Original Article

The effect of adding platelet-rich plasma to fibrin glue on release of platelet growth factors and its stability

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Abstract:

Background: *Fibrin glue is a commonly used hemostatic agent.* ***Objective:*** *The aim of the study was to determine the effect of adding platelets to fibrin glue concerning the release of platelet growth factors (PGF) and its stability.* ***Methods:*** *Platelet-rich plasma (PRP) was added to a fibrinogen solution and mixed with 250 IU/mL thrombin solution to form fibrin glue. The contents in the completely formed glue were squeezed out at 5, 60, 120, and 300 minutes to determine PGF. Then the stability of fibrin glue with and without platelets in plasma, urine and saliva was studied.* ***Results:*** *Fibrin glue with platelets showed increased amounts of platelet-derived growth factor (PDGF)-AB and PDGF-BB by time-dependent fashion. The fibrin glue with platelets showed less reduced mass, reflecting better stability, compared with that without platelets in plasma, urine and saliva.* ***Conclusion:*** *Adding PRP to the fibrin glue released PGF and enhanced its stability.*

Keywords : ● *Platelet growth factors* ● *Platelet-rich plasma* ● *Fibrin glue*

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นิพนธ์ต้นฉบับ

ผลการเติมพลาสมาที่มีเกล็ดเลือดสูงในกาวไฟบรินต่อการหลั่ง growth factors จากเกล็ดเลือดและความคงทนของกาวไฟบริน

สุทธิกานต์ สันติวัฒนา วีระศักดิ์ ศาสนกุล ประกายวรรณ เกษเกษม และ อำไพวรรณ จวนสัมฤทธิ์
ภาควิชากุมารเวชศาสตร์ คณะแพทยศาสตร์โรงพยาบาลรามาธิบดี มหาวิทยาลัยมหิดล

บทคัดย่อ

บทนำ *กาวไฟบรินเป็นสารห้ามเลือดที่ใช้บ่อย* ***วัตถุประสงค์*** *ศึกษาผลของการเติมเกล็ดเลือดในกาวไฟบริน ต่อการหลั่ง growth factors และความคงทนของกาวไฟบริน* ***วิธีการศึกษา*** *เติมเกล็ดเลือดในไฟบริโนเจนก่อนนำไปผสมกับธร็อมบิน 250 ยูนิต/มล. ทำให้เกิดกาวไฟบรินขึ้น หลังจากนั้นบีบเค้นของเหลวจากกาวไฟบรินที่เวลา 5, 60, 120 และ 300 นาที นำไปวัดระดับ platelet growth factor (PGF) และศึกษาความคงทนของกาวไฟบรินที่เติม และไม่เติมเกล็ดเลือดในพลาสมา ปัสสาวะและน้ำลาย* ***ผลการศึกษา*** *กาวไฟบรินที่เติมเกล็ดเลือด มีระดับ PDGF-AB และ PDGF-BB เพิ่มขึ้นตามระยะเวลา และเปรียบเทียบความคงทนของกาวไฟบรินที่เติมเกล็ดเลือด และไม่เติมเกล็ดเลือดในพลาสมา ปัสสาวะ และน้ำลาย ปรากฏว่า กาวไฟบรินที่เติมเกล็ดเลือด มีความคงทน แสดงได้จากน้ำหนักกาวไฟบรินที่ลดลงน้อยกว่ากาวไฟบรินที่ไม่เติมเกล็ดเลือด* ***สรุป*** *การเติมเกล็ดเลือดในกาวไฟบรินจะเพิ่มการหลั่ง PGF และมีความคงทนมากขึ้น*

คำสำคัญ : ● *Platelet growth factors* ● *Platelet-rich plasma* ● *Fibrin glue*

วารสารโลหิตวิทยาและเวชศาสตร์บริการโลหิต 2561;28:443-8.

Introduction

Fibrin glue is a blood-derived topical hemostatic agent commonly used in various procedures to control bleeding.[1-5] Similar to the propagation phase of the coagulation pathway, fibrin glue is obtained by mixing a fibrinogen solution with a thrombin solution. Many formulations of fibrin glue also contain antifibrinolytic agents such as tranexamic acid or aprotinin to prolong its stability.[6] Fibrin glue is nontoxic, biodegradable and does not induce tissue necrosis.[7] Commercial fibrin glue is expensive, approximately 50 USD/mL[8]; therefore, its application may be limited.

