Growth Factor Levels in the Platelet-rich Plasma Produced by 2 Different Methods: Curasan-type PRP Kit Versus PCCS PRP System

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Purpose: Potential treatments using autologous thrombocyte growth factors are an important reason to improve methods for isolating platelet-rich plasma (PRP). Two methods for extracting PRP directly by the surgeon are currently available; this study was conducted to compare the growth factor levels in the resulting PRP. Materials and Methods: Whole blood was drawn from 46 healthy donors (17 men, 29 women) aged 20 to 59 years (29.9 ± 7.8). PRP was then separated from each sample by both the PCCS (3i) and Curasan (PRP Kit, Curasan) methods. Results: The growth factor content differed significantly for TGF-β1 (PCCS 467.1 ng/mL; Curasan 79.7 ng/mL) (sign test P < .0001) and PDGF-AB (PCCS 251.8 ng/mL; Curasan 314.1 ng/mL) (P < .0001); this was less significant for IGF-I (PCCS 91.0 ng/mL; Curasan 69.5 ng/mL) (P < .02). The higher platelet count in the PCCS PRP (PCCS 2,232,500/μL; Curasan 1,140,500/μL) seemed to correlate with a higher level of TGF-β1 (Spearman's correlation coefficient, rS = 0.7), whereas the higher leukocyte count in the Curasan PRP (PCCS 1,140,500/μL; Curasan 314.1 ng/mL) had only a minor correlation with higher levels of PDGF-AB (rS = 0.46). Discussion: The PCCS end product has both a higher platelet count and a higher total content of the growth factors investigated. Nevertheless, the biologic effect of the evaluated growth factor levels remains unknown. The amount of PRP necessary to achieve the intended biologic effects still remains unclear. Conclusion: PRP contains growth factors in high concentrations. Precise predictions of growth factor levels based on the thrombocyte counts of whole blood or PRP appeared limited. There are different sources for growth factors (platelets, leukocytes, plasma). (INT J ORAL MAXILLOFAC IMPLANTS 2002;17:184–190)

Key words: autologous, buffy coat, growth factor level, platelet-rich plasma, thrombocyte concentrate

Platelets contain a number of different growth factors, including platelet-derived growth factor (PDGF), transforming growth factor beta 1 (TGF-β1), transforming growth factor beta 2 (TGF-β2), insulin-like growth factor (IGF), epidermal growth factor (EGF), epithelial-cell growth factor (ECGF), and a growth factor for hepatocytes.1 Using thrombocyte concentrates (platelet-rich plasma, PRP) as a source of autologous growth factors, Marx et al showed an increase in bone formation and bone density (both radiographically and histologically) 6 months after autologous bone grafting in 44 patients.2 The use of analogous recombinant growth factors in combination with different bone-regeneration materials for alveolar crest augmentation has been much debated in recent years.3-10 Treatment with PRP to support the osseointegration of endosseous dental implants has also been described.11 To date there have been some promising case reports but no controlled studies.

The clinical use of platelet concentrates obtained from transfusion institutes (by the discontinuous cell separation method) as a source of endogenous thrombocyte growth factors is limited by the high levels of cardiovascular stress they produce in elderly patients and the high production costs. However, 2 methods that allow surgeons to produce small amounts of PRP have recently become commercially available: the Curasan PRP kit and the PCCS system. Both of these methods are more acceptable to the patient, because they produce less...
The thrombocyte counts of the whole blood, platelet concentrate, and self-concentrated PRP were determined automatically (Cell Dyn 3500, Abbott, Wiesbaden-Erbenheim, Germany).

The PRP samples were stored in Eppendorf tubes at –78°C. Immediately before an assay to analyze growth factor content was performed at room temperature (RT), the samples were thawed and centrifuged for 10 minutes at 10,000 rpm in a microcentrifuge. Commercial enzyme-linked immunosorbent assay (ELISA) kits (R&D Diagnostics, Wiesbaden, Germany) were used to quantify the concentrations of TGF-β1, PDGF-AB, and IGF-I. ELISAs were performed according to the manufacturer’s instructions, as described below.

