Vox Sanguinis (2009)

ORIGINAL PAPER

DOI: 10.1111/j.1423-0410.2009.01188.x

Not every PRP-gel is born equal **Evaluation of growth factor availability for tissues through four PRP-gel preparations: Fibrinet®, RegenPRP-Kit®, Plateltex® and one manual procedure**

L. Mazzucco,[1] V. Balbo,[1] E. Cattana,[1] R. Guaschino[1] & P. Borzini*

[1]*Centro Trasfusionale e Laboratorio Biotecnologie, Ospedale 'SS Antonio e Biagio', Alessandria, Italy*

Vox Sanguinis

Background The rationale for using topical platelet gel therapy is to provide the healing tissues with concentrated platelet-derived factors. Several systems are available to prepare platelet-rich plasma (PRP) and from these, the platelet gel. These systems produce two- to six-fold platelet and growth factor-enriched concentrations. The bioavailability of growth factors in tissue healing depends on the amount of growth factors stored in platelets but a portion of these is lost during platelet manipulation. Very few data have been reported on the kinetics of growth factor release from PRP-gels. The aim of this study is to assess the growth factor recovery and its bioavailability to tissues in four different PRP and PRP-gel preparation techniques.

Materials and methods Three commercially available devices (Fibrinet®, RegenPRP-Kit®, Plateltex®) and one manual procedure (home made, HM) were evaluated with reference to resulting platelet concentration, growth factor content and the kinetics of growth factor release from gel.

Results Platelet concentration increased from 1·65- to 4·4-fold in comparison with whole blood initially used. The final platelet concentration ($\times 10^3/\mu l$) was: Fibrinet 1358 ± 419, Regen 430 ± 109, HM 1196 ± 188, and Plateltex 1160 ± 164. A high variation (5- to 27-fold) was found in growth factor concentration in relation to the method used and also a high variation in the kinetics of growth factor release from gels.

Conclusions Similar methods for platelet gel preparation revealed different performances concerning growth factor recovery and the kinetics of its release from the gel. It is unclear whether these noticeable differences are important for clinical management.

Received: 2 October 2008, revised 12 March 2009, accepted 15 March 2009

Key words: Growth factor, platelet gel preparation, thrombin-activated gel, batroxobin-activated gel.

Introduction

In the last few years a variety of manual, semiautomatic and fully automated systems for platelet gel preparation have become commercially available. The rationale for the therapeutic use of platelets is to make platelet-derived factors locally available for tissue to be healed [1,2].

Correspondence: Dr Laura Mazzucco, Centro Trasfusionale e Laboratorio Medicina Rigenerativa, Az. Ospedaliera SS Antonio e Biagio e C. Arrigo, Via Venezia 16, 15100 Alessandria, Italy.
E-mail: lmazzucco@ospedale.al.it
*Retired

For prior topical application, platelets must be harvested and concentrated from whole blood and transformed into an easy to handle gel. Methods and devices used to prepare platelet-rich plasma (PRP) and gel are aimed to obtain a high yield of harvested platelets. Platelet-rich plasma is defined as the volume of the plasma fraction from autologous blood having platelet concentration above baseline (200000 platelets/μl). Studies have shown that clinical efficacy can be expected with a minimum increase of four- to six-fold from this baseline (1 million platelets/μl) [3,4].

The platelet growth factor content is quite variable among individuals and it is not necessarily proportional to the platelet count [5,6]. Activation and release of growth factors

also occur during platelet processing. Hence, the sole platelet count cannot be predictive of the growth factor content in individual PRP preparations.

Platelets are extremely sensitive to any kind of process-induced stress, from blood extraction to PRP gel production [7–9]. Thus the amount of platelet-derived factors available at the end of the manipulation process depends on cumulative effects over platelets, starting from phlebotomy and ending with gel formation. Gel formation is induced by pro-coagulant enzymes. The physical quality of the platelet-embedding network of polymerized fibrin depends on the fibrinogen concentration of the PRP. This is variable among individuals and this variability modifies the availability of growth factors to the tissue to be healed. In fact, fibrin first absorbs then releases platelet-derived growth factors the same way as extracellular matrix does. The kinetics of this absorption/release process is relevant to growth factors availability to tissues. Beside individual fibrinogen concentration, these kinetics depend on fibrin structure diversity which depend on the pro-coagulant enzyme used to induce the gel formation.

