Curasan PRP kit vs. PCCS PRP system
Collection efficiency and platelet counts
of two different methods for the preparation of
platelet-rich plasma

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

Abstract: An important reason to improve methods of isolating platelet-rich plasma (PRP) is the potential use of autologous thrombocyte growth factors. In addition to discontinuous cell separation, two methods for extracting PRP that can be performed directly by the surgeon are now available. This study compared the suitability of these two methods for the preparation of PRP. Whole blood was drawn from 47 healthy donors (18 men, 29 women) aged 20–59 years (mean 29.9, SD 7.7). For each donor, PRP was separated by the PCCS method (PCCS Kit, 3i Implant Innovations, Palm Beach Gardens, FL, USA) and by the Curasan method (analogous to the PRP kit, Curasan, Kleinostheim, Germany). Thrombocyte counts differed significantly (sign test \( P = 0.001 \)) between the donor blood (mean 290,000/\( \mu l \), SD 86,000/\( \mu l \)), the PCCS PRP preparation (mean 2,209,000/\( \mu l \), SD 901,000/\( \mu l \)), and the Curasan PRP (mean 1,075,000/\( \mu l \), SD 636,000/\( \mu l \)). The correlation between the thrombocyte count in the PRP and the thrombocyte count in the donor whole blood was greater for the PCCS PRP (Spearman's correlation coefficient \( r_S = 0.60 \)) than for the Curasan PRP (\( r_S = 0.34 \)). A slight, clinically irrelevant, influence of gender on thrombocyte concentration in whole blood was found, but no influence of age was detected.

Platelets contain a number of different growth factors, including platelet-derived growth factor (PDGF), transforming growth factor beta 1 (TGF \( \beta 1 \)), transforming growth factor beta 2 (TGF \( \beta 2 \)), insulin-like growth factor (IGF), epidermal growth factor (EGF), epithelial cell growth factor (ECGF), and a growth factor for hepatocytes (Kiuru et al. 1991). Using thrombocyte concentrates (platelet-rich plasma, PRP) as a source of autologous growth factors, Marx et al. (1998) showed an increase in bone formation and bone density (radiographically and histologically) six months after autologous bone grafting in 44 patients. 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 (Anitua 1999; Becker et al. 1992; Cho et al. 1995; Lynch et al. 1991a; 1991b; Park et al. 1995; Rutherford et al. 1992; Rutherford et al. 1993). Treatment with PRP to support osseointegration of anodense dental implants has also been described (Rutherford et al. 1992). 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 because of high levels of cardiovascular stress (Westphal 1984) for the most elderly patients and high production costs (Singbartl & Schle-
inzer 1999). However, two methods that produce small amounts of PRP have recently become commercially available. These are the Curasan PRP kit (Fa. Curasan, Kleinostheim, Germany) and the PCCS method (Si Implant Innovations, Palm Beach Gardens, FL, USA). These methods are both more acceptable to the patient because they produce less stress on the cardiovascular system and can be done in minutes. However, it has not yet been proven whether there are any differences in their ability to produce PRP with respect to the resulting platelet concentration and collection efficiency. The possible influence of patients’ age, gender, and preoperative thrombocyte count also remains unclear.

Therefore, this study analyzed the ability of the two named methods in preparing PRP, and the influence of the donors’ age, gender, and platelet count on thrombocyte concentration (respective collection efficiency) in the platelet concentrates (PRP).

Material and methods

Between 23 October and 7 November 2000, blood samples were collected from 47 donors without relevant diseases (18 men, 29 women) aged 20-59 years (mean 29.9, SD 7.7, for age distribution see Fig.1). All donors included in this study had thrombocyte counts > 150,000/μl.

For the study, we used the Platelet Concentrate Collection System kit (PCCS) with ACD-A (6-ml ampoules, 3i) and the PRP kit (Curasan).

After volunteers had given written informed consent, as required by our institute’s ethics board, 6ml of ACD-A solution were drawn up in a 60-ml syringe. Venipuncture was done with an 18G apheresis needle from the PCCS set, to fill, in order, the 60-ml syringe with 54ml of whole blood slowly, an 8.5-ml CPDA monovette (Sarstedt, Cat. no. 01.1610.001), and a 2.7-ml EDTA monovette (Sarstedt, Cat. no. 05.1167). The blood-filled syringe and CPDA monovette were inverted five or six times to ensure that the anticoagulant (ACD-A and CPDA) was evenly dispersed. With the 60-ml syringes, PRP was prepared as recommended by the manufacturer of the PCCS kit (for the method see below).

The 8.5-ml CPDA monovette was used for preparation of 0.4ml PRP by a method analogous to the PRP kit (see below for method).

