CORRESPONDENCE

Growth Factor Variation in Two Types of Autologous Platelet Biomaterials: PRP Versus PRF

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Abstract Autologous platelet biomaterials represent a key source of cytokines and growth factors extensively used for clinical and surgical applications involving tissue regeneration; wound healing and tissue repair. In this communication we discuss the growth factors released by activated platelet rich plasma (PRP) and platelet rich fibrin (PRF) releasate. Our study highlights that significantly higher growth factors (TGF- β 1) are released by activated PRP as compared to releasate of PRF. The various growth factors released by both platelet products are significantly higher than the baseline concentration in the whole blood and have different bio-mechanism hence should be individualized as per the clinical indication.

Keywords Platelet rich plasma (PRP) · Platelet rich fibrin (PRF) · Growth factors

Autologous platelet biomaterials represent an interesting source of cytokines and growth factors extensively used for clinical and surgical applications involving tissue regeneration; wound healing and tissue repair [1–3]. These varieties of platelet derivatives represent a promising therapeutic product for various periodontal, oral, maxillo-facial, orthopedic and dermatological procedures [4, 5]. Our correspondence represents part of initial results from our study on standardization of platelet rich plasma prepared in lab with quantification of growth factors released by them [6]. In this communication we discuss the growth

factors released by activated platelet rich plasma (PRP) and platelet rich fibrin (PRF) releasate.

Total 16 volunteers were enrolled; 8 were enrolled each for PRP and PRF preparation. Volunteers included in the study had not taken any platelets inhibitors (last 48 h) and anti coagulants and not suffering from any bleeding disorder. 10 mL of whole blood was collected from all the volunteers. PRP was prepared from whole blood (WB) with CPDA as the anticoagulant (AC) in the ratio of 10:1.4 mL (WB:AC). The algorithm adopted for preparation of both the product is elaborated in Fig. 1. PRP was prepared as explained in our recent study [6] where as PRF was prepared as explained in our previous communication [7]. The EDTA anticoagulated blood samples were collected from all the volunteers prior to procedure and cell counts were done using an automated cell counter (Sysmex KX-21, Japan) as well as supernatant (plasma) was stored at -70°C for base line growth factor quantification. Calcified Thromboplastin (Uniplastin by Tulip Diagnostics (P) Ltd. India) was used as an activator of PRP. Supernatant of PRF (releasate) and activated PRP were quantified for growth factors such as PDGF-AB, TGF- β 1 and VEGF. Commercial sandwich ELISA kits were used to quantify the concentrations of PDGF-AB (Platelet Derived Growth Factors-AB; Qayee-Bio, China), TGF- β 1 (Transforming growth factor- β 1; DRG; Germany) and VEGF (Vascular *endothelial growth factor*; Boster Immuno Leader, Boster Biological Technology, USA) in these specimens. These ELISAs were performed according to the manufacturer's instructions.

There was no statistical difference (Table 1; $p > 0.05$) in age, gender and baseline blood counts of volunteers in both the groups. Mean platelet count of the volunteers enrolled for PRP and PRF preparation [PRP group $218.6 \pm 72.2 \times 10^3/\mu\text{L}$; PRF group $234.7 \pm 46 \times 10^3/$

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Fig. 1 Algorithm adopted for preparation of both the product **Table 1** Demographic details of the volunteers

Parameter	PRP group	PRF group
Age (mean; years)	26.4	24.2
Gender	M:F-7:1	M:F-6:2
Cigarette smoking	None	None
RBC counts ($\times 10^6/\mu\text{L}$)	4.23 ± 0.7	4.11 ± 0.9
Platelets counts ($\times 10^3/\mu\text{L}$)	218 ± 72.4	234.7 ± 46
WBC counts ($\times 10^3/\mu\text{L}$)	6.38 ± 1	6.12 ± 0.8

μL]. The mean baseline concentration of growth factors present in the whole blood (EDTA anticoagulated) were 10.6 ± 12.7 ng/mL of PDGF-AB, 15.1 ± 16.4 ng/mL of TGF-β1 and VEGF 0.66 ± 0.4 ng/mL. The mean PDGF-AB, TGF-β1 and VEGF released by both the type of products and there statistical analysis (paired t-test) are discussed in Table 2.

TGF-β1 release was significantly higher from activated PRP than PRF where as concentration of PDGF-AB and VEGF release from both the product did not show any statistical difference. (Table 2) However both PDGF-AB and VEGF showed significant increase over the baseline whole blood values when released by activated PRP as compared to PRF (Fig. 2).