This study evaluated the growth factors made available from platelet gel. The results and the influence of several phases of the production process were considered and discussed: (i) blood collection; (ii) platelet harvesting by centrifugation; (iii) platelet concentration by further centrifugation; (iv) induction of gel formation; and (v) absorption and release of the growth factors under selected experimental conditions.

Three commercially available devices (Fibrinet®, RegenPRP-Kit®, Plateltex®) and a manual procedure (home-made process, HM) have been evaluated.

Materials and methods

Platelet-rich plasma preparation

Three legally marketed commercial devices for preparation of autologous platelet gel were studied (RegenPRP-Kit®, RegenLab, Mollens-VD, CH; Fibrinet®, Cascade Medical Enterprises LLC, Wayne, NJ, USA; Plateltex®, Bratislava, Slovakia) and one HM method customized at the Blood Transfusion Centre, Alessandria Hospital, Italy.

Thirty millilitres of human blood were collected from healthy consenting blood donors and distributed into specific tubes. PRP was prepared by differential centrifugation according to instructions provided by the manufacturers (Table 1). The platelet count was performed using ABX Micros 60 cytometer (Horiba ABX, Shefford, UK).

Gel preparation

According to instructions, Fibrinet gel and supernatant were achieved through centrifugation at 1500 *g* for 15 min in the Fibrinet gel-inducing tube (with 0·1 ml calcium chloride). Centrifugation causes a mechanical stress; calcium ions lead to thrombin formation which transforms the fibrinogen in fibrin. Calcium and gravitational force act together inducing thrombin- and platelet-dependent clot formation.

Regen gel is formed by mixing PRP and autologous thrombin (ratio 9 : 1). Regen autologous thrombin is obtained treating the patient's whole blood (Regen ATS tube) with calcium clorure (1 ml) and ethanol (1 ml). Thrombin is recovered after centrifugation of ATS tube.

Plateltex gel kit includes a vial of liophylized batroxobin, a fibrinogen cleaving enzyme for a rapid fibrin clot formation. Batroxobin (5 BU) is dissolved in 1 ml of calcium gluconate (Monico, Italy) immediately before use (PRP/activator ratio 9 : 1) [10].

HM method induces gel formation through the use of autologous thrombin obtained by recalcification of patient's platelet poor plasma (PPP) with calcium gluconate 1000 mg/ml (Bioindustria LIM, Italy – ratio 5 : 1). Thrombin is recovered after centrifugation: PRP/activator ratio 9 : 1 is used to induce gel formation.

Sample preparation

To assess the dynamics of growth factor release after gelation four types of samples were prepared: PRP1, GS1, GS2 and GS3.

PRP1 was obtained by centrifugation of whole blood (see PRP preparations). One millilitre of PRP1 was centrifuged at 14 000 *g* for 30 min (Heraeus Biofuge Stratos, Langenselbold, Germany), divided into aliquots and stored frozen at –80°C until testing.

GS1 was supernatant of platelet gel. Briefly, 1 ml of PRP1 was treated as previously described (see gel preparation) in

Table 1 Synopsis of the preparation process

Devices	Processed blood	Anticoagulant	Centrifugation 1th step	Centrifugation 2th step	Activator
Fibrinet	7 ml	ACD + separator gel	1100 *g* × 10 min	1500 *g* × 15 min	Ca^{++}, high speed centrifugation
Home made	5 ml	ACD	160 *g* × 10 min	1200 *g* × 10 min	Ca^{++}, autologous thrombin
Plateltex	6 ml	Sodium citrate	180 *g* × 10 min	1000 *g* × 10 min	Ca^{++}, batroxobin
Regen	8 ml	ACD + separator gel	1500 *g* × 10 min	–	Autologous thrombin

order to obtain platelet gel. After gel formation (20 min) gel was centrifuged at 14 000 *g* for 30 min, divided into aliquots and stored frozen at –80°C until testing.

