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Comparison of three different preparations of platelet concentrates for growth factor enrichment

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Abstract: The aim of the present study was to compare three different systems for preparing platelet concentrates: two commercially available bed-side techniques (Curasan system and PCCS) and a procedure used routinely in transfusion medicine. Platelet concentrates were prepared from venous blood of 12 healthy male volunteers using the three different systems. Platelet and leucocyte counts were performed and platelet derived growth factor and transforming growth factor beta were assayed by enzyme linked immunoassay. Handling was also considered. The three systems were able to collect 19.0 ± 16.6% (laboratory system), 41.9 ± 9.7% (Curasan system) and 49.6 ± 21.0% (PCCS) of the absolute number of platelets which were originally in the venous blood volume within the platelet concentrate. Due to the amount of plasma which is left in the platelet concentrate portion, the platelet concentration could be increased between 1.4 ± 1.3 times (laboratory system), 5.0 ± 2.3 times (PCCS) and 11.7 ± 2.4 times (Curasan system) compared to the venous blood. The amount of growth factors correlated with the number of platelets within the platelet concentrates. The two systems for intraoperative use are similar in their effects on the platelets. The absolute gain of platelets seems to be the highest with the PCCS; the highest concentration of platelets per μL is gained with the Curasan system. The laboratory system may offer an alternative if an intraoperative system is not available.

Platelets isolated from peripheral blood are an autologous source of growth factors. Therefore, the application of platelet concentrates (PCs) has undergone a significant increase in oral and maxillofacial surgery (Marx et al. 1998; Anitua 1999; Marx & Garg 1999; Marx 1999). Growth factors are a class of natural biologic mediators which regulate key cellular events in tissue repair, including cell proliferation, chemotaxis, differentiation and matrix synthesis via binding to specific cell-surface receptors (Mohan & Baylink 1991; Marx et al. 1998; Kassolis et al. 2000). The clinical importance of these growth factors is demonstrated by the finding that recombinant DNA-derived polypeptide growth factors significantly accelerate healing of soft tissue (Ross et al. 1986; Pierce et al. 1992). Focussing on periodontal regeneration, preclinical animal as well as clinical studies have demonstrated that growth factors have the capacity to stimulate new bone, periodontal ligament and cementum formation in periodontal lesions (Lynch et al. 1989, 1991; Dennison et al. 1994; Giannobile et al. 1994; Wang et al. 1994; Howell et al. 1995, 1997; Committee on Research, Science and Therapy of the American Academy of Periodontology 1996). Recent clinical and histological findings suggest that the use of platelet concentrates has

technical benefits and may enhance bone regeneration when used in conjunction with autologous bone grafts (Marx et al. 1998), freeze-dried bone allografts (Kassolis et al. 2000) and, possibly, alloplastic bone substitution substances. Although the mechanisms have not been elucidated in detail, today's understanding of bone regeneration emphasises the importance of growth factors (Centrella et al. 1986; Kübler 1997; Marx et al. 1998; Kassolis et al. 2000).

Platelet concentrates (although other terms such as platelet rich plasma (PRP), autologous platelet gel (APG) and plasma very rich in platelets (PVRP) are used for the same preparations, platelet concentrate (PC) is the hematologically correct term) are produced from autologous blood using various forms of gradient density centrifugation or cell separation principles. The clinician who intends to use PCs has to consider whether to use a system for intraoperative use or a service provider who prepares the PC prior to surgery.

In the present investigation two commercially available bed-side techniques for the preparation of platelet concentrates (Curasan system, Curasan AG, Kleinostheim, Germany and 3i PCCS, 3i Implant Innovations Inc., Palm Beach Gardens, FL, USA) were compared with a standard regimen (Roto Silenta RP, Hettich Zentrifugen, Tuttlingen, Germany) used by the Institute of Experimental Hematology and Transfusion Medicine, University of Bonn, with regard to efficacy and handling. All the devices use the general principle of centrifuge technology. For quantification of the efficacy, platelet counts and the levels of platelet derived growth factor (PDGF-AB) and transforming growth factor beta (TGFβ1) generated from the concentrates, prepared with the three methods, were compared. In addition, the amount of loading with white blood cells was determined.

Material and methods

Venous blood (586 mL) was obtained from 12 healthy male volunteers, aged 22–40 years. Informed consent was given according to the Declaration of Helsinki. Of the total volume, 2 mL was used for standard machine blood count and as a standard for determination of the growth factor levels, 30 mL for the Curasan system, 54 mL for the PCCS and 500 mL for the laboratory system. With the Curasan system, the initial venous blood volume can be varied from 15 up to 60 mL for one centrifugation turn, depending on the number of monovettes used. The PCCS and the laboratory system use fixed volumes of 54 and 500 mL, respectively.

