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ORIGINAL ARTICLE

Quantification of growth factors in different platelet concentrates

Jing Qiao[1], Na An[2], & Xiangying Ouyang[3]

[1]Department of Periodontology, The First Clinical Division, Peking University School and Hospital of Stomatology, Beijing, China, [2]Department of General Dentistry II, Peking University School and Hospital of Stomatology, Beijing, China, and [3]Department of Periodontology, Peking University School and Hospital of Stomatology, Beijing, China

Abstract

Concentrated growth factor (CGF), a new generation of platelet concentrate product, appears to have more abundant growth factors because of its special centrifugation process. However, there are few studies supporting this. This study was designed to evaluate the contents of major growth factors in CGF and compare them with those found in PRP (platelet-rich plasma) and PRF (platelet-rich fibrin). PRP, PRF, and CGF were obtained from the same samples of peripheral blood. Concentrations of five representative growth factors in platelets were measured with enzyme-linked immunosorbent assay (ELISA): platelet-derived growth factor-BB (PDGF-BB), transforming growth factor β-1 (TGF-β1), insulin-like growth factor-1 (IGF-1), vascular endothelial growth factor (VEGF), and basic fibroblast growth factor (bFGF). The results showed that the bFGF levels in CGF and PRF were significantly higher than that in activated PRP. For other growth factors, such as PDGF-BB, TGF-β1, IGF-1, and VEGF, the levels did not differ significantly among activated PRP, PRF, and CGF. Our findings extended the currently available data on the release and measurement of growth factors in CGF and other platelet gels. In future studies, we need more data to find the proper therapeutic doses for platelet concentrates suitable for different clinical applications.

Keywords

Concentrated growth factors, growth factors, platelet concentrates, platelet-rich fibrin, platelet-rich plasma

History

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Introduction

Periodontitis is an inflammatory disease that leads to the loss of tooth-supporting tissues. The ultimate goal of treatment of periodontitis is complete and predictable periodontal tissue regeneration. As signaling molecules, polypeptide growth factors control each stage of tissue regeneration [1]. In vitro recombination of exogenous growth factors is time-consuming and expensive, and its safety is still under research [2]. Besides, the complex interaction and network between endogenous growth factors cannot be simulated in vitro [3]. Therefore, how to get abundant endogenous growth factors has become one of the focuses in the field of tissue engineering.

Platelet is one of the major resources of autogenous growth factors [4]. Platelet-rich plasma (PRP) was the first generation of platelet gels for periodontal regeneration therapy [5]. While the potential benefits of this procedure have been criticized, many of the discrepancies are likely more related to the lack of more suitable standardization methods and definition of different PRP preparations than to any functional deficiencies, as the protocols and biological and surgical techniques differ widely between different research groups [6,7].

Platelet-rich fibrin (PRF), the second generation of platelet concentrates, has the same properties as PRP with the advantages of osteogenicity [8,9]. The preparation process of PRF is simple and without the addition of bovine thrombin and anticoagulant drugs, because PRF is derived from autologous blood [10,11].

Concentrated growth factor (CGF) is a novel generation of platelet concentrate product [12]. CGF is made by centrifuging blood samples at alternating and controlled speeds using a special centrifuge (Medifuge, Silfradentsrl, Italy) [13]. Different centrifugation speeds permit the isolation of a much larger and denser fibrin matrix with abundant growth factors. Rodella et al. observed the presence of transforming growth factor β-1 (TGF-β1) and vascular endothelial growth factor (VEGF) in CGF and red blood cell layers [14].

In theory, CGF appears to have more abundant growth factors because of its special centrifugation process. However, there are few studies supporting this. The purpose of this study was to evaluate the contents of major growth factors in CGF and compare them with those found in PRP and PRF.