The thrombocyte counts of the whole blood and the platelet concentrates were automatically determined (Cell Dyn 3500, Abbott, Wiesbaden-Erbenheim, Germany).

For the Curasan method, additional measurements of the thrombocyte counts were made using the first sediment (erythrocytes) and the second supernatant (platelet-poor plasma, PPP).

Preparation of platelet concentrates using PCCS

Platelet Concentrate Collection System – PCCS

The PCCS consists of the following: a modified IEC Centra CL-2 centrifuge (IEC Model 7427, International Equipment Company, Needham Heights, MA, USA) with a four-place swinging bucket rotor for specially designed inserts, a 6-ml ACD-A ampoule, and a Platelet Concentration Collection System Set. The latter is delivered in a sterile box and contains:

1. a plastic device consisting of two flexible plastic bags, 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, one for collecting whole blood and one for transferring the supernatant between the bags;
4. an 18-gauge apheresis needle set, for collecting whole blood;
5. a 10-ml syringe for collecting platelet concentrate.

PRP preparation using PCCS

To produce PRP, 60ml 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 filled with an equivalent amount of water. The blood was then centrifuged for 3min 45s at 3000r.p.m., using 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 #2 until 1cm³ of red cells had followed the plasma through the transfer line. This ensures catching the precursor thrombocyte cells as well. To form a single thrombocyte pellet, a second centrifugation step was performed for 13min at 3000r.p.m. By pumping 35cm³ of air into valve #3 after reopening the clamp, platelet-poor plasma refilled section one of the collection bag, and about 5ml of PPP remained in section two with the thrombocyte pellet. The thrombocytes were suspended in the residual plasma by carefully ‘massaging’ the cell mass between the thumb and forefinger for approximately 3min. Finally, the entire contents of bag section two were transferred to a 10-ml syringe via valve #4.

Fig.1. Distribution of age and gender of the donors (n = 47).
After 15 min, the PRP was added to Eppendorf tubes for later thrombocyte count analysis.

**Preparation of platelet-rich plasma using a PRP kit**

The Curasan PRP kit

The PRP kit consists of the following parts from Sarstedt (Nümbrecht, Germany):

1. a ‘multifly set’ (Cat. no. 85.1637.005),
2. two ‘multiadapters’ (Cat. no. 93552213),
3. one 8.5-ml CPDA monovette (Cat. no. 01.1610.001),
4. one 9-ml monovette (Cat. no. 02.1726.001),
5. one 7.5-ml monovette (Cat. no. 02.1726.001),
6. one 1-ml syringe (Ref. no. 9161406F),

and the following parts from Braun (Melsungen, Germany):

1. two 0.8×120-mm injection needles (Ref. no. 4665643),
2. one 0.8×80-mm injection needle (Ref. no. 4665465),
3. two intake air cannulas (Ref. no. 4190017).

To reduce the cost of materials, the components of the PRP kit were not ordered from the distributor (Curasan), but were purchased directly from the manufacturers. As the samples were not for clinical use, we employed intake air cannulas without sterile filters (Ref. no. 4665457, Braun).

**PRP preparation using the PRP kit**

To produce PRP extracts, 8.5ml of citrated blood (8.5-ml CPDA monovette, Sarstedt, Cat. no. 01.1610.001) were centrifuged in a standard laboratory centrifuge (Heraeus Labofuge 300, Kendro Laboratory Products, Osterrode, Germany) for 10 min at 2400 r.p.m. 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 this second monovette for 15 min at 3600 r.p.m. The plasma supernatant (containing relatively few cells) was then reduced to approximately 0.4ml (again with a long cannula and an air-intake cannula). The thrombocyte pellet was resuspended in the residual 0.4ml plasma using a conventional shaker (MS1 Mini-shaker, IKA, Staufen, Germany) and transferred to an Eppendorf tube for later analysis.

**Statistical methods**

All quantitative measurements are described using summary statistics (n, mean, standard deviation, median, minimum, maximum, and other quantiles). The three thrombocyte counts (donor whole blood, PCCS PRP, and Curasan PRP) were compared using the sign test for nonparametric, paired data. Scatter plots and Spearman’s correlation coefficient (r_S) were used to demonstrate the relationship between the whole blood and PRP thrombocyte counts, and to evaluate the possible influence of

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Table 1. Descriptive statistics for the platelet counts in the respective blood fractions of the different preparations