**Production of Platelet Concentrates Using the PCCS Kit**

The PCCS kit consists of 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 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.

**Production of PRP Using the PCCS Kit**

To produce PRP using the PCCS kit, 60 mL of anticoagulated whole blood were transferred into the polyvinylchloride collection bag via valve 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 minutes and 45 seconds at 3,000 rpm in an IEC Centra CL-2 centrifuge. The clamp was then opened to transfer the platelet-containing plasma into the opposite section of the collection bag, and air was blown through valve 2 until 1 cc of red cells had followed the plasma through the transfer line. This ensured that the precursor thrombocyte cells were included. To form a single thrombocyte pellet, a second centrifugation step was performed for 13 minutes at 3,000 rpm. Pumping 35 cc of air into valve 3 after reopening the clamp caused platelet-poor plasma (PPP) to refill section 1 of the collection bag and about 5 mL of PPP to remain in section 2 with the

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thrombocyte pellet. The thrombocytes 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 4. After 15 minutes, the PRP was added to Eppendorf tubes for later thrombocyte count analysis.

Production of PRP Using the Curasan PRP Kit

The Curasan PRP kit consists of the following parts from Sarstedt: a multi set (Catalog No. 85.1637.005), 2 multi-adapter (Catalog No. 93552213), an 8.5-mL CPDA monovette (Catalog No. 01.1610.001), a 9-mL monovette (Catalog No. 02.1726.001), a 7.5-mL monovette (Catalog No. 02.1726.001), and a 1-mL syringe (Reference No. 9161406F). The kit also contains the following parts from Braun (Melsungen, Germany): 2 injection needles, 0.8 × 120 mm (Reference No. 4665643), a 0.8 × 80-mm injection needle (Reference No. 4665465), and 2 intake air cannulas (Reference No. 4190017).

The components of the PRP kit were purchased directly from the manufacturers, not from the distributor (Curasan). Since the samples were not for clinical use, intake air cannulas without sterile filters (Reference No. 4665457, Braun) were used.

Production of PRP Using the PRP Kit

To produce PRP extracts, 8.5 mL of citrated blood (8.5-mL CPDA monovette, Sarstedt, Catalog No. 01.1610.001) were centrifuged in a standard laboratory centrifuge (Heraeus Labofuge 300, Kendro Laboratory Products, Osterrode, Germany) for 10 minutes at 2,400 rpm. Subsequently, the yellow plasma (containing the thrombocytes) was taken up into a monovette with a long cannula, using an additional air-intake cannula. To combine the platelets into a single pellet, a second centrifugation step was performed with the second monovette for 15 minutes at 3,600 rpm. The plasma supernatant (containing relatively few cells) was then reduced to approximately 0.4 mL (again with a long cannula and an air-intake cannula). The thrombocyte pellet was resuspended in the residual 0.4 mL plasma using a conventional shaker (MSI Minishaker, IKA, Staufen, Germany) and transferred to an Eppendorf tube for later analysis.

Measurement of PDGF-AB Levels

Samples were assayed for PDGF-AB using commercially available Quantikine ELISA kits (DHD00, R&D Systems, Minneapolis, MN). The lower detection limit (sensitivity) of this assay, as reported by the manufacturer, is 8.4 pg/mL. The samples and standards were prepared in duplicate, according to the manufacturer’s protocol. The plates were incubated for 2 hours, washed, and incubated with enzyme-conjugated antibodies directed against PDGF-AB for an additional 2 hours at RT. The wells were then washed and substrate was added for 20 minutes at RT. Stop solution was added to each well, and the absorbance at 450 nm was determined for each using a microtiter plate reader.