Preparation of platelet concentrates

The Curasan system consists of a standard laboratory centrifuge with inserts for up to eight monovettes, a vortex mixer and a kit with disposable material. Each monovette is filled with 8.5 mL solution (7.5 mL blood and 1 mL citrate-phosphate–dextrose–adenine (CPDA) solution for anticoagulation). The number of chosen monovettes is placed into the inserts and the first spin is performed at 2400 r.p.m. for 10 min. This procedure divides the blood into three basic components red blood cells, platelet rich plasma (PRP) and platelet poor plasma (PPP). The red blood cell layer forms at the lowest level, the PRP layer in the middle and the PPP layer at the top. PRP and PPP are drawn off with a second monovette, which is then placed in the centrifuge. The second spin is done at 3600 r.p.m. for 15 min. The platelet pellet accumulates at the bottom of the monovette, the PPP on top. The PPP is drawn off so that the PRP remains in the monovette. After resuspending the platelet pellet within the remaining volume of plasma with the vortex mixer, the PC can be drawn up with a syringe for use. This procedure has to be done for each set of monovettes – up to eight – for one centrifugation turn.

The PCCS uses a custom designed centrifuge consisting of a four place swinging bucket rotor and two specially designed container inserts for loading with the disposable platelet concentration set. The latter consists of two flexible plastic processing bags bonded to the underside of a clear plastic cap. The principle of the procedure is the same, only the individual steps are different. After loading the collection set with 60 mL (54 mL blood and 6 mL Na-citrate for anticoagulation), the blood is first centrifuged at 3000 r.p.m. for 3:45 min. PRP and PPP are transferred into the second bag by adding air to the first bag. The second spin is performed at 3000 r.p.m. for 13 min. PPP is then transferred to the first bag again, so that the PC remains in the second and, after resuspending the platelet pellet into the remaining plasma fraction, can be drawn up in a syringe for use.

Single-donor platelet concentrates were prepared by the Institute of Experimental Hematology and Transfusion Medicine from buffy coats according to a standardized technique that is routinely used in transfusion medicine. Briefly, the whole blood donations were separated in red cells, buffy coat and plasma by centrifugation. Subsequently, PCs were prepared from buffy coat by slow centrifugation.

Blood counts

An aliquot of the venous blood and each PC preparation was removed and platelet, white cell and red cell machine counts were measured (Sysmex GmbH, Norderstedt, Germany). The PC preparations were diluted by adding saline solution at a ratio of 1 : 5, because the number of corpuscular elements was too high for undiluted machine counting. The venous blood as well as the PC samples were then stored at −30 °C for further use.

Determination of growth factors PDGF and TGFβ

To confirm the amount of growth factors within the different PC preparations, PDGF-AB and TGFβ1 were chosen as examples. Concentrations of PDGF-AB and TGFβ1 were measured using commercially available sandwich enzyme immunoassay technique kits from R & D Systems (Quantikine™, R & D Systems, Wiesbaden-Nordenstadt, Germany). The PDGF-AB assay uses a microplate, which is coated with monoclonal antibody to PDGF-AA. The TGFβ1 microplate is coated with TGFβ receptor II antibody. Preparation and dilution of samples and standards were performed as suggested by the manufacturer with the exception that PC was diluted 1 : 5 and 1 : 10, respectively. The plates were read on a MRX Dynatex microplate reader for ELISA at 450 nm. A standard curve was generated and the levels of PDGF-AB and TGFβ1 were determined for PPP and the three PC preparations.

In addition, the time needed for preparation of the PC samples with each method was recorded and handling procedures of the different systems were judged.

Table 1. **Number of volunteers (n), means (m) and standard deviations (Δm) for the percentage of platelets in the PCs referred to the number of platelets in the initial blood volume, percentage of PC volume, referred to the initial blood volume, platelet counts per μL and concentration of the PC, referred to the initial blood volume and leucocyte counts per μL**

	Venous blood ($n = 12$) m ± Δm	Curasan ($n = 12$) m ± Δm	PCCS ($n = 12$) m ± Δm	Hematology ($n = 12$) m ± Δm
% of platelets	100	41.9 ± 9.7	49.6 ± 21.0	19.0 ± 16.6
% of volume	100	3.6 ± 0.3	10.3 ± 1.7	14.4 ± 1.6
Platelets ($10^3/\mu l$)	212.4 ± 39.6	2519.6 ± 834.3	1073.0 ± 533.4	270.9 ± 218.3
Concentration	1	11.7 ± 2.4	5.0 ± 2.3	1.4 ± 1.3
Leucocytes ($10^3/\mu l$)	4.8 ± 0.9	14.8 ± 17.3	5.5 ± 7.7	0.1 ± 0.1

Results

Platelet count study

When comparing the efficacy of PC devices, several parameters of 'concentration' have to be taken into consideration. An essential parameter is the total number of platelets which can actually be obtained in the PC from the venous blood volume drawn from the patient. Therefore it is necessary to relate the absolute number of platelets contained in the volume of the venous blood to the absolute number of platelets contained in the PC. Secondly, the concentration of the platelets within the PC (number of platelets per μL) has to be related to the concentration within the original venous blood. And thirdly, the total volume of the PC has to be compared to the volume of blood drawn from the patient. The last two parameters are proportional and both give an indication of the volume of the plasma fraction, in which the platelets are 'diluted'.