<table>
<thead>
<tr>
<th></th>
<th>Platelet count in whole blood (1000/μl)</th>
<th>Platelet count in PRP – PCCS (1000/μl)</th>
<th>Platelet count in PRP-Curasan (1000/μl)</th>
<th>Difference in platelet count (PCCS-Curasan) (1000/μl)</th>
<th>Platelet count in erythrocyte sediment Curasan (1000/μl)</th>
<th>Platelet count in PPP-Curasan (1000/μl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>47</td>
<td>47</td>
<td>47</td>
<td>47</td>
<td>46</td>
<td>46</td>
</tr>
<tr>
<td>Mean</td>
<td>290</td>
<td>2209</td>
<td>1075</td>
<td>1133</td>
<td>75</td>
<td>14</td>
</tr>
<tr>
<td>95% Confidence interval</td>
<td>265–316</td>
<td>1944–2473</td>
<td>889–1262</td>
<td>891–1374</td>
<td>64–87</td>
<td>11–17</td>
</tr>
<tr>
<td>Median</td>
<td>272</td>
<td>2249</td>
<td>1155</td>
<td>1139</td>
<td>66</td>
<td>12</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>86</td>
<td>901</td>
<td>636</td>
<td>845</td>
<td>38</td>
<td>11</td>
</tr>
<tr>
<td>Minimum</td>
<td>173</td>
<td>500</td>
<td>64</td>
<td>–1057</td>
<td>32</td>
<td>2</td>
</tr>
<tr>
<td>Maximum</td>
<td>709</td>
<td>4424</td>
<td>2510</td>
<td>3434</td>
<td>182</td>
<td>76</td>
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<td>Percentile 10</td>
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<td>103</td>
<td>52</td>
<td>40</td>
<td>6</td>
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<td>1827</td>
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<td>583</td>
<td>48</td>
<td>9</td>
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<tr>
<td>75</td>
<td>326</td>
<td>2717</td>
<td>1577</td>
<td>1656</td>
<td>88</td>
<td>16</td>
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<tr>
<td>90</td>
<td>365</td>
<td>3394</td>
<td>1807</td>
<td>2228</td>
<td>146</td>
<td>21</td>
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</tbody>
</table>
Fig. 3. Scatter plot of the relationship between whole blood and the respective PRP thrombocyte counts.

Fig. 4. Collection efficiency of PCCS, collection efficiency of the PRP-kit, and the difference in the collection efficiency (PCCS – PRP kit).

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 have to be considered as tentative.

Results

For the PCCS and Curasan PRP kits, the respective volumes of whole blood collected, excluding anticoagulant, were 54 and 7.5 ml with a hematocrit of 41.9 ± 5.4% (mean ± SD). The preparation of PRP required about 30 min using the PCCS kit and 40 min using the Curasan method. The whole-blood platelet count averaged 290,000 ± 86,000/μl (see Fig. 2). The thrombocyte counts differed significantly between the donor blood, the PCCS PRP preparation (220,000 ± 90,000/μl), and the Curasan PRP (107,000 ± 63,000/μl) (sign test: whole blood vs. PCCS PRP P = 0.001; whole blood vs. Curasan PRP P = 0.001; PCCS PRP vs. Curasan PRP P = 0.001) (descriptive statistics, see Table 1).

The correlation between the thrombocyte concentration of the PCCS PRP and the thrombocyte count in the donor whole blood (Spearman's correlation coefficient r = 0.60) was higher than for Curasan PRP (r = 0.34) (see Fig. 3 and Table 2). A slight positive trend was observed for the influence of gender on thrombocyte concentration in whole blood (Fig. 2), whereas no age-specific correlation was found (for Spearman's correlation coefficients see Table 2).

The collection efficiency from the PCCS (68.5 ± 22.1%) and PRP (17.6 ± 9.9%) kits differed significantly (sign test P = 0.001) (Table 3, Figs 4 and 5).

For the Curasan PRP-kit, the platelet counts in the erythrocyte fraction averaged 75,000 ± 38,000/μl, and in the platelet poor plasma (PPP) 14,000 ± 11,000/μl (Table 1). This means that 4.9 ± 3.3% of all platelets were found in the erythrocyte fraction, 2.9 ± 2.3% in the platelet-poor plasma (PPP), and 74.4 ± 12.1% could not be located in these three fractions (Table 3, Fig. 6).
Discussion

The two analyzed methods for preparation of PRP directly by a surgeon differ mainly with regard to preparation methods and results. After preparing 47 PRP specimens, we consider the PCCS method better, not only on the basis of the results, but also because of the ease of clinical handling. The advantages include:

1. The end product has a higher platelet count, which is considered a criterion for the quality of PRP.
2. The platelet collection efficiency is higher, so the surgeon can use more of the drawn thrombocytes.
3. The volume of PRP produced by the PCCS method is sufficient for most dentoalveolar procedures. Using the Curasan method, up to 10 monovettes are required to produce an equal volume of PRP; even more would be necessary to achieve the same quantity of platelets. This would increase the number of working steps during PRP production.
4. The preparation time needed is shorter using PCCS than using the Curasan method.
5. PCCS is a needle-free system. Once the blood has been drawn by the doctor, there is no risk of injury to the staff from contaminated needles.
6. PRP preparation using PCCS is more standardized and needs less training, diminishing the possibility of mistakes by staff.
7. The PCCS system has been developed and licensed as a therapeutic instrument, whereas the components of the PRP kit (monovettes and laboratory centrifuge) are considered diagnostic tools.

It still remains unclear why the platelet collection efficiency of the Curasan method is only about one-third that of the PCCS method. To ensure that counting errors did not cause the difference, all samples were measured using the same mechanical counter, and as a control, double measurements of some specimens were made and showed repeatable results. One possible reason for the difference in thrombocytes collected might be that differences in the construction of the two systems might result in different degrees of platelet damage during the preparation process, or different degrees of adhesion of platelets to the walls of the respective processing bags.

When examining the construction of the PCCS kit, the similarity to collection-bag systems used in transfusion institutes is conspicuous. When separating whole blood into components, transfusion institutes use bags with outlets at the top and bottom (Hogman et al. 1988). After centrifugation of the bags in oval-shaped cups, the units are squeezed out in an extractor (a fully automated machine with upper and lower presses) and the components flow out from the top and bottom. The separation efficiencies (67.7–70.8%) of these optimized blood-bank systems (Kretschmer et al. 1990) are similar to our PCCS results. For this reason, we postulate that the high values for PCCS PRP are based on careful handling of the platelets, ensured by the

Table 3. Descriptive statistics of the collection efficiencies in the respective blood fractions of the different preparations

<table>
<thead>
<tr>
<th></th>
<th>Collection efficiency</th>
<th>Collection efficiency</th>
<th>Difference in collection efficiency (PCCS-Curasan)</th>
<th>Platelet recovery</th>
<th>Platelet recovery</th>
<th>Platelet missing</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PCCS (%)</td>
<td>Curasan (%)</td>
<td>(PCCS-Curasan) (%)</td>
<td>PPP-Curasan (%)</td>
<td>erythrocyte fraction</td>
<td>Curasan (%)</td>
</tr>
<tr>
<td>n</td>
<td>47</td>
<td>47</td>
<td>47-46</td>
<td>46</td>
<td>46</td>
<td>46</td>
</tr>
<tr>
<td>Mean</td>
<td>68</td>
<td>17.6</td>
<td>50.9</td>
<td>3.0</td>
<td>5.0</td>
<td>74.4</td>
</tr>
<tr>
<td>95% Confidence interval</td>
<td>62-75</td>
<td>15-21</td>
<td>45-57</td>
<td>2.3-3.6</td>
<td>4.0-5.9</td>
<td>70.8-78.0</td>
</tr>
<tr>
<td>Median</td>
<td>71</td>
<td>17</td>
<td>53.4</td>
<td>2.7</td>
<td>5.7</td>
<td>72.2</td>
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<tr>
<td>Standard deviation</td>
<td>22</td>
<td>10</td>
<td>21.7</td>
<td>2.3</td>
<td>3.3</td>
<td>12.1</td>
</tr>
<tr>
<td>Minimum</td>
<td>15</td>
<td>1</td>
<td>-5.8</td>
<td>0.0</td>
<td>0.0</td>
<td>54.1</td>
</tr>
<tr>
<td>Maximum</td>
<td>100</td>
<td>33</td>
<td>86.2</td>
<td>16.4</td>
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<td>96.8</td>
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<tr>
<td>Percentile</td>
<td>10</td>
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<td>16</td>
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<td>0.210</td>
<td>60.5</td>
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<tr>
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<td>78.3</td>
<td>4.4</td>
<td>8.9</td>
<td>95.4</td>
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</table>
optimized material and production process, which ensure reduced thrombocyte damage and diminish thrombocyte wall adhesion.

Comparing PCCS to the Curasan method, one major difference is in the material from which the monovettes are made. PCCS is made of polyvinylchloride with some additional ingredients, while the Sarstedt monovettes consist of polyethylene and polypropylene. Some of the missing platelets might adhere to the inner wall of the monovettes.