Materials & methods

Volunteers

Twenty healthy volunteers (12 males and 8 females with an average age of 41.3 years) were included in this study. All volunteers were informed of the nature of this study and signed an informed consent prior to their inclusion. The study was performed in accordance with the Helsinki Declaration of 1975, as revised in 2000, and the study protocol was reviewed and approved by the university ethical board (Peking University, School and Hospital of Stomatology). Exclusion criteria consisted of donors with systemic diseases, pregnant and/or lactating women, patients taking any drug known to affect the number or

Correspondence: Xiangying Ouyang, Department of Periodontology, Peking University School and Hospital of Stomatology, 10008122 Zhongguancun South Avenue, Haidian District, Beijing, China. E-mail: kqouyangxy@bjmu.edu.cn

function of platelets in the past 3 months, and patients with abnormal platelet counts.

PRP preparation

PRP was obtained through two separate centrifuge phases. Firstly, 10 mL of venous whole blood was drawn from each donor by venepuncture of the antecubital vein under the condition of empty stomach in the morning. The blood was collected in a sterile centrifuge tube (Greiner Bio-One, GmbH, Kremsmunster, Austria) containing 10% trisodium citrate solution as an anticoagulant. Then, the tubes were centrifuged at 1600g for 6 min (Silfradent, Italy), which resulted in the separation of two layers. The upper layer was plasma, platelets, and white blood cells (WBC), followed by the red blood cell (RBC) layer at the bottom. Then, the top layer and 3 mm of the RBC layer were aspirated with a Pasteur pipette and centrifuged at 900g for 10 min (Silfradent, Italy). Two basic fractions were obtained: platelet-poor plasma (PPP) on the top, followed by PRP (a mixture of platelet concentrates and a small amount of red blood cells and white blood cells) at the bottom.

Most PPP was aspirated and stored at -80°C for use. The remaining 2 mm PPP and PRP were mixed and collected. Then, 10% calcium chloride solution containing 1000U/mL bovine thrombin (Sigma) was added at the ratio of 10:1, stored in 4°C overnight and then centrifuged at 1000 g for 20 min (Eppendorf centrifuge, Germany). The supernatant was aspirated and stored at -80°C for the measurements of contents of growth factors.

CGF preparation

CGF was produced as follows: 9 mL of blood was drawn from each donor by venepuncture of the antecubital vein under the condition of empty stomach in the morning. The blood was collected in sterile Vacuette tubes (Greiner Bio-One, GmbH, Kremsmunster, Austria) without anticoagulant solutions. These tubes were then immediately centrifuged (Medifuge, Silfradentsrl, Sofia, Italy) using a program with the following characteristics: 30 sec acceleration, 2 min at 2700 rpm, 4 min at 2400 rpm, 4 min at 2700 rpm, 3 min at 3000 rpm, and 36 sec deceleration and stop. At the end of the process, three blood fractions were created:(1) a superior phase represented by the serum (blood plasma without fibrinogen and coagulation factors, platelet-poor plasma, PPP); (2) an interim phase represented by a very large and dense polymerized fibrin block containing the CGF, white blood cells and stem cells; and (3) the lower red blood cell (RBC) layer.

The PPP layer was aspirated and stored at -80°C. The fibrin block with red interface was cut out as CGF. Then, obtained CGF was minced into pieces of 1~2 mm and transferred into cryogenic vials. The vials were immersed in liquid nitrogen for 5 min and quickly warmed at 37°C for 5 min, three times [15]. Vials were then centrifuged at 1000 g for 20 min at 4°C (Eppendorf centrifuge, Germany). The supernatant was aspirated and stored at −80°C for the measurement of growth factors.

PRF preparation

About 9 mL blood was drawn from each donor by venepuncture of the antecubital vein under the condition of empty stomach in the morning. The blood was collected in sterile Vacuette tubes (Greiner Bio-One, GmbH, Kremsmunster, Austria) without anticoagulant solutions. PRF was developed by centrifuging the blood for 12 minutes at 2700 rpm (Eppendorf, Germany). The centrifuge process resulted in the fibrin clot (PRF) in the middle of the tube, between the RBC layer at the bottom and PPP at the top.