A series of gels was prepared for longitudinal kinetics study. All gels were incubated in a moist chamber at 37°C. Half of them were centrifuged (1250 *g* per 15 min) after 24 h. The supernatants from this centrifugation (early gel supernatants, GS2) were harvested after additional six more days of incubation in moist chamber. The second half of the gels were incubated in moist chamber for 7 days and then centrifuged. These supernatants (late gel supernatants, GS3) were harvested immediately after centrifugation. GS2 and GS3 were stored at –80°C until testing.

Human growth factors: PDGF-BB, TGF-β1, b-FGF, VEGF, EGF and IGF-I were measured in PRP1, GS1, GS2 and GS3.

Since small amount of bone morphogenetic proteins (BMPs) have been localized in megakaryocytes and platelets and since these proteins are strongly involved in bone cell pathophysiology [11,12], we also studied PRP1, GS1, GS2 and GS3 to find BMP-2.

The growth factor concentration was dosed with Quantikine® ELISA kits (R&D Systems, Abingdon, UK) according to producer's instructions. It was assessed diluting both PRP and supernatant samples as follows: PDGF-BB 1 : 20; TGF-β1 1 : 40; b-FGF 1 : 1; VEGF 1 : 1; EGF 1 : 20; IGF-I 1 : 20; and BMP-2 1 : 1. Immediately before testing, the frozen aliquots were thawed at room temperature.

Statistical analysis

The data are presented as arithmetic mean and standard deviation (SD). They were analysed using regression analysis, *t*-test, ANOVA and the Bonferroni's multiple comparison test (Prism5 for Windows, version 5·01, GraphPad Software, San Diego, CA, USA). Minimum confidence interval of 95% ($P < 0{\cdot}05$) was considered for statistical significance of differences.

Results

The volume of whole blood of PRP and of PRP1; the platelet concentration in whole blood in PRP and in PRP1; the growth factor concentration in PRP and PRP1 are reported in Table 2. According to instruction, PRP from Regen was not re-centrifuged while PRP from Fibrinet, Plateltex, and HM underwent a second centrifugation (Table 1). After this centrifugation phase the mean fold increment of the platelet concentration varied from 1·65 to 4·4 ($P < 0{\cdot}001$).

High individual variability of growth factor concentration was found. Such variability was proven by the high SD values. BMP-2 was absent in all samples, thus BMP-2 values were not included in table. Growth factor concentration and platelet concentration in PRP1 did not correlate.

When treating a lesion with platelet gel, the amount of growth factors made bioavailable depends on the amount of

Table 2 Volume, platelet and growth factor concentration during the platelet enrichment process, (mean and mean ± SD). Table 2a, WB: starting whole blood; PRP: platelet suspension after the first centrifugation step. Table 2b, PRP1: platelet suspension prior gelation

(a)

	WB		PRP							
	Vol ml	plts 10^6/ml	Vol ml	plts 10^6/ml	PDGF-BB ng/ml	TGF-β ng/ml	b-FGF pg/ml	VEGF pg/ml	EGF pg/ml	IGF-I ng/ml
Fibrinet	7·0	263 ± 70	4·8	346 ± 105	3·6 ± 2·2	8·8 ± 5·0	32 ± 27	366 ± 377	1432 ± 1266	27 ± 11
Regen	8·0	263 ± 70	5·0	430 ± 109	2·3 ± 1·9	6·2 ± 4·0	13 ± 10	133 ± 121	112 ± 98	36 ± 14
Home made	5·0	263 ± 70	3·0	558 ± 158	3·0 ± 1·5	8·1 ± 2·6	28 ± 27	172 ± 135	123 ± 67	32 ± 11
Plateltex	6·0	263 ± 70	3·0	557 ± 155	4·4 ± 3·0	12·2 ± 3·2	1·3 ± 2·7	225 ± 153	505 ±237	26 ± 8

(b)