The relevant parameters, percentage of platelets in the PC referred to the number of platelets in the initial blood volume, percentage of PC volume referred to the initial blood volume, platelet counts per μL, concentration of the PC referred to the initial blood volume and leucocyte counts per μL are shown in Table 1 for the venous blood and the three PC preparations. The absolute number of platelets in the PC preparation is the most meaningful parameter because it is a measure of the ability of the system to obtain as many of the platelets within the PC as possible. The PCCS, on average, is able to collect 49.6 ± 21.0% of the platelets, which had originally been contained in the initial blood volume. The Curasan system follows with 41.9 ± 9.7% and the laboratory system with 19.0 ± 16.6%. These values confirm the ability of all three methods to collect the platelets within a certain volume of plasma (Fig. 1).

Based on the amount of plasma left in the PC portion, the latter is more or less 'diluted'. The Curasan system leaves the least amount of plasma in the PC portion, so that the volume of the PC portion is only 3.6 ± 0.3% (1.1 ± 0.1 mL) of the blood volume, drawn from the patient. For the PCCS 10.3 ± 1.7% (5.5 ± 0.9 mL) of the original blood volume remains in the PC portion, for the laboratory system 14.4 ± 1.6% (72.1 ± 7.7 mL) (Fig. 1).

Platelet counts done on each volunteer yielded a mean platelet count value of $212.4 \pm 39.6 \times 10^3/\mu L$. The PC mean platelet count was $2519.6 \pm 834.3 \times 10^3/\mu L$ for the Curasan system, $1073.0 \pm 533.4 \times 10^3/\mu L$ for the PCCS and $270.9 \pm 218.3 \times 10^3/\mu L$ for the laboratory system. This corresponds to a platelet concentration 11.7 ± 2.4 times greater for the Curasan system, 5.0 ± 2.3 times greater for the PCCS and 1.4 ± 1.3 times greater for the laboratory system (Fig. 2).

PDGF-AB and TGFβ1 immunoassays

The results of the PDGF-AB and TGFβ1 immunoassays are shown for the PPP and the three PC preparations in Fig. 3. On the basis of the content of platelets and the differences in volume, the preparations from the Curasan system turned out to contain the highest values for growth factors, 295.2 ± 142.7 ng/mL for PDGF-AB and 499.8 ± 388.6 ng/mL for TGFβ1. For the PCCS preparations PDGF-AB was measured with 157.2 ± 53.7 ng/mL and TGFβ1 with 144.7 ± 70.4 ng/mL, for the laboratory system PDGF-AB with 142.5 ± 37.6 ng/mL and TGFβ1 with 136.1 ± 31.1 ng/mL. Compared to the values measured within the PPP probes of the volunteers – PDGF-AB 10.2 ± 4.5 ng/mL and TGFβ1 12.8 ± 5.2 ng/mL – there is an increase by a factor of 14 for the laboratory system, 16 for the PCCS and 29 for the Curasan system concerning PDGF-AB and an increase by a factor of 10 for the laboratory system, 11 for the PCCS and 39 for the Curasan system with respect to TGFβ.

White blood cells

For determining the contamination with white blood cells, cell counts were taken for the venous blood and the three PC preparations (Table 1). With $14.8 \pm 17.3 \times 10^3/\mu L$ for the Curasan system and $5.5 \pm 7.7 \times 10^3/\mu L$ for the PCCS the values

Figure 1: Percentage of Platelets and Volume

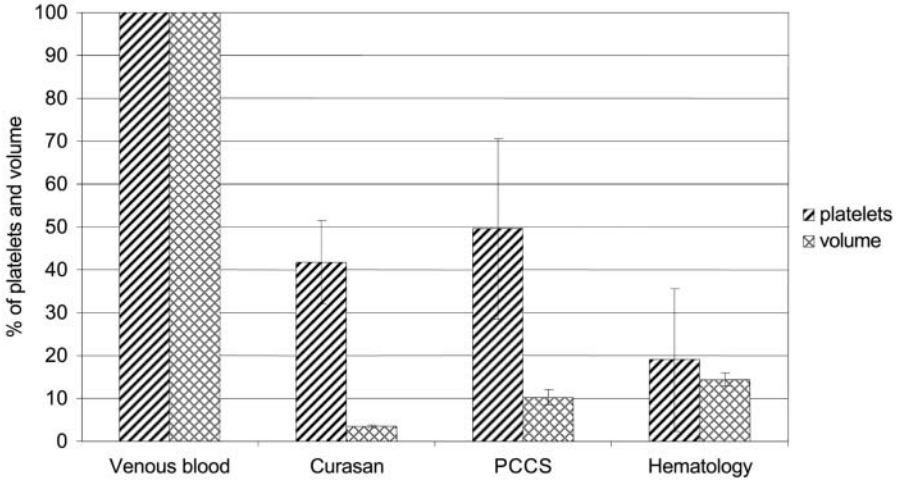

Fig. 1. Percentage of platelets and reduction of volume of the three platelet concentrate preparations referred to the initial number of platelets and venous blood volume.