Thrombin not only activates fibrinogen to fibrin but also activates platelets. Through physiologic wound healing and the repairing process, platelet activation results in aggregation and degranulation releasing various growth factors necessary for wound repair. For example, platelet-derived growth factor (PDGF), transforming growth factor-β (TGF-β) and vascular endothelial growth factor (VEGF) are able to stimulate cell proliferation, matrix remodeling and angiogenesis.[9] By combining platelet-rich plasma (PRP) with calcified thrombin, the soft, gelatin-like biomaterial called platelet gel is formed. The use of platelet gel is restricted by its reduced strength, and unlike fibrin glue, platelet gel is produced exclusively from donor platelet concentrates causing questionable consistency.[10,11]

This study aimed to determine the effect of adding platelets to fibrin glue concerning the release of PDGF and TGF, the two most abundant platelet growth factors (PGF), and its stability in human body fluids.

Materials and Methods

The study was approved by the Faculty Ethics Committee (ID 02-57-09). All participants provided written consent before enrollment. Fibrinogen and human thrombin solutions were provided by TISSEEL Fibrin Sealant Kit (Baxter Healthcare Corporation, CA, USA). The fibrinogen solution contained 149 mg/mL of fibrinogen and 3,000 KIU/mL of aprotinin. Platelet-rich plasma (PRP) was prepared by centrifuging the citrate whole blood at 180 g for 10 minutes from one healthy volunteer. In PRP, fibrinogen content was 2.0 mg/mL and platelet count was 212 x10^9/L. Then 2 mL of fibrinogen solution were mixed gently with an equal volume of PRP at room temperature before using as fibrinogen solution with platelets. Equal volume of PRP was used to demonstrate the effect of a 50% reduction of the use of commercial fibrin glue solution. Another 4 mL of fibrinogen solution without platelets was used as control. Additionally, 4 mL of 500 IU/mL thrombin solution was diluted with an equal volume of 40 mM calcium solution resulting in 250 IU/mL. The study consisted of two parts: part 1, determining PGF in the fibrin glue and part 2, determining the stability of fibrin glue in human body fluids.

Part 1 Determining PGF in fibrin glue

Four samples of each fibrin glue were prepared from 100 mcL of fibrinogen solution with platelets mixed with 100 mcL of thrombin solution in sterile polypropylene tubes placed in a 37°C water bath. The content from each completely formed glue was squeezed out at the specific times of 5, 60, 120, and 300 minutes, successively and stored at -80°C until determining PGF using specific Quantikine, enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, MN, USA.). The detection limit was 1.14 pg/mL for PDGF-AB, 15 pg/mL for PDGF-BB, 4.61 pg/mL for TGF-β1 and 7.0 pg/mL for TGF-β2. The PRP sample from the same volunteer was used as control, and the assays were determined in duplicate.

Part 2 Stability of fibrin glue in human body fluids

Four healthy individuals (2 males, 2 females) with a median age of 27 years old were enrolled in the study. Plasma, urine and saliva samples were obtained in the morning after withholding oral food and water overnight. Blood was drawn freely without applying pressure using the two-syringe technique. The first syringe of 3 mL of blood was collected in EDTA for complete blood count (CBC) and the second syringe of 10 mL of blood was collected in citrate buffer at a 9:1 ratio. Plasma was separated after centrifugation at 2,000 g for 15 minutes. Midstream urine samples were obtained and filtrated

Table 1 The amount of platelet-derived growth factor (PDGF)-AB, PDGF-BB, transforming growth factor (TGF)-β1 and TGF-β2 in the squeezed-out content of fibrin glue with platelets; platelet-rich plasma (PRP) was used as control

Platelet growth factors	5 minutes		60 minutes		120 minutes		300 minutes		*P**
	Mean	Control	Mean	Control	Mean	Control	Mean	Control	
PDGF-AB (pg/dL)	607.7	27.1	902.3	30.2	851.5	32.4	947.1	36.6	0.012
PDGF-BB (pg/dL)	176.3	225.5	418.6	177.6	395.5	221.0	503.0	238.1	0.036
TGF-β1 (pg/dL)	3,604.8	18,065.3	3,582.2	15,393.4	3,478.5	14,425.4	3,375.4	14,825.7	0.012
TGF-β2 (pg/dL)	171.4	49.9	156.8	44.0	124.5	40.8	156.8	45.5	0.012

*comparison between fibrin glue with platelets and control

through 0.2 micron filter paper. Saliva samples of 10 mL were collected in a sterile container. Specific gravity and pH of each sample were determined using a standard refractometer and pH strip, respectively.

Two mL of fibrinogen solution with platelets and 2 mL of thrombin solution were mixed. Another mixture of 2 mL of fibrinogen solution without platelets and 2 mL of thrombin solution was used as control. The mixing process was performed in a square block, and fibrin glues with and without platelets of 5 mm thickness were formed. After 15 minutes, the well-formed glues were cut in cubic shapes of 5 mm for width, length and height. One piece of glue was put in each tube of 1 mL of plasma, urine and saliva samples from each subject and kept in a 37°C water bath. Glue in a blank tube without any fluid was used as control. The weight of each glue sample was measured before and at 24-hour intervals for the dissolution of glue totaling five days. All tests and controls were performed in duplicate.