	PRP1								
	Vol ml	plts 10^6/ml	Fold increment	PDGF-BB ng/ml	TGF-β ng/ml	b-FGF pg/ml	VEGF ng/ml	EGF ng/ml	IGF-I ng/ml
Fibrinet	1·2	1358 ± 419	3·9 ± 0·44	3·6 ± 2·4	8·8 ± 5·0	31 ± 27	0·3 ± 0·3	1·4 ± 1·2	27 ± 11
Regen	5·0	430 ± 109	1·65 ± 0·17	2·3 ± 1·9	6·2 ± 4·0	13 ± 10	0·1 ± 0·1	0·1 ± 0·1	36 ± 14
Home made	1·9	1196 ± 188	4·4 ± 0·48	11·4 ± 6·7	29·8 ± 13·5	95 ± 102	0·6 ± 0·5	0·4 ± 0·2	112 ± 44
Plateltex	2·0	1160 ± 164	4·4 ± 0·36	14·3 ± 11·3	40·4 ± 14·9	3·5 ± 8	0·7 ± 0·4	1·6 ± 0·7	88 ± 34

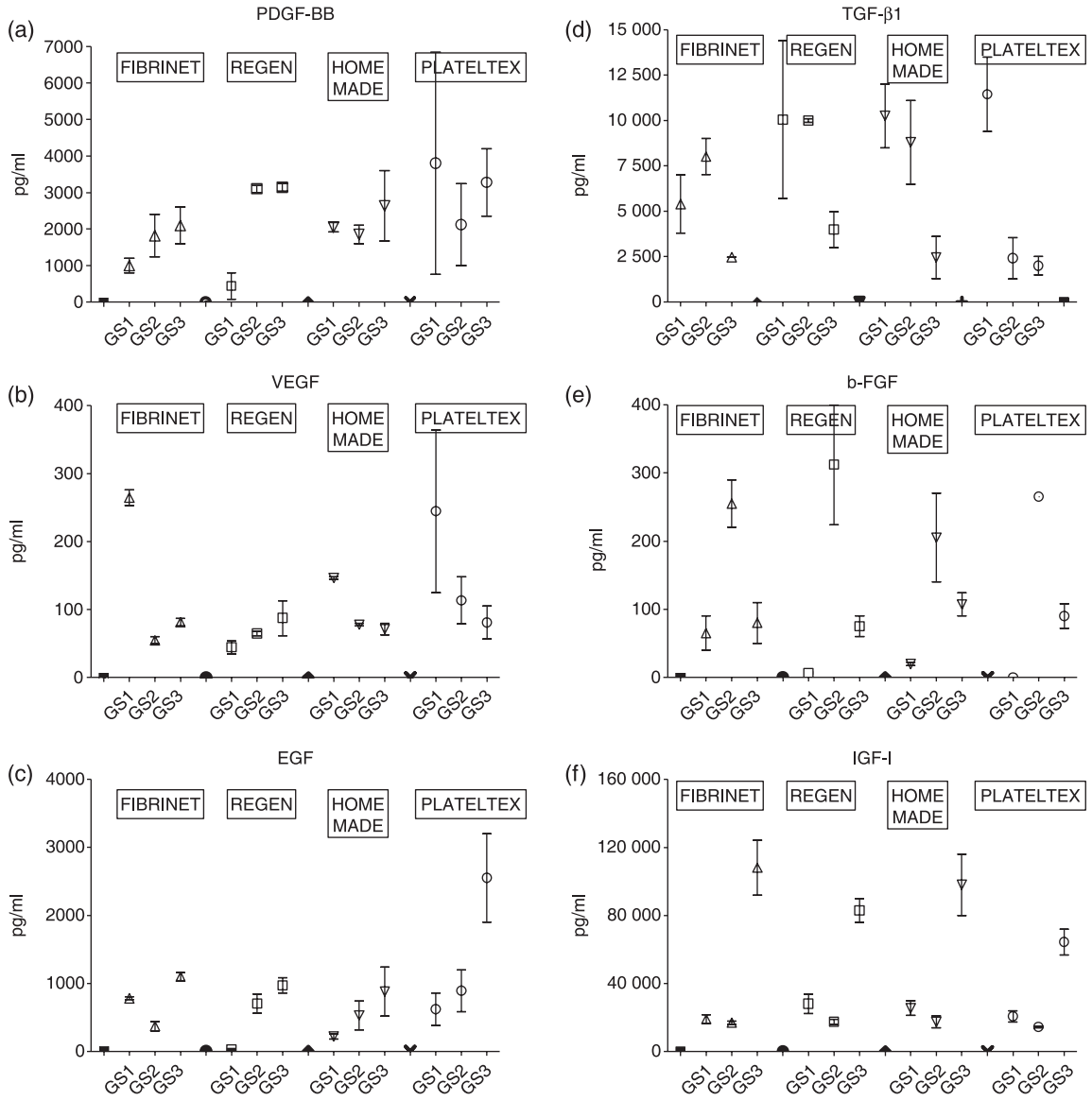

Fig. 1 Kinetics of growth factor release in gel supernatants GS1, GS2 and GS3.

growth factors stored in platelets but also on those released in plasma moiety of PRP during platelet isolation. However, the net amount of growth factors available in the blood product is not necessarily equivalent to that made available to tissue, such availability depends also on the kinetics of growth factor release from gel. It is very likely that gel-like material (mainly, but not only, polymerized fibrin) is actively involved as a growth factor-dispensing apparatus, mainly through its absorbance/release properties. In this view, we have studied time and the mode of growth factor release from PRP-gel measuring growth factor concentration in GS1, GS2 and GS3 in order to represent the relative amount of growth factors made available to tissue over time (1 week). The kinetics of such release is reported in Fig. 1. These illustrate different profiles of growth factor release. Profiles A, B and C show that the release of PDGF-BB, VEGF and EGF was variously influenced by process/device used (technique-dependent). On the contrary, profiles D, E and F show that the release of TGF-β1, b-FGF and IGF-I, although all factors having an identical profile, was not influenced by process/device used (technique-independent). From the results depicted in Fig. 1 the sequence of bioavailability of growth factors to tissue is variable from method to method. While profiles C, D, E and F clearly illustrate that TGF-β1 is released promptly, b-FGF is released within 24 h, EGF and IGF-I are released later, timing of release of PDGF-BB and VEGF are

Table 3 Sequence of growth factor release from the gels

	1st wave (GS1)	2nd wave (GS2)	3rd wave (GS3)
Fibrinet	VEGF	PDGF-BB	PDGF-BB
	EGF	TGF-β1	EGF
		b-FGF	IGF-I
Regen	TGF-β1	PDGF-BB	PDGF-BB
		TGF-β1	VEGF
		EGF	EGF
		b-FGF	IGF-I
Home made	PDGF-BB	PDGF-BB	PDGF-BB
	VEGF	TGF-β1	EGF
	TGF-β1	EGF	IGF-I
		b-FGF	
Plateltex	PDGF-BB	PDGF-BB	PDGF-BB
	VEGF	VEGF	EGF
	TGF-β1	EGF	IGF-I
		b-FGF	

more technique-dependent. Time-differentiated release of growth factor has been summarized in Table 3 showing that growth factors made available to tissue during three successive waves represented by GS1, GS2 and GS3. Major differences among methods are evident in very early phase of growth factor release (GS1), while intermediate and final phases are characterized by similar qualitative feature of growth factor availability.

Total amount of growth factors released from a single tube of blood processed through the methods under investigation is reported in Table 4.

Albeit the four procedures can be said substantially equivalent, considering gel-released growth factors per tube of blood processed, substantial differences were evident. Recovery of PDGF-BB, TGF-β, and IGF-I was low when using Fibrinet compared with that achieved using other techniques. Regen displayed high recovery of TGF-β, b-FGF and IGF-I, and Plateltex achieved high EGF recovery. Cross-tabulation of significant differences of growth factor recovery among the four techniques is reported as well (Table 4b).