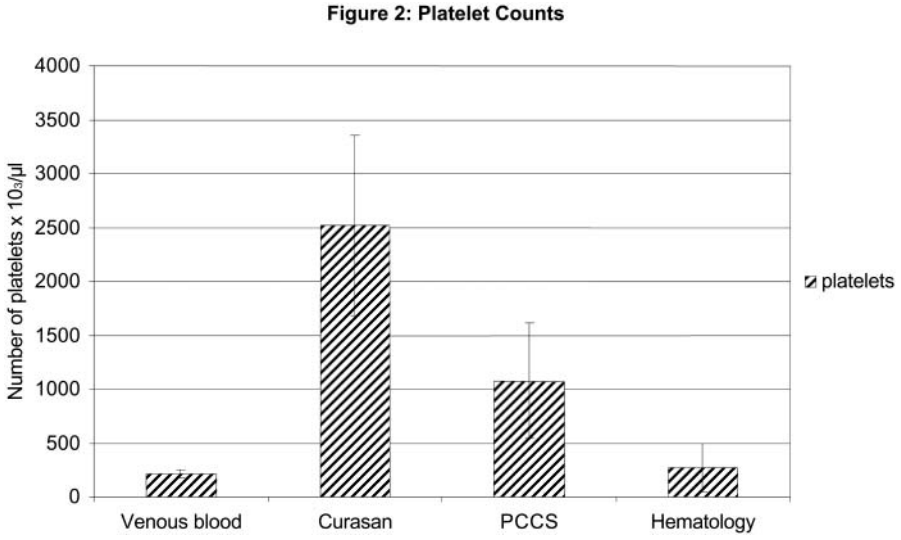

Fig. 2. Number of platelets per µL within the venous blood and the three platelet concentrate preparations.

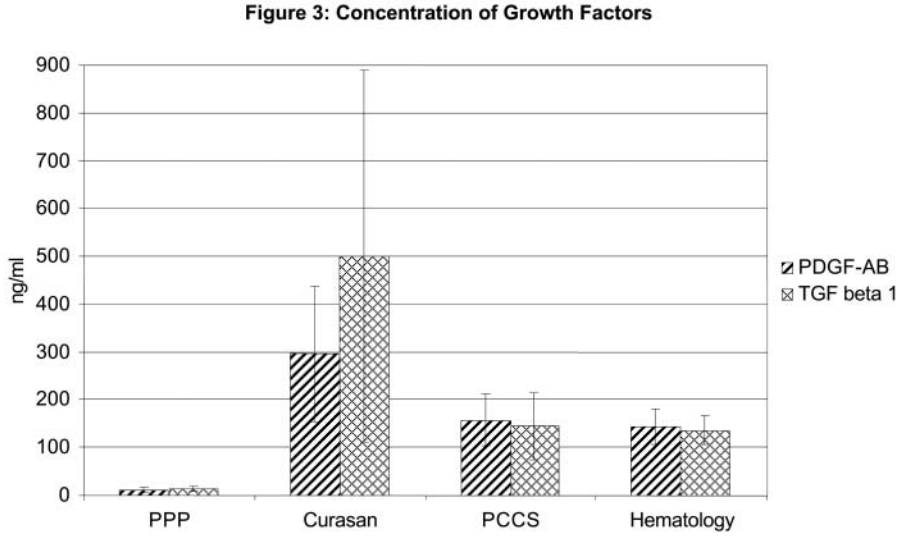

Fig. 3. Concentration of growth factors PDGF-AB and TGFβ1 within the platelet poor plasma and the three platelet concentrate preparations.

for the concentration of leucocytes were higher than in the venous blood with $4.8 \pm 0.9 \times 10^3/\mu L$. In contrast, due to the method of preparation, the laboratory system with $0.1 \pm 0.1 \times 10^3/\mu L$ contained only a very few leucocytes.

Preparation time

The overall time needed for getting the PC ready for use with the office setting devices is made up of the time for getting the disposable material ready, obtaining the venous blood, performing the two centrifugation turns and the procedures for transferring and separating the blood components.

For the Curasan system, a standard procedure, using the maximum capacity of eight monovettes, corresponding to 1.6–2.4 mL of PC, takes about 70 min for the whole procedure. Above all, the procedure of drawing off the individual blood components was the most time-consuming, as it had to be performed $3 \times 8 = 24$ times. The actual spin turns take $10 + 15 = 25$ min. If more than the above-mentioned 1.6–2.4 mL of PC is required, as in the case of a extensive sinus augmentation for both sides, two turns of centrifugation are necessary, consequently requiring more than 2 h.

The spin turns for the PCCS take $3{:}45 + 13 = 16{:}45$ min. Obtaining the blood from the patient as well as transferring it is much more efficient, because there is only one syringe (60 mL) used for every step, reducing the overall time needed for preparing between 4.5 and 6.5 mL PC to about 35 min

In the laboratory system, the clinician does not actually have to spend any time preparing the PCs. The patient donates the blood the day before the surgery and the PC is ready for use in the evening. The procedure itself is fully automatic and takes about 2 h.