The PPP layer was aspirated and stored at -80°C. The fibrin block with red interface was cut out as PRF. Then, obtained PRF was minced into pieces of 1~2 mm and transferred into cryogenic vials. The vials were immersed in liquid nitrogen for 5 min and quickly warmed at 37°C for 5 min, three times [15]. Vials were then centrifuged at 1000 g for 20 min at 4°C (Eppendorf centrifuge, Germany). The supernatant was aspirated and stored at −80°C for the measurement of growth factors.

Quantification of growth factors

The levels of five representative growth factors in platelets were evaluated using double antibody sandwich enzyme-linked immunosorbent assay (ELISA) (R & D, Minneapolis, MN, USA): platelet-derived growth factor-BB (PDGF-BB), transforming growth factor β-1 (TGF-β1), insulin-like growth factor-1 (IGF-1), vascular endothelial growth factor (VEGF), and basic fibroblast growth factor (bFGF). OD values were measured under 450 nm wavelength absorbance using microplate reader (Bio-Rad, USA). Growth factor concentrations were measured according to the manufacturer's instructions. Triplicates were performed for all assays.

Statistical analysis

All reported values are the means of triplicate samples, and tests were repeated twice. Data were analyzed using SPSS version 10.0 (Chicago, IL, USA). Statistical analysis of the data was performed by one-way analysis of variance (ANOVA) followed by the Tukey's post hoc test for comparisons. The levels of growth factors in PPP from each preparation procedure, activated PRP, PRF, and CGF were compared with significance assigned at the $p \leq 0.05$ level.

Results

The PDGF-BB levels

There was no significant difference between the levels of PDGF-BB in PPP from each group ($p > 0.05$) (Figure 1). In activated PRP, the PDGF-BB level was 155.20 ± 57.67 ng/mL, which was 1.85 times higher than that in PPP (54.40 ± 28.95 ng/mL) ($p < 0.001$). In PRF and CGF, this growth factor level was 146.36 ± 52.31 ng/mL and 175.10 ± 57.09 ng/mL, respectively, which were 1.79 times and 2.84 times condensed than in PPP ($p < 0.001$). There was no significant difference between the levels in activated PRP, PRF, and CGF ($p > 0.05$).

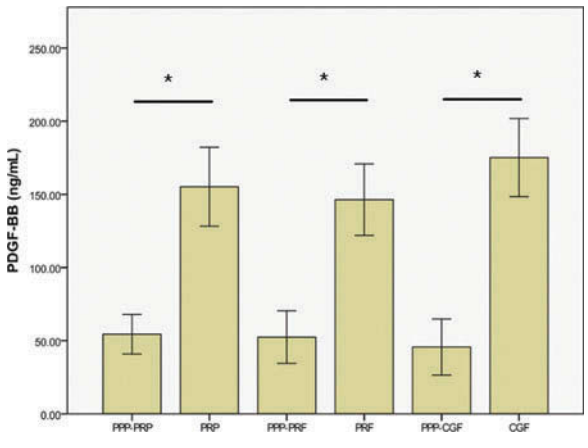

Figure 1. PDGF-BB concentrations in PRP, PRF, and CGF. *: $p < 0.001$.

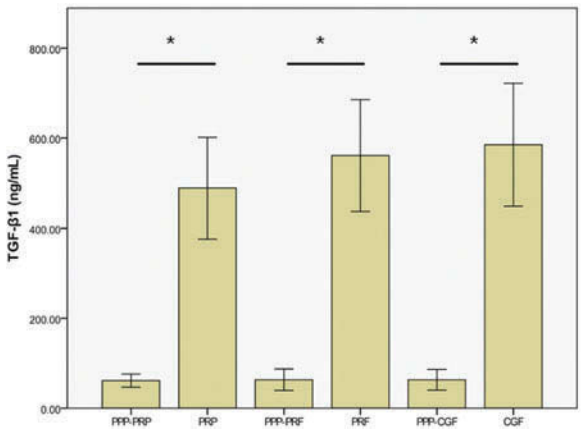

Figure 2. TGF-β1 concentrations in PRP, PRF, and CGF. *: $p < 0.001$.