It is well established now that platelet derivatives play a key role in soft or hard tissue regeneration and healing [1]. The biological activity of these individual platelet biomaterials varies with respect to the type of product prepared, the methodology adopted to prepare them as well as their interplay with the biological tissue where they are administered for a clinical applications [7]. Centrifugation is one of the most critical steps in preparation of all these type of products from whole blood [6]. In our work we have analyzed the growth factors released by two types of platelet biomaterials (PRP and PRF) prepared at similar time and almost similar centrifugal force (440 vs 400 g).

PRF consists of an autologous leukocyte-platelet-rich fibrin clot, composed of a tetra molecular structure, with cytokines and platelets within it, which acts as a biodegradable scaffold (Fig. 3b) that favours the development of vascularization and is able to guide epithelial cell migration to its surface [8]. Release of PRF is the supernatant of the membrane of the PRF clot. The difference from natural blood clot and PRF is that the latter clot will offer more stable, easy to handle and homogeneous clot which can be placed locally at the indicated site [9]. Compared to platelet rich plasma is concentrated form of platelets prepared from whole blood, suspended in plasma

Table 2 Mean and SD of growth factor quantification and platelet counts of PRP and PRF

Growth factor	Baseline whole blood	Platelet rich plasma	Platelet rich fibrin
PDGF-AB (ng/mL)	10.6 ± 12.7	20 ± 7.5*	19.8 ± 9.9
TGF-β1 [#] (ng/mL)	15.1 ± 16.4	34.1 ± 16.3 [#]	2.3 ± 0.6
VEGF (ng/mL)	0.66 ± 0.4	2.7 ± 2.1*	1.3 ± 0.2
Platelet Counts (×10 ³ /μL)	218.6 ± 72.2	404.5 ± 171	Zero

[#] Signifies statistically higher ($p \leq 0.05$) growth factors released between both the products (activated PRP and PRF)

* Signifies statistically higher ($p \leq 0.05$) growth factors released by the platelet product compared with the base line values

Fig. 2 Analysis of growth factors released

and various other cellular components based on the method used to prepare them. The therapeutic action is provided by either activating them prior to administration or injecting them as it is and anticipating their activation on exposure to the tissue factor at the clinical site. Figure 3a represents the PRP clot post-activation by thromboplastin and overnight incubation at 4 °C. Thromboplastin activates the platelets in the PRP suspension and overnight incubation causes the clot to retract and release the growth factors in the supernatant.

Even with similar preparation protocols (Fig. 1) these products are used for variable clinical set of indication. PRF provides appropriate surface for tissue regeneration and healing hence have gained enormous amount of popularity among the dentistry [10, 11]. PRF also offers other advantages as it has a standard protocol for its preparation which gives a uniformity for results comparison whereas preparation protocols of PRP have seen great amount of variation in the scientific literature. PRF also does not require any external agent for activation which initially was used in the case of PRP.

The significant enrichment of PDGF-AB over the baseline by activated PRP plays a vital role when administered for wound healing and re-vascularization. PDGF stimulates bone cell replication as well as bone collagen synthesis [12, 13]. TGF-β1 is physiologically majorly released by platelets and bones. TGF-β1 stimulates osteoblast precursor cells as well as modulates bone matrix synthesis. It also regulates PDGF release as well as together plays an important role in new bone formation [8, 14]. VEGF in addition promotes angiogenesis and promote healing of chronic wounds and aid in endochondral ossification [9].

Some of the recent work [15, 16] comparing platelet derived biomaterial showed consistent results as our study, showing an increase of growth factors released by both of these biomaterials when compared with baseline but quantification of each growth factors varies with our results due to variation in preparation protocols used.

The exact biology of these products is still evolving in the current literature. Their exact understanding of interplay with the targeted tissue of therapeutic value is still to be completely understood. Our study highlights that

Fig. 3 **a** Platelet rich plasma (PRP clot post activation and overnight incubation), **b** platelet rich fibrin (PRF)

A Retracted Platelet Rich Plasma (PRP) clot post overnight incubation at 4–8 °C.
B Platelet Rich Fibrin (PRF) clot

significantly higher growth factors are released by activated PRP as compared to releasate of PRF. The various growth factors released by both platelet products are significantly higher than the baseline concentration in the whole blood. Moreover these biomaterials possess different bio-mechanism even when prepared similarly hence should be individualized as per the clinical indication.

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Compliance with Ethical Standards

Conflict of interest None.

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