Discussion

The idea of adding autologous fibrin adhesive to cancellous bone during mandibular reconstruction goes back to Tayapongsak et al. (1994). Marx et al. (1998) showed that PRP (he uses the term PRP, synonymous with the hematological definition of PC used in this article) increases platelet concentration when placed into grafts and proved the presence of three growth factors (PDGF, TGFβ1 and TGFβ2) as well as the receptors for these growth factors of the cancellous marrow cells. PRP increased the bone formation and enhanced the density of the bone formed at 6 months. They also presented a model of bone regeneration illustrating the mechanism by which PRP may enhance bone regeneration both in rate and amount. The amount of PRP was about 70 mL, the mean platelet count was $785 \times 10^3/\mu L$ with a range of $595–1100 \times 10^3/\mu L$. Marx (2000) proposes that a PRP concentrate should approximate 400% (4×) of the peripheral blood platelet count. Anything less than this concentration is PRP diluted with PPP. Consequently, the volume of PPP as the true platelet concentrate should be about 10% of the whole blood volume drawn from the patient.

Using the Curasan system, we obtained a volume of 1.1 ± 0.1 mL of PC out of 30 mL of venous blood. With the PCCS we obtained 5.5 ± 0.9 mL PC out of 54 mL venous blood. This corresponds to $3.6 \pm 0.3\%$ of the whole blood volume for the Curasan system and $10.3 \pm 1.7\%$ for the PCCS. Although the percentage of platelets in the concentrates was $57.3 \pm 33.5\%$ for the PCCS and thus higher than the

41.9 ± 9.7% for the Curasan system, the results of the platelet counts were 2520 ± 834 × 10^3/μL for the Curasan system and thus more than double as high as the 1073 ± 533 × 10^3/μL for the PCCS. Thus both systems for intraoperative use fulfil the definition of Marx (2000). The values of the growth factors within the two preparations corresponded well to the number of platelets. The values for PDGF-A3 and TGFβ1 were more than double as high for the preparation of the Curasan system compared to the PCCS. This is due to the different concentrations resulting from the amount of plasma left in the PC preparations. The PCs resulting from the PCCS are much more diluted than the PCs from the Curasan system.

Landsberg et al. (2000) compared two methods of preparing PRP gel and the levels of PDGF and TGFβ in each preparation. Using the upper half of the preparation as PPP and the lower half as PRP, the platelet percentage enrichments for the PRP – before generating the gel – were only between 174 and 222%. The levels of PDGF (between 42.1 and 46.0 ng/mL) and TGFβ (between 40.0 and 41.1 ng/mL) were similar regardless of the method used for clot formation. However, compared to our findings, these were between 3 and 10 times less than measured for our systems.

When used together with alloplastic bone substitution substances, which contain calcium, such as β-tricalciumphosphate, the PC application can be used without adding any further agents. A gel preparation is gained about 10–12 min after adding the PC to the bone substitution substance. When used in combination with cancellous bone alone, the PCs require initiating the coagulation process with 10% calcium chloride, mixed at a ratio of 1:5 up to 1:3 with the PCs. We do not see any necessity of adding (bovine) thrombin, as suggested by Marx et al. (1998).

As a further use of the PC fraction, a membrane like structure was described by Sonnleitner et al. (2000). A mixture of PC and the fibrinogen component of Tisseel™ fibrin adhesive (Baxter Healthcare Corporation, Deerfield, IL, USA) in a 1:1 ratio is allowed to flow onto a glass plate or a small flat cup and is consolidated by coating with thrombin. This creates a flat, membrane-like structure, which can be used to cover fenestrations and small defects.

An advantage of the Curasan system seems to be the flexibility in terms of PC volumes obtained, which can be adapted to the actual needs in the clinical situation. Consequently, the quantity of blood used can be varied from 15 up to 60 mL. Additionally, compared to the PCCS, the concentration of platelets and growth factors is twice as high. Disadvantages are the complicated and time-consuming preparation of the PC and the theoretical potential of bacterial contamination if not prepared under strictly aseptic conditions.

However, the greatest advantage of the PCCS is a relatively easy and less time-consuming preparation of the PC in a closed bag system. This also offers better security regarding potential bacterial contamination. Because of the larger amount of plasma left in the PC portion, the concentration of platelets and growth factors is less than with the Curasan system. The quantity of blood used cannot be adapted to the individual requirement of the surgical procedure. Sometimes, 4.5–6.5 mL is more than is normally necessary for periodontal and dentoalveolar surgery. On the other hand, 54 mL of blood loss seems negligible.

Which concentration of platelets and growth factors is most effective for bone regeneration, is not yet known. With both systems, the PCs were prepared according to the manufacturer's guidelines. If more were known about the mechanisms and the ideal concentration of platelets and growth factors in the PC fraction, it would easily be possible to vary the concentration for both intraoperative systems used in our study simply by increasing or reducing the amount of plasma left in the PC portion.

