature concerning the biologic effect (respective efficacy) of PRP in animal and human studies might be explained by the different kinds of PRP preparations. Only a few articles have described the composition of the used PRP.[2,12]

CONCLUSIONS

Self-produced platelet concentrates are not standardized products, which results in a high scattering of the cellular and growth factor contents. In this investigation, PCCS collected much greater concentrations of platelets and leukocytes than the PRGF kit. This resulted in significantly higher growth factor levels. Further in vivo studies should demonstrate whether these differences in the analyzed platelet concentrates result in different clinically biologic effects.

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REFERENCES

1. Kiuru J, Viinikka L, Myllyla G, Pesonen K, Perheentupa J. Cytoskeleton-dependent release of human platelet epidermal growth factor. Life Sci 1991;49:1997–2003.
2. Marx RE, Carlson ER, Eichstaedt RM, Schimmele SR, Strauss JE, Georgeff KR. Platelet-rich plasma: Growth factor enhancement for bone grafts. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1998;85:638–646.
3. Zechner W, Tangl S, Tepper G, et al. Influence of platelet-rich plasma on osseous healing of dental implants: A histologic and histomorphometric study in minipigs. Int J Oral Maxillofac Implants 2003;18:15–22.

4. Kim SG, Chung CH, Kim YK, Park JC, Lim SC. Use of particulate dentin–plaster of Paris combination with/without platelet-rich plasma in the treatment of bone defects around implants. Int J Oral Maxillofac Implants 2002;17:86–94.
5. Kim SG, Kim WK, Park JC, Kim HJ. A comparative study of osseointegration of Avana implants in a demineralized freeze-dried bone alone or with platelet-rich plasma. J Oral Maxillofac Surg 2002;60:1018–1025.
6. Terheyden H, Roldan-Ossa JC, Miller J, Jepsen S, Acil Y. Platelet-rich plasma in der Knochenregeneration—Erste Ergebnisse zweier experimenteller Studien. Implantologie 2002;10: 195–205.
7. Weibrich G, Kleis WGK, Hafner G, Hitzler WE. Growth factor levels in platelet-rich plasma and correlations with donor age, sex, and platelet count. J Craniomaxillofac Surg 2002;30:97–102.
8. Anitua E. Plasma rich in growth factors: Preliminary results of use in the preparation of future sites for implants. Int J Oral Maxillofac Implants 1999;14:529–535.
9. Menitove JE. Standards for Blood Banks and Transfusion Services, ed 19. Bethesda, MD: American Association of Blood Banks, 1999.
10. Weibrich G, Kleis WKG, Hafner G, Hitzler WE, Wagner W. Comparison of platelet, leukocyte, and growth factor levels in point-of-care platelet-enriched plasma, prepared using a modified Curasan kit, with preparations received from a local blood bank. Clin Oral Implants Res 2003;14:357–362.
11. Roberts AB, Sporn MB. The transforming growth factor-bs. Peptide growth factors and their receptors. In: Sporn MB, Roberts AB (eds). Peptide Growth Factors and Their Receptors (Handbook of Experimental Pharmacology, vol 95/1). New York: Springer, 1990:419–472.
12. Weibrich G, Hansen T, Kleis W, Buch R, Hitzler WE. Effect of platelet concentration in platelet-rich plasma on peri-implant bone regeneration. Bone 2004;34:665–671.