For this reason, Barthelmai (1969) used siliconized glass tubes and needles to prepare 1.5ml PRP out of 30ml whole blood. His first centrifugation was for 10min at 1000 r.p.m., and the second was at 3000 r.p.m. for 15min. The resulting collection efficiency was 72% (±7.7, n = 40), which equals the PCCS system analyzed here. He lost 23% of the platelets in the red-cell fraction (Curasan PRP lost 5% in the erythrocyte fraction) and 3% in the platelet-poor plasma (as did the Curasan PRP). Even using siliconized glass tubes, some platelets were missing (2%); these were probably damaged during the preparation process. Since the Curasan and Barthelmai preparation processes are similar, it can be assumed that some of the missing thrombocytes in the Curasan PRP may also be damaged.

Conclusions

Comparing the PCCS system and the Curasan PRP kit with regard to preparation methods, the ease of handling and preparation time of the PCCS system are more acceptable. Considering thrombocyte collection efficiency, the PCCS system yields significant higher thrombocyte counts than the Curasan PRP kit. Further studies are needed to evaluate whether there are more reasons for the missing thrombocytes, and to analyze the mechanism of damage to thrombocytes, such as split thrombocytes that aren’t recognized and counted by the counting machine, or activation and fibrous aggregation of the platelets, which might indicate slight thrombocyte damage. Growth factor levels in the PRP preparations and the amount of PRP needed to achieve the intended biological effects should also be examined.

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Zusammenfassung

Eine wichtiger Grund zur Verbesserung der Methoden zur Isolierung von plättchenreichem Plasma (PRP) liegt in der Tatsache, dass autologe Thrombozytenwachstumsfaktoren verwendet werden könnten. Zusätzlich zur diskontinuierlichen Selbsterkennung stehen heute zwei Methoden zur Extraktion von PRP zur Verfügung, welche direkt vom Behandler angewendet werden können. Die Studie verglich die Eignung dieser Zwei Methoden zur Isolierung von PRP. Von 47 gesunden Spendern (18 Männern, 29 Frauen) im Alter von 20 bis 59 Jahren (Mittelwert 29,9, SD 7,7) wurde Vollblut entnommen. Für jeden Spender wurden PRP mittels der PCCS Methode (PCCS Kit, 3 Implant Innovations, Palm Beach Gardens, Florida 33410, USA) und mittels der Curasan Methode (kurz zum PRP Kit, Curasan, D-63801, Kleinostheim, Deutschland) separiert. Die Thrombozytenzahlen unterscheiden sich signifikant (sign test P = 0,003) zwischen dem Spen-
der (Mittelwert 29,9, SD 8,6) und dem Curasan PRP (Mittelwert 29,9, SD 7,7). Die Korrelation der Plättchenzahl in PRP und der Thrombozytenzahl im Vollblut war größer für das PCCS PRP (Spearman’s Korrelationskoeffizient r = 0,60) als für das Curasan PRP (r = 0,34). Ein leichter, aber klinisch irrelevanter Einfluss des Geschlechts auf die Thrombozytenzahlen im Vollblut konnte gefunden werden, jedoch konnte kein Einfluss des Alters ermittelt werden.

Résumé

Une raison importante d’améliorer les méthodes d’isolation du plasma riche en plaquettes (PRP) est l’utilisation potentiel de facteurs de croissance thrombocytes. En plus de la séparation cellulaire discontinue, deux méthodes d’extraction de PRP utilisables directement par les chirurgiens sont à présent disponibles. Cette étude compare les qualités de ces deux méthodes pour la préparation du PRP. Du sang a été prélevé chez 47 donneurs sains, 18 hommes et 29 femmes, âges de 20 à 59 ans. Chez chaque donneur, le PRP a été séparé par la méthode PCCS (kit PCCS, 3, Floride) et par la méthode de Curasan (semblable au kit PRP, Allemande). Les comptages de thrombocytes étaient significativement différents (P = 0,001) entre le sang du donneur (290,000 ± 85,000 µl), la préparation PCCS PRP (2,209,000 ± 901,000 µl) et le PRP de Curasan (1,075,000 ± 636,000 µl). La relation entre les concentrations et comptages thrombocytes dans le sang complet du donneur était plus importante chez le PCCS PRP (coefficient de corrélation de Spearman r = 0,60) que chez le PRP de Curasan (r = 0,34). Une faible influence, mais cependant non-importante cliniquement, du sexe sur la concentration thrombocytaire du sang total a été établie, mais aucune influence de l’âge n’a été pu être mise en évidence.

Resumen

Una razón importante para mejorar los métodos de aislar plasma rico en plaquetas (PRP) es el uso potencial de factores autólogos trombocíticos de crecimiento. Además de la separación continua celular, están ahora disponibles dos métodos para la extracción de PRP que se pueden llevar a cabo directamente por el cirujano. Este estudio...


References


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