Regarding the pro and cons of PC prepared by means of the laboratory system, it should be noted that the normal purpose for using the laboratory preparation of PC is promotion of coagulation. Besides the platelets, which are only one of the coagulation promoting substances, the plasmatic coagulation factors are of importance. Thus, the volume of plasma must be left higher than for the other systems studied. The relatively high amount of 500 mL venous blood represents a regular blood donation, which is separated in three preparations: a concentrate of erythrocytes, PC and fresh frozen plasma. The PC obtained by this method is actually a part of the plasma fraction of the blood containing a higher amount of platelets as the venous blood. For the laboratory system, 500 mL of venous blood resulted in 72.1 ± 7.7 mL of PRP, corresponding to 14.4 ± 1.6% of the volume. The percentage of platelets contained in the PC (19.0 ± 16.6%) as well as the platelet counts (270.9 ± 218.3 × 10^3/μL) were comparatively low. This is a result of the requirement of an (almost) erythrocyte- and leucocyte-free concentrate and is demanded as a quality standard for hemotherapy, the original purpose of the PCs prepared in the Institute of Experimental Hematology and Transfusion Medicine. It is still a matter of speculation, to what extent the relatively high concentration of leucocytes within the preparations of the intraoperative systems (higher than the leucocyte counts within the venous blood) is of negative significance for bone regeneration.

Within the limits of this study and from the facts discussed above it may be concluded that the two platelet-processing techniques for intraoperative use are similar in their effects on the platelets. We have confirmed a strong correlation between the number of platelets and the amount of growth factors PDGF-A3 and TGFβ1. The absolute gain of platelets within the PCs and consequently PDGF-A3 and TGFβ1 compared to the venous blood seems to be higher with the PCCS. On the other hand, due to the smaller amount of plasma left in the PC portion, the concentration of platelets per μL is higher using the Curasan system. At the moment, however, it cannot be stated whether this is an advantage, because the optimal dose of growth factors needed for bone regeneration has not been determined yet. Consequently, which technique to use is also a question of economics and the amount of PC needed. If one uses PCs frequently and in comparatively larger quantities, the PCCS seems to be advantageous. However, if one uses PCs less frequently and wants to vary the amount produced, the Curasan system seems to be favourable. If a centrifuge for intraoperative use is not available, the laboratory system may offer an alternative for making PCs available. Future studies have to assess the ideal concentration of the different growth factors, to characterise other physiochemical factors that may be present in PC and explain the beneficial effect of PC treatment in bone regeneration.

Résumé

Le but de cette étude a été de comparer trois systèmes différents de préparation de concentrés de plaquettes : deux techniques accessibles commercialement (système Curasan et PCC*TM*) et un processus habituellement utilisé lors des transfusions. Les concentrés de plaquettes ont été préparés à partir de sang veineux de douze hommes volontaires sains en utilisant ces trois systèmes différents. Les comptages de plaquettes et de leucocytes ont été effectués et le facteur de croissance dérivé des plaquettes (PDGF) et le facteur béta de croissance transformant (TGFß) ont été évalués par ELISA. De plus, le maniement a été pris en considération. Les trois systèmes ont été capables de collecter 19 +/- 17% (système de laboratoire), 42 +/-10% (système Curasan) et 50 +/-21% (PCCS*TM*) du nombre absolu de plaquettes qui provenaient du volume sanguin veineux à l'intérieur du concentré de plaquettes. Dû à la quantité de plasma qui est laissée dans la portion de concentré de plaquettes, la concentration de plaquettes pouvait être augmentée entre 1,4 +/-1,3 fois pour le système de laboratoire, 5,0 +/-2,3 fois pour le PCCS*TM* et 11,7+/-2,4 fois pour le système Curasan comparé au sang veineux. La quantité de facteurs de croissance était en corrélation avec le nombre de plaquettes à l'intérieur des concentrés de plaquettes. Les deux systèmes étaient semblables pour leur utilisation durant l'opération. Le gain absolu de plaquettes semblait être plus élevé avec le PCCS*TM*, La concentration la plus importante de plaquettes par µl était obtenue avec le système Curasan. Le système de laboratoire pourrait offrir une alternative si un système intra-opération n'est pas disponible.