Measurement of TGF-β1 Levels

TGF-β1 levels were assayed using a commercially available Quantikine ELISA kit (DB100, R&D Systems). A dilution series of TGF-β1 standards (#890207) was prepared in 100-µL volumes in 96-well microtiter plates coated with TGF-β-receptor II. The lower detection limit of TGF-β1 was 7 pg/mL. Since a large proportion of the TGF-β1 in biologic samples is often present in a latent form,12 conversion of TGF-β1 to its active form is necessary to estimate total TGF-β1. To do this, 0.5 mL of the PRP samples was mixed with 0.1 mL of 1 N HCl, incubated at RT for 10 minutes, neutralized by addition of 0.1 mL of 1.2 N NaOH/0.5 M HEPES, and centrifuged. The supernatant fraction was then assayed for total TGF-β1 content. Aliquots (200 µL) were added in duplicate to the microtiter plate, which was then covered and incubated for 3 hours at RT. The wells were then washed, enzyme-conjugated polyclonal antibody to TGF-β1 was added, and incubation continued for 1.5 hours at RT. The wells were washed, substrate was added, and the plates were incubated for an additional 20 minutes at RT. Stop solution (50 µL) was added to each well, and the absorbance at 450 nm was determined for each using a microtiter plate reader.

Measurement of IGF-I Levels

IGF-I levels were estimated using a Quantikine ELISA kit (DG100, R&D Systems). A dilution series of IGF standards (#890775) was prepared in 100-µL volumes in 96-well microtiter plates coated with a monoclonal antibody specific for IGF-I. The lower detection limit of IGF-I was 0.026 ng/mL, as reported by the manufacturer, is 8.4 pg/mL. The samples and standards were prepared in duplicate, according to the manufacturer’s protocol. The plates were incubated for 2 hours, washed, and incubated with enzyme-conjugated antibodies directed against PDGF-AB for an additional 2 hours at RT. The wells were then washed and substrate was added for 20 minutes at RT. Stop solution was added to each well, and the absorbance at 450 nm was determined for each using a microtiter plate reader.
the plates were incubated for 30 minutes at RT. Stop solution (50 µL) was added to each well, and the absorbance at 450 nm was determined for each using a microtiter plate reader.

Statistical Methods

All quantitative measurements are described using summary statistics (n, mean, standard deviation, median, minimum, maximum, and other quantiles).

The 3 thrombocyte counts (donor whole blood, PCCS PRP, and Curasan PRP) and their respective leukocyte counts and growth factor content were compared using the sign test for nonparametric, paired data. Scatter plots and Spearman’s rank correlation coefficient ($r_S$) were used to demonstrate the relationship between whole blood and PRP thrombocyte counts and PRP growth factor levels.

To account for multiplicity, the $P$ values for the respective sign tests of the different platelet count measurements were compared with the Bonferroni-adjusted significance level of 0.05/3 = 0.0167. All other $P$ values should be considered tentative.

RESULTS

For the PCCS and Curasan PRP kits, the respective volumes of whole blood collected, excluding anticoagulant, were 54 and 7.5 mL, respectively, with a hematocrit of 41.9 ± 5.4% (mean ± SD). Production of PRP required about 30 minutes using the PCCS kit and 40 minutes using the Curasan method.

The whole-blood platelet count showed a median of 269,000/µL (Table 1, Fig 1). The thrombocyte counts differed significantly between the donor blood, the PCCS PRP preparation (median 2,232,500/µL), and the Curasan PRP (1,140,500/µL) (sign test: whole blood vs PCCS PRP, $P = .001$; whole blood vs Curasan PRP, $P = .001$; PCCS PRP vs Curasan PRP, $P = .001$).

Correlation between the thrombocyte concentration and the thrombocyte count in the donor whole blood was higher for the PCCS PRP ($r_S = 0.596$) than for the Curasan PRP ($r_S = 0.393$).

The whole-blood leukocyte count showed a median of 6,420/µL (Table 1, Fig 2). The leukocyte counts differed significantly between the donor blood, the PCCS PRP preparation (median 15,300/µL, and the Curasan PRP (33,150/µL) (sign test: whole blood vs PCCS PRP, $P < .001$; whole blood vs Curasan PRP, $P < .001$; PCCS PRP vs Curasan PRP, $P < .001$).