The TGF-β1 levels

As shown in Figure 2, there was no significant difference between the levels of TGF-β1 levels in PPP from each group ($p > 0.05$). In activated PRP, the TGF-β1 level increased to 488.76 ± 240.77 ng/mL, which was 6.97-fold higher than that in PPP (61.30 ± 31.06 ng/mL) ($p < 0.001$). TGF-β1level was increased 7.87-fold from PRF (560.81 ± 265.91 ng/mL) compared with PPP (63.25 ± 51.07 ng/mL) ($p < 0.001$). And TGF-β1 level in CGF was 8.25 times higher (584.89 ± 292.50 ng/mL) than that in PPP (63.20 ± 49.31 ng/mL) ($p < 0.001$). There was no significant difference in TGF-β1 levels between activated PRP, PRF, and CGF ($p > 0.05$).

The IGF-1 levels

The IGF-1 level in PPP from preparation of PRP was 77.59 ± 44.24 ng/mL, which did not differ significantly among PPP from preparation of PRF and CGF (82.58 ± 54.82 and 80.26 ± 51.50 ng/mL, respectively) ($p > 0.05$) (Figure 3). In contrast, the IGF-1 levels in activated PRP, PRF, and CGF were 236.07 ± 222.10, 274.36 ± 212.14, and 321.42 ± 150.30 ng/mL, respectively, which were 3.04, 3.32, and 4 times higher than that in PPP, respectively ($p < 0.001$). The levels of IGF-1 did not differ significantly among different platelet concentrates ($p > 0.05$).

The VEGF levels

The VEGF levels in activated PRP, PRF, and CGF were 242.29 ± 97.64, 259.39 ± 172.79, and 238.14 ± 149.89 pg/mL, respectively, with no statistically significant difference ($p > 0.05$) (Figure 4). In contrast, VEGF levels in PPP from different preparation were 51.68 ± 44.59, 54.19 ± 43.30, and 57.92 ± 56.55 pg/mL, respectively.

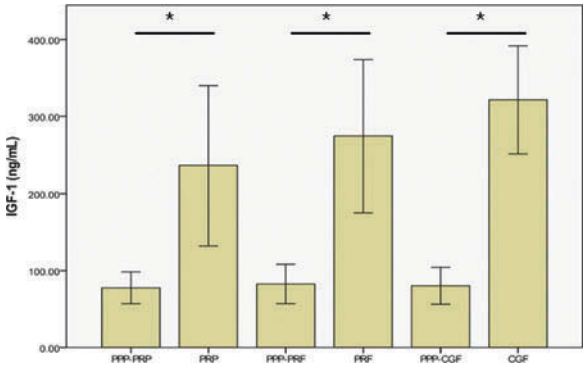

Figure 3. IGF-1 concentrations in PRP, PRF, and CGF. *: $p < 0.001$.

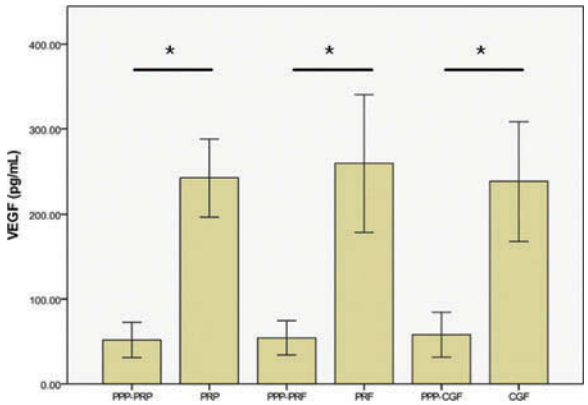

Figure 4. VEGF concentrations in PRP, PRF, and CGF. *: $p < 0.001$.