Discussion

The aim of the topical application of platelet derivatives is to provide tissues with concentrated growth factors to induce or to accelerate the healing process. Platelets are natural providers of platelet-derived factors therefore PRP is a concentrate with the 'right' factors in the 'right' proportions

Table 4 Total amount (ng) of growth factors made available to tissue after processing a single tube of blood (table 4a) and significance cross tabulation (mean ± SD); statistical differences *$P < 0{\cdot}05$; **$P < 0{\cdot}01$; ***$P < 0{\cdot}005$ (table 4b)

(a)

	PDGF-BB	TGF-β	b-FGF	VEGF	EGF	IGF-I
Fibrinet	5·9 ± 4·5	19·0 ± 9·9	0·48 ± 4·0	0·48 ± 0·4	2·7 ± 1·5	105·9 ± 42
Regen	18·7 ± 11·2	67·3 ± 42	3·9 ± 3·0	0·53 ± 0·4	4·7 ± 4·5	359·6 ± 140
Home made	12·4 ± 6·9	49·8 ± 25	0·63 ± 7·0	0·57 ± 0·5	3·1 ± 1·7	267·9 ± 89
Plateltex	18·4 ± 12·1	31·7 ± 13·9	0·71 ± 1·1	0·83 ± 0·6	8·1 ± 3·8	199·2 ± 88

(b)

	Fibrinet	Regen	Home made	Plateltex
Fibrinet		PDGF* TGF-β*** b-FGF** IGF-I***	PDGF* TGF-β** IGF-I***	PDGF* TGF-β* VEGF*** IGF-I***
Regen	PDGF* TGF-β*** b-FGF** IGF-I***		b-FGF*	TGF-β* b-FGF* IGF-I*
Home made	PDGF* TGF-β** IGF-I***	b-FGF*		EGF*
Plateltex	PDGF* TGF-β* VEGF*** IGF-I***	TGF-β* b-FGF* IGF-I*	EGF*	

necessary for the healing process [13]. The arbitrary target of platelet concentration of 0·8–1·0 × 10^9 platelets per millilitre, that is three to six folds platelet concentration in whole blood, has been proven as clinically effective by several studies.

We have evaluated four systems providing PRPs with platelet concentration in range considered clinically effective. PRPs and gels were prepared using three commercially available devices and one HM procedure which has been developed in our laboratory in 1999 and which is conceptually similar to commercially available PRGF [14]. The aim of our study was to evaluate using *in vitro* analysis the release of platelet-derived factors from PRP-gels. These PRP-gels were produced with commonly used systems which are regarded as substantially equivalent and we have considered both the process of preparation and the intended clinical use.

The relationship among platelet concentration, growth factor concentration and clinical effectiveness is far from being clear. Few clinical studies did specify platelet concentration in PRP used to treat patients. Most of the studies claiming clinical efficacy of concentrated PRPs were not controlled trials and/or reported small group of patients [15]. Furthermore, manipulation of platelets and way of gel activation were not given enough emphasis as variables relevant to growth factor release from PRP-gels [16,17].

In Tables 1–4 preparation processes and results obtained through each step of the process are reported. All preparations were carried out using the same starting blood samples and the mode and amount of growth factor release from gels were end-points.

The volume of processed blood (Table 1) varied from a minimum of 5 ml (HM) to a maximum of 8 ml (RegenKit). One should expect that such starting difference might greatly influence the end-point but looking at Table 4, which summarized the total amount of growth factor released after processing a single tube of blood, this assumption was verified in less than 50% of cases. This implies that other variables were important to/for the end-point.

A relevant variable was the second centrifugation step. RegenKit was the only system which did not include a second centrifugation step to further concentrate platelets by removing part of PPP (Table 1). Suggesting that platelet and growth factor concentration run parallel, we suppose that second centrifugation step affected platelet concentration and final volume of PRP1. As for RegenKit, which has a relatively low platelet concentration in PRP1 and this assumption was sufficiently verified. However, this was not demonstrated at all considering HM, Plateltex and Fibrinet that had highest platelet concentration and lowest growth factor concentration. It is likely that this result was due to high gravitational force applied to second centrifugation step and to relatively large volume of PPP removed. Albeit HM and Plateltex showed similar platelet concentration in PRP1, substantial differences in growth factor concentration were found between these systems.