High levels of all 3 growth factors were found in both types of PRP, but the proportions of the individual growth factors differed (Table 1, Fig 3). TGF-$\beta$-1 was the dominant growth factor in the PCCS PRP (median PCCS PRP 467.1 ng/mL; Curasan PRP 79.7 ng/mL), whereas PDGF-AB was the major growth factor in the Curasan PRP (PCCS PRP 251.8 ng/mL; Curasan PRP 314.1 ng/mL).

IGF-I levels in the 2 types of PRP differed less (PCCS PRP 91.0 ng/mL; Curasan PRP 69.5 ng/mL).

Because of the limited number of specimens (n = 17 for males), the influence of sex on growth factor levels in the PRP was not analyzed. Spearman’s rank correlation coefficient revealed no clinically relevant age-specific correlation with growth factor levels in PRP.

Scatter plots of thrombocyte counts and their respective PRP growth factor levels showed a good correlation for TGF-$\beta$-1 content in the PCCS PRP, only slight correlations for PDGF-AB content in both types of PRP, and no correlation for IGF-I content (or for the TGF-$\beta$-1 content in the Curasan PRP; Figs 4 and 5).

The Spearman’s rank correlation coefficients for whole blood platelets, PRP platelets, and growth factor content demonstrated little relationship between these parameters ($r_S = 0.5$), except that the platelet count in the PCCS PRP correlated with the TGF-$\beta$-1 and PDGF-AB content ($r_S = 0.70$ and 0.62, respectively).

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Descriptive Statistics for the Platelet Counts, Leukocyte Counts, and Growth Factor Levels in the Respective Blood Fractions of the Different Preparations (n = 46)</th>
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<tr>
<td>Minimum</td>
<td>Maximum</td>
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<td>----------</td>
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</tr>
<tr>
<td>Leukocytes (1,000/µL)</td>
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<td>TGF-$\beta$1 (ng/mL)</td>
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<td>PDGF-AB (ng/mL)</td>
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<td>IGF-I (ng/mL)</td>
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<td>Curasan</td>
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Fig 1 Platelet counts in whole blood, PCCS PRP, Curasan PRP, and the difference (PCCS PRP minus Curasan PRP) (n = 46).

Fig 2 White blood cell counts in whole blood, PCCS PRP, and Curasan PRP (n = 46).

Fig 3 Growth factor content in the respective PRP preparations (n = 46).

Fig 4 Scatter plot of growth factor content vs platelet count in PCCS PRP.

Fig 5 Scatter plot of growth factor content vs platelet count in Curasan PRP.
DISCUSSION

The 2 methods for obtaining PRP differ mainly in regard to preparation methods and results. The quality of the PRP produced is the main concern for the surgeon, so that predictable results can be achieved. For this limited number of specimens, the PCCS end product had both a higher platelet count and a higher total content of the growth factors investigated (TGF-β1, PDGF-AB, and IGF-I). Nevertheless, the biologic effect of the evaluated growth factor levels remains unknown, and might be affected by the different balance of growth factors in the PRP preparations.

To ensure that measurement errors did not cause the differences in the PRP preparations, the same mechanical thrombocyte counter was used for all samples. Furthermore, as a control, some of the specimens were measured twice, and the results were repeatable. All growth factor measurements were performed in duplicate, using validated commercially available ELISA kits, and no relevant scattering (< 10%) was observed. All growth factor analyses were performed using specimens that had been stored frozen. Deep-freezing is a common exception for releasing intracellular thrombocyte growth factors,13–15 and it has been shown that freezing specimens does not affect the levels of biologically active PDGF detected.