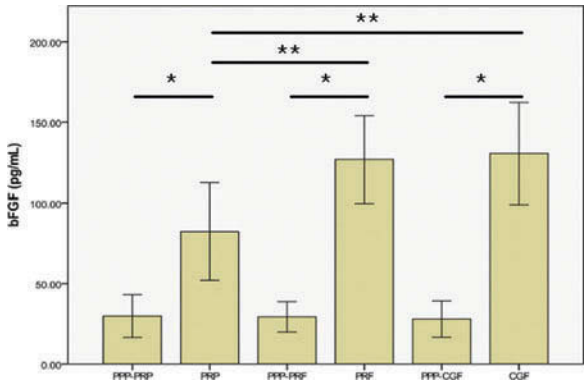

Figure 5. bFGF concentrations in PRP, PRF, and CGF. *: $p < 0.001$; **: $p < 0.01$.

Thus, the fold increase in activated PRP, PRF, and CGF was determined as 3.69-, 3.79-, and 3.11-fold ($p < 0.001$).

The bFGF levels

The bFGF levels were also significantly concentrated in activated PRP, PRF, and CGF ($p < 0.001$) (Figure 5): 82.24 ± 64.51, 126.86 ± 58.08, and 130.56 ± 67.66 pg/mL, respectively. While the average levels of bFGF in PPP from different preparation were 29.81 ± 28.53, 29.30 ± 20.31, and 27.93 ± 24.18 pg/mL, respectively, with no statistically significant difference ($p > 0.05$). Furthermore, the bFGF levels in PRF and CGF were significantly higher than that in activated PRP ($p < 0.01$), and the bFGF level in PRF did not differ significantly from that in CGF ($p > 0.05$).

Discussion

CGF has been thought, but not well demonstrated, to contain higher concentrations of certain growth factors due to its special centrifuge. Our findings extend the currently available data on the release and measurement of growth factors in CGF and other platelet gels.

In the present study, we have clearly demonstrated that CGF is a mixture of concentrated growth factors. However, in the growth factors we detected, only the bFGF levels in CGF and PRF were significantly higher than that in activated PRP. For other growth factors, such as PDGF-BB, TGF-β1, IGF-1, and VEGF, the levels did not differ significantly among activated PRP, PRF, and CGF.

Discussion of the methods

First, it may be due to the difference in the secretion mechanism from platelets or to the small sample size.

PRP works via the rapid clotting process of blood initiated by exogenous bovine thrombin and begins within 10 minutes after clotting. More than 95% of the presynthesized growth factors are secreted within 1 hour [16].

Unlike PRP, CGF does not dissolve rapidly following application. Instead, the strong fibrin gel in the matrix addition is slowly remodeled in a similar manner to a natural blood clot. Platelets are activated in contact with the glass wall initially and release the coagulation cascade [17]. The alternated and controlled speed centrifugation speed permits the isolation of a larger and denser fibrin matrix compared to PRF. A large number of platelets are caught in the fibrin network, which make the release of growth factors more slowly. Thus, CGF prolongs the duration of growth factor activity. Dohan et al found that PRF could continue to release growth factors for at least one week [18]. Qin et al. proved that CGF could release TGF-β1 over a sustained period of time (at least 13 days) [19]. Furthermore, the differential centrifugation steps during preparation process of CGF can make constant collision and ruptures of platelets, which will also improve the release of growth factors [20]. In theory, CGF could become a powerful bio-scaffold with an integrated reservoir of growth factors.

Kim et al evaluated the effect of PRP, PRF, and CGF on bone healing. Their results showed that the addition of PRP, PRF, and CGF had significantly increased bone formation at the 6th week, and the effect of PRP, PRF, and CGF was similar [21]. Further studies should be performed to evaluate the effects of different platelet concentrates in tissue regeneration and find the relevance between growth factor concentrations and final results.