The origin of these differences is less clear. We did not find any correlation between platelet concentration and growth factor concentration in PRP. Some authors reported a correlation between such parameters [18], some other did not [19]. In theory several factors might contribute to this lack of correlation: manipulation-induced platelet stress; variable susceptibility of platelets to stress; and microaggregates in PRP affecting an accurate platelet count. Furthermore, it cannot be excluded that growth factor-absorbing proteins (fibrin monomers, thrombospondin and other proteins released from platelets during high speed centrifugation phase) might affect both platelet count and growth factors measurement [20,21]. One common feature reported in Table 2 was variability of growth factors concentration among individuals, showed by high SD values and this variability has also been shown by other authors [6,22]. The gel moiety of PRP-gel is not an inert release system. It is a natural scaffold with biodynamic properties inherent to its physical constitution and to the site where it is placed. It is not only a fibrin net where platelets are meshed in releasing their factors outwards. It is a biomaterial absorbing and releasing water, salts, factors and other molecules prior to being dissolved by proteolytic enzymes and phagocytic cells. Therefore, we have considered that counting platelets and measuring platelet-derived factors in PRP might be neither representative nor predictive of the actual growth factor delivery to tissues from PRP-gels. We aimed to find out a simple *ex vivo* method representative of some dynamic aspect of the growth factor release from PRP-gels across time. In Fig. 1, growth factor determination in GS1, GS2 and GS3 represented the growth factors that had been released respectively after 20 min, 1 h, and 7 days after gel formation had occurred. This timing is to be considered a hypersimplified representation of duration of a clinically applied PRP-gel. The growth factor determination at these times aimed to prove mode and time of growth factor release in clinical-like conditions. We are aware that this is an oversimplification of much more complex events occurring *in vivo*.

Figure 1 shows the mode of growth factor release at the arbitrary times described above. In profiles A, B and C growth factor release appears relatively correlated to (dependent on) the system used to produce PRP-gel. More intriguing features were the following: (i) Fibrinet-produced PRP-gel released promptly (within 20 min, GS1) large amounts of VEGF; (ii) RegenKit-produced PRP-gel released nearly no PDGF-BB and EGF immediately after gel formation, the release of these factors occurring after some delay (GS2, GS3); Plateltex-produced PRP-gel promptly released large amounts of PDGF-BB and of VEGF; this gel also released large amount of EGF only belatedly (GS3). The nature of these technique-dependent features was not clear. Yet, no specific literature exists about

the release of growth factors from gel; thus, our following attempts of explanation of these features are just inductive. Fibrinet did not employ exogenous thrombin to induce gelation. PRP was placed in calcium chloride-containing tubes and it was immediately centrifuged at high gravitational force. Owing to the presence of calcium, thrombin formation occurs in Fibrinet tube during the centrifugation phase. Thrombin formation takes some time at room temperature; thus platelet stress owed to high spin centrifugation occurs during thrombin formation and prior to fibrin polymerization. Since this was the major difference between Fibrinet and the other systems, one may speculate that prompt release of VEGF from Fibrinet PRP-gel might be somehow related to lack of polymerized fibrin during the centrifugation phase. The reason for delayed release of PDGF-BB and of VEGF from RegenKit gel seems still less clear. RegenKit was the only system among those investigated that did not employ the second centrifugation step. It is likely that less mechanically stressed platelets were embedded into the gel and this might be related to poor release of some factors. A more convincing hypothesis has to do with the calcium/ACD ratio. Since calcium was only added in the Regen thrombin ATS-tube, the calcium/ACD ratio in the PRP1 prior to gel activation was smaller than that in other PRPs. Since active release of many factors is calcium-dependent, some factor (PDGF-BB and EGF) may require more time to be released completely. The features observed in Plateltex, might have to do with the fibrinogen-cleaving enzyme, batroxobin. Since batroxobin cleaves only fibrinopeptide-a (while thrombin cleaves also fibrinopeptide-b), at initial step of fibrin polymerization (before added calcium induces formation of autologous thrombin) the structure of polymerized fibrin induced by batroxobin is likely to be slightly different from that induced by thrombin. This might be sufficient for prompt release of some factors (PDGF-BB and VEGF). Elucidation of late EGF release from Plateltex PRP-gel is somehow more complex. Hypothetically, biochemical basis for this high release might be inherent to complex relationship among fibrin-bound plasminogen, metalloproteinases, plasminogen activators (t-PA; u-PA) and EGF [23]. The fibrin–plasminogen–EGF interactions in batroxobin-induced fibrin might be slightly different from those occurring in thrombin-induced fibrin. These differences might make EGF more susceptible to protease-related release, late after gel formation have occurred (GS3), as suggested in a study reporting the release of EGF from fibrin glue in post-surgery healing [24].