The levels of platelet-derived growth factors in PRP might be expected to depend on the number of platelets involved. The present data did show a statistically significant correlation between the platelet count and growth factor levels in the PCCS PRP (for TGF-β1 and PDGF-AB) and in the Curasan PRP (for TGF-β1 only). However, no statistically significant correlation was seen between the platelet count and the IGF-I or PDGF-AB content of the Curasan PRP. This result might be explained by sources of growth factors other than thrombocytes.

The major source of TGF-β1 might be platelets (high platelet counts in PCCS corresponded to high TGF-β1 levels, whereas lower platelet counts in Curasan PRP corresponded to lower TGF-β1 levels); however, leukocytes still seemed to release a smaller amount of TGF-β1, except when there was a low number of thrombocytes in the PRP (Curasan PRP: correlation of leukocyte count vs TGF-β1, r = 0.42).

The PDGF-AB level was higher in the Curasan PRP. Even if some of the PDGF-AB came from platelets (PCCS PRP: correlation of platelet count vs PDGF-AB, r = 0.62), the higher PDGF-AB levels might be explained by the much higher leukocyte content in the Curasan PRP. Leukocytes might have also released some of the PDGF-AB, in spite of limited statistical correlations (PCCS PRP: correlation of leukocytes vs PDGF-AB, r = 0.33; Curasan PRP: correlation of leukocytes vs PDGF-AB, r = 0.24). A higher leukocyte count in the Curasan PRP corresponded to a higher PDGF-AB content, while a lower leukocyte count in PCCS PRP corresponded to a lower PDGF-AB content.

The IGF-I levels of the 2 types of PRP preparation were nearly similar. No relevant correlation was found for platelet or leukocyte counts in whole blood or PRP. This suggests that the major source of IGF-I might be other components of both PRP preparations (e.g., plasma).

The measured correlation of donor whole-blood and PRP thrombocyte counts with the resulting growth factor levels (r < 0.5) was slightly lower than expected, except for the PCCS PRP thrombocyte count and the respective TGF-β1 and PDGF-AB levels (r = 0.70 and 0.62, respectively), which correlated to a higher degree. The level of correlation of whole-blood and PRP platelet counts with growth factor levels suggests that neither can be used to predict the PRP growth factor content likely to be produced by either method. A possible exception is an estimation of the TGF-β1 content of the PCCS PRP, based on the PCCS PRP thrombocyte count. Further studies with more specimens are needed to evaluate this point.

Knowledge (or at least reliable prediction) of growth factor levels in PRP samples is necessary to ensure reliable and reproducible use of PRP for clinical treatment, since the regenerative potency of PRP undoubtedly depends on its growth factor levels. Unfortunately, there is no simple procedure available for obtaining preoperative estimates of individual growth factor levels in PRP samples. The data obtained in this study demonstrate that neither whole-blood nor PRP platelet counts are generally predictive of the resultant growth factor levels in PRP. Limited predictions of TGF-β1 and PDGF-AB content based on thrombocyte counts might be possible in the PCCS PRP.

CONCLUSIONS

Growth factor levels in PRP preparations obtained using the PCCS system and the Curasan PRP kit were evaluated. PDGF-AB, TGF-β1, and IGF-I were found in high concentrations (median 69.5 to 467.1 ng/mL). Precise prediction of growth factor levels based on the thrombocyte counts of whole blood or PRP appeared limited.

TGF-β1 was the dominant growth factor in the PCCS PRP, while PDGF-AB was dominant in the
Curasan PRP. The different proportions of growth factors seemed to correspond to differences in cellular proportions (ie, thrombocyte counts, leukocyte counts) in the PRP preparations. Thus, there are probably different sources for the growth factors analyzed in the PRP, (eg, platelets, leukocytes, and plasma). Further studies on this topic are needed.

The total growth factor content was higher in the PCCS PRP, although the biologic significance of the different proportions of growth factors in these PRP preparations remains unclear. This point is worthy of further investigation, and the amount of PRP necessary to achieve the intended biologic effects should also be examined.

Furthermore, a technique to rapidly assess the exact growth factor content in PRP would be of therapeutic benefit.

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