Second, freeze–thaw method was used in the present study to activate platelets in PRF and CGF thoroughly. It is indicated that, as a kind of pure physical method, freeze–thaw method can activate platelets safely and effectively [22]. Li et al compared the effect of freeze–thaw-activated PRP and bovine thrombin-activated PRP on the release of growth factors. They found no significant difference between the concentrations of PDGF-AA and TGF-β1 in the two kinds of PRP [23]. And in another study, Wen et al proved that both freeze–thaw-activated and thrombin-activated PRP could promote the proliferation of human dental pulp cells. Besides, the former showed stronger stimulatory effects on cell proliferation than the latter [24]. However, some authors found that the activation methods of platelets differed in terms of growth factor output. Textor et al activated PRP by four methods: autologous thrombin, bovine thrombin, calcium chloride ($CaCl_2$), or freeze–thaw. The resultant PDGF-BB and TGF-β1 levels in PRP releasates were compared. Their results showed that $CaCl_2$ activation of PRP yielded significantly greater PDGF-BB release than did any other method. TGF-β1 release was comparable after PRP activation by four different methods [25]. More sophisticated studies are needed to identify or explain the discrepancy between different literatures. However, it is possible that freeze–thaw method used in the present study might partly affect the output or evaluation of growth factors in PRF and CGF. More effective method should be explored to reflect the levels of growth factors in PRF and CGF accurately.

Third, previous reports have showed that the distribution of growth factors is not uniform in PRF and CGF. Nishimoto et al found that concentrations of growth factors were the highest at bottom part of PRF. Their histological observation revealed that platelets were dense at interface of yellow and red part of the PRF [26]. Rodella et al evaluated TGF-β1 and VEGF expression in CGF, and the immunohistochemical results showed a widespread immunostaining in both CGF and RBC layers [12]. In the present study, the fibrin block with red interface was cut out as PRF or CGF. Most part of RBC layer was discarded before freeze–thaw activation method avoiding hemolysis. The growth factors stored in RBC layer might be partly lost, which could affect the result.

Discussion of the results

In the present study, we have clearly demonstrated that PRP, PRF, and CGF contain abundant growth factors. It is well known that these growth factors, in turn, set the stage for tissue healing and regeneration. They play roles at very specific levels.

In a study, various concentrations of PDGF-BB were added, and fibroblast adherence and cell morphology were determined after 24 hours. The result showed that the optimal concentration of PDGF-BB for inducing the periodontal ligament fibroblasts to adhere to periodontitis-affected root surfaces was 50 ng/mL [27]. IGF-1 also had dose-dependent chemotactic effects on osteoblasts [28]. There are also similar trends in PRP, PRF, and CGF. In vitro, there is a dose–response relationship between platelet concentration in PRP and the proliferation of human adult mesenchymal stem cells, the proliferation of fibroblasts, and the production of type I collagen [29,30]. In vivo, PRP may exert positive effects on intestinal anastomotic healing in a dose-dependent manner up to a certain level, but adverse effects occur when it is highly concentrated [31]. In a previous study, we demonstrated that in a range of concentrations, CGF significantly promoted the proliferation and alkaline phosphatase activity of human periodontal ligament cells in a dose-dependent manner [32]. In future studies, we need more data to find the proper therapeutic doses for platelet concentrates suitable for different clinical applications.

Conclusion

Within the limitation of this study, it can be concluded that PDGF-BB, TGF-β1, IGF-1, VEGF, and bFGF are surely concentrated in CGF. Furthermore, bFGF levels in CGF and PRF were significantly higher than that in activated PRP. As a storage vehicle for growth factors, CGF is a new application of tissue engineering and regeneration. The ease and rapidity of processing CGF holds promise for clinical applications. More well-designed studies are needed to provide solid evidence of CGF's capacity for wound healing and tissue regeneration.

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Declaration of interest statement

The authors declared that there is no conflict of interest in this study. This work was supported by National Natural Science Foundation of China No. 81600868.

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