Zusammenfassung

Es war das Ziel vorliegenden Studie, drei verschiedene Systeme zur Herstellung von Plättchenkonzentraten zu vergleichen. Es wurden zwei auf dem Markt erhältliche Systeme zur intraoperativen Anwendung (Curasan System und PCCS®) und ein Verfahren, welches routinemässig in der Transfusionsmedizin verwendet wird, miteinander verglichen. Aus dem venösen Blut von zwölf gesunden männlichen Freiwilligen wurden mit den drei verschiedenen Systemen Plättchenkonzentrate hergestellt. Es wurde eine Auszählung der Plättchen und Leukozyten durchgeführt. Der Wachstumfaktor der Plättchen (PDGF) und der transformierende Wachstumsfaktor beta (TGFß) wurden mittels enzymgebundenem Immuntest (ELISA) bestimmt. Zusätzlich wurde auch die Handhabung beurteilt. Die drei Systeme waren in der Lage, 19.0 +/− 16.6% (Laborsystem), 41.9 +/− 9.7% (Curasan System) und 49.6 +/− 21.0% (PCCS®) der absoluten Anzahl an Plättchen, welche ursprünglich im entsprechenden Volumen der venösen Blutproben vorhanden waren, zu sammeln. Aufgrund der Menge an Plasma, welche in den Proben der Plättchenkonzentrate belassen wurde, konnte die Konzentration der Plättchen im Vergleich zu venösem Blut um 1.4 +/− 1.3 mal (Laborsystem), um 5.0 +/− 2.3 mal (PCCS®) und um 11.7 +/− 2.4 mal (Curasan System) erhöht werden. Die Menge an Wachstumsfaktoren korrelierte mit der Anzahl Plättchen in den Konzentraten. Die zwei Systeme für den intraoperativen Gebrauch haben einen ähnlichen Effekt auf die Plättchen. Der absolute Gewinn von Plättchen scheint bei der PCCS® am höchsten zu sein. Die höchste Konzentration an Plättchen pro µl wird mit dem Curasan System gewonnen. Das Laborsystem könnte eine Alternative darstellen, wenn ein intraoperatives System nicht zur Verfügung steht.

Resumen

La intención del presente estudio fue comparar tres diferentes sistemas de preparar concentrados de plaquetas: dos técnicas comercialmente disponibles intraoperatorias (sistema Curasan y PCCS™) y un procedimiento usado rutinariamente en medicina de transfusiones. Los concentrados de plaquetas se prepararon de sangre venosa de doce voluntarios varones voluntarios usando los tres diferentes sistemas. Se llevaron a cabo recuentos de leucocitos y se analizaron por medio de inmunoensayos ligados a enzimas (ELISA) el factor de crecimiento derivado de las plaquetas (PDGF) y el factor beta transformador del crecimiento (TGFβ). Se consideró manipulación adicional. Los tres sistemas fueron capaces de recolectar 19.0 ± 16.6% (sistema de laboratorio), 41.9 ± 9.7% (sistema Curasan) y 49.6 ± 21.0% (PCCS™) del numero absoluto de plaquetas que estaban originalmente en el volumen de sangre venosa respecto al concentrado de plaquetas. Debido a la cantidad de plasma que queda en la porción de concentrado de plaquetas, la concentración de plaquetas se podría incrementar entre 1.4 ± 1.3 veces (sistema de laboratorio), 5.0 ± 2.3 veces (PCCS™) y 11.7 ± 2.4 veces (sistema Curasan) comparado con la sangre venosa. La cantidad de factor de crecimiento se correlacionó con el número de plaquetas dentro de los concentrados de plaquetas. El incremento absoluto de plaquetas parece ser mayor con el PCCS™, la mas alta concentración de plaquetas por µl se ganó con el sistema Curasan. El sistema de laboratorio puede ofrecer una alternativa, si el sistema intraoperatorio no esta disponible.

要旨

本研究は、血小板濃縮血液を精製するための３つの異なるシステムを比較した：すなわち２つの市販されているベッドサイド・テクニック（Curasan systemと PCCS®）と医科の輸血にルーティンに用いられている術式である。血小板濃縮血液は、１２名の健常男性ボランティアの静脈血から、３つの異なるシステムを用いて精製した。血小板数と白血球数を数え、酵素免疫法（ELISA）によって血小板由来成長因子（PDGF）とトランスフォーミング成長因子β（TGFβ）を評価した。取り扱いについても考慮した。３つのシステムは、血小板濃縮血液において、元の静脈血中の血小板絶対数の各々１９．０±１６．６％（検査室システム）、４１．９±９．７％（Curasanシステム）及び４９．６±２１．０％（PCCS®）を採取できた。血小板濃縮血液に残った血漿量のために、静脈血に比べて血小板濃度は、１．４±１．３倍（検査室システム）、５．０±２．３倍（PCCS®）及び１１．７±２．４倍（Curasanシステム）増加できた。成長因子の量は、血小板濃縮血液において血小板数と相関していた。術中に使用される２つのシステムの、血小板に対する影響は類似していた。血小板の絶対獲得量は、PCCS®が最高で、µlあたりの血小板濃度はCurasanシステムが最高であった。検査室システムは、術中使用できるシステムがない場合の代替法となる可能性がある。

References

Anitua, E. (1999) Plasma rich in growth factors: Preliminary results of use in the preparation of future sites for implants. *International Journal of Oral and Maxillofacial Implants* **14**: 529–535.

Centrella, M., Massague, J. & Canalis, E. (1986) Human platelet-derived tranforming growth factor-beta stimulates parameters of bone growth in fetal rat calvariae. *Endocrinology* **119**: 2306–2312.

Committee on Research, Science and Therapy of the American Academy of Periodontology (1996) The potential role of growth and differentiation factors in periodontal regeneration. *Journal of Periodontology* **67**: 545–553.