In Fig. 1, profiles C, D and F showed individual dynamics of growth factors release which depends exclusively on type of growth factor and not on method. Each factor displayed a typical pattern: TGF-β (D); b-FGF (E); and IGF-I (F). Released TGF-β was mostly detectable in GS1 and, excepting Plateltex, in GS2. The release of IGF-I seemed specular to that of TGF-β; it was nearly undetectable in GS1 and in GS2, while high concentrations were detected in GS3. These particular patterns seem to reflect specific biodynamic of gel uptake and release of individual factors. Some hypotheses can be made. Early (GS1, GS2) bioavailability of TGF-β might be owed to the biochemical relationship between gel-bound fibronectin and TGF-β. Fibronectin also could be advocated to explain the feature of bioavailability of b-FGF. A role for FGF-binding protein (FGF-BP) might be assumed as well [25] although FGF-BP is known to be a tissue cell-produced proteins and few is known about a plasma protein form. Since many adhesion/binding activities are known to be conformational and calcium-dependent [26], we cannot exclude that process-induced variations of free-calcium ions modulated growth factor release from gels. IGF-binding proteins (IGFBPs) are high-affinity not-covalently binding proteins for IGF that regulate its activity at the cell level [27]. It is generally assumed that IGF-I found in PRP is that originally contained in the plasma moiety of PRP [28]. Our results confirmed in part such assumption. Nevertheless, in late supernatant (GS3) the concentration of IGF-I was higher than that determined in the PRP. It is probable that a time-dependent release of IGF-I from binding proteins occurred after gel formation, leading to large amounts of growth factors availability. Several IGFBPs proteinases are known to regulate the IGF bioavailability in the bloodstream and at the cellular level. Some of these proteinase can be constitutively associated to gel structure (serine proteinases, plasmin, thrombin, cathepsins, metalloproteinases) [29,30]. The protease-dependent bioavailability of IGF-I might explain why IGF-I was found at particularly high concentration in late gel supernatant (GS3). The intricate dynamics described so far leads to differentiated growth factor supply to tissues (Table 3). Early after PRP-gel generation, Fibrinet provided tissues mostly with VEGF and EGF; RegenKit with TGF-β; HM and Plateltex with blended PDGF-BB, VEGF and TGF-β. Providing PDGF-BB immediately after application of gel (HM and Plateltex) seems to be of clinical value considering the chemotactic and cell proliferation-inducing activity of these factors. After 24 h up to 7 days, PRP-gels from all systems have been shown to provide tissues with a mix of most platelet-derived growth factors. It is likely that this phase might mimic the physiological growth factor release which occurs during platelet-releasing reaction after acute wound. Heavy amounts of IGF-I were finally provided by all systems in late phase of growth factor release from PRP-gels. The total amount of growth factors provided by PRP-gels by systems under investigation over a period of 7 days after having processed a single tube of blood is resumed in Table 4. Fibrinet provided smaller amount of growth factors; Regen higher amount. Plateltex and HM provided approximately 30% less growth factor than RegenKit, with two consistent exceptions: b-FGF provided by Plateltex and by HM was 50% less than that provided by Regen; EGF provided by

Plateltex was significantly more than that provided by other techniques.

In conclusion the methods evaluated in this study were substantially similar. Each one of these methods had peculiar technical characteristic (disparity of tubes, centrifugation force, gel-inducing enzyme) cumulatively affecting the amount and the kinetics of the release of platelet-derived growth factors. In principle, from clinical point of view, a dose–response relationship over cell proliferation is expected [31] and relative amount of growth factors made available from platelet derivative seems to play a important role [32,33]. If the differences of the kinetics of growth factors availability to tissues in this study are clinically relevant, it is questionable. Albeit several significant differences have been shown in Table 4, clinical significances of these differences remain to be ascertained. It is possible that different kinetics of growth factors bioavailability, such as those seen in this study, might be more or less appropriate to treat different kinds of lesions or different kinds of tissues.

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