Dennison, D.K., Vallone, D.R., Pinero, G.J., Rittman, B. & Caffesse, R.G. (1994) Differential effect of TGF-beta 1 and PDGF on proliferation of periodontal ligament cells and gingival fibroblasts. *Journal of Periodontology* **65**: 641–648.

Giannobile, W.V., Finkelman, R.D. & Lynch, S. (1994) Comparison of canine and non-human primate animal models for periodontal regenerative therapy: results following a single administration of PDGF/IGF-I. *Journal of Periodontology* **65**: 1158–1168.

Howell, T.H., Fioellini, J.P. & Paquette, D.W. (1995) Evaluation of platelet derived growth factor-BB/purified insulin-like growth factor-I in patients with periodontal disease. *Journal of Dental Research* **74**: 253 (Abstract 1039).

Howell, T.H., Fiorellini, J.P., Paquette, D.W., Offenbacher, S., Giannobile, W.V. & Lynch, S.E. (1997) A phase I/II clinical trial to evaluate a combination of recombinant human platelet-derived growth factor-BB and recombinant human insulin-like growth factor-I in patients with periodontal disease. *Journal of Periodontology* **68**: 1186–1193.

Kassolis, J.D., Rosen, P.S. & Reynolds, M.A. (2000) Alveolar ridge and sinus augmentation utilizing platelet-rich plasma in combination with freeze-dried bone allograft: Case series. *Journal of Periodontology* **71**: 1654–1661.

Kübler, N.R. (1997) Osteoinduktion und -reparation. *Mund-, Kiefer- und Gesichtschirurgie* **1**: 2–25.

Landsberg, R., Roy, M. & Glickman, R.S. (2000) Quantification of growth factor levels using a simplified method of platelet-rich plasma gel preparation. *Journal of Oral and Maxillofacial Surgery* **58**: 297–300.

Lynch, S.E., de Castilla, G.R., Williams, R.C., Kiritsy, C.P., Howell, T.H., Reddy, M.S. & Antoniades, H.N. (1991) The effects of short-term application of a combination of platelet-derived and insulin-like growth factors on periodontal wound healing. *Journal of Periodontology* **62**: 458–467.

Lynch, S.E., Williams, R.C., Polson, A.M., Howell, T.H., Reddy, M.S., Zappa, U.E. & Antoniades, H.N. (1989) A combination of platelet-derived and insulin-like growth factors enhances periodontal regeneration. *Journal of Clinical Periodontology* **16**: 545–548.

Marx, R.E. (1999) Platelet-rich plasma: A source of multiple autologous growth factors for bone grafts. In: Lynch, S.E., Genco, R.J. & Marx, R.E., eds. *Tissue Engineering: Application in Maxillofacial Surgery and Periodontics*, 71–82. Chicago: Quintessence.

Marx, R.E. (2000) Discussion. *Journal of Oral and Maxillofacial Surgery* **58**: 300–301.

Marx, R.E., Carlson, E.R., Eichstaedt, R.M., Schimmele, S.R., Strauss, J.E. & Georgeff, K.R. (1998) Platelet-rich plasma. Growth factor enhancement for bone grafts. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontics* **85**: 638–646.

Marx, R.E. & Garg, A.K. (1999) Bone graft physiology with use of platelet-rich plasma and hyperbaric oxygen. In: Jensen, O.T., ed. *The Sinus Bone Graft*, 183–189. Chicago: Quintessence.

Mohan, S. & Baylink, D.J. (1991) Bone growth factors. *Clinical Orthopaedics* **263**: 30–43.

Pierce, G.F., Tarpley, J., Yanagihara, D., Mustoe, T.A., Fox, G.M. & Thomason, A. (1992) Platelet-derived growth factor (BB homodimer), transforming growth factor-beta 1 and basic fibroplast growth factor in dermal wound healing. Neovessel and matrix formation and cessation of repair. *American Journal of Pathology* **140**: 1375–1388.

Ross, R., Raines, E. & Bowen-Pope, D.F. (1986) The biology of platelet-derived growth factor. *Cell* **46**: 155–169.

Sonnleitner, D., Huemer, P. & Sullivan, D.Y. (2000) A simplified technique for producing platelet-rich plasma and platelet concentrate for intraoral bone grafting techniques: a Technical Note. *International Journal of Oral and Maxillofacial Implants* **15**: 879–882.

Tayapongsak, P., O'Brien, D.A., Monteiro, C.B. & Arceo-Diaz, L.Y. (1994) Autologous fibrin adhesive in mandibular reconstruction with particulate cancellous bone and marrow. *Journal of Oral and Maxillofacial Surgery* **52**: 161–165.

Wang, H.-L., Pappert, T.D., Castelli, W.A., Chiego, D.J. Jr, Shyr, Y. & Smith, B.A. (1994) The effect of platelet-derived growth factor on the cellular response of the periodontium: An autoradiographic study on dogs. *Journal of Periodontology* **65**: 429–436.