Statistical analysis

Statistical analyses were performed using SPSS Software, Version 22.0. The comparison of the amounts of PGF and reduced mass of fibrin glues were determined using the Mann-Whitney *U* test and paired T-test, respectively. Statistical significance was accepted at a p-value < 0.05.

Results

The levels of PDGF-AB, PDGF-BB and TGF-β2 in the squeezed-out content of fibrin glue with platelet were statistically significant higher than those of PRP, used as control, as shown in Table 1. Levels of PDGF-AB and PDGF-BB increased in time-dependent fashion while the level of TGF-β2 was also increased but without significant increase over time. No increase of TGF-β1 was observed in glue with platelet but was significantly lower than those of PRP. PRP had all measurable levels of growth factors but without increase of each factor over time.

Normal results of CBC, coagulogram and urinalysis were found among the four healthy subjects. The platelet counts and fibrinogen in the plasma ranged from 1,000 to 7,000/mcL and 1.65 to 2.26 mg/mL, respectively. Specific gravity and pH of urine samples ranged between 1.010-1.020 and 5 to 9, respectively. Specific gravity and pH of saliva ranged between 1.001-1.002 and 7.5 to 8, respectively.

In plasma, the reduced mass of fibrin glue without platelets was significantly higher than that with platelets on Day 1 (8.4% *vs.* 3.2%, $p = 0.003$). At the end of the experiment (Day 5), the reduced mass of fibrin glue without platelets was still significantly higher than that with platelets (15.9% *vs.* 9.5%, $p = 0.002$) as shown in Figure 1. Similar results were also shown in urine samples. On Day 1, the reduced mass of fibrin glue without platelets was significantly higher than that with platelets (6.7% *vs.* 0.05%, $p = 0.003$). On Day 5, the reduced mass of glue without platelets was still higher than that with platelets (15.2% *vs.* 0.6%, $p = 0.041$). For the saliva samples, both glues with and

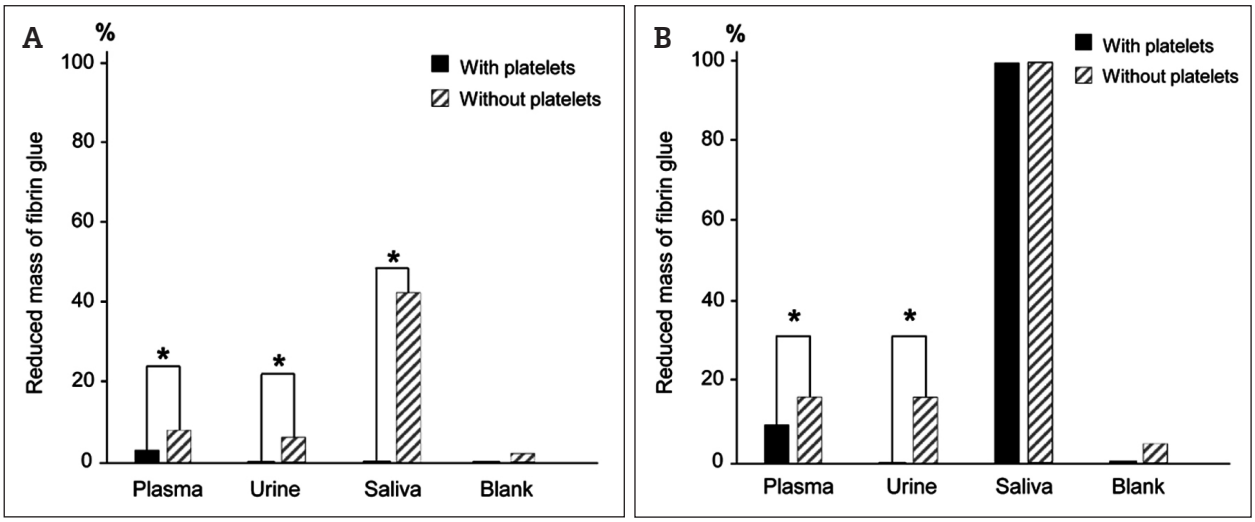

Figure 1 Reduced mass of fibrin glues with or without platelets in plasma, urine, saliva and blank at (**A**) 24 hours and (**B**) 120 hours. (*p value < 0.05)

without platelets completely resolved within five days. However, the rate of dissolution was faster for the glue without platelets. On Day 1, the reduced mass of glue without platelets was significantly higher than that with platelets (43.5% *vs.* 3%, $p = 0.013$) and completely resolved on Day 4 and Day 5, respectively.

Discussion

Fibrin glue was introduced several decades ago. Now it has become a common surgical sealant used in various types of operations to stop bleeding, seal suture lines and air leaks and support wound repair or graft placement. It has proved useful during surgical procedures among patients with bleeding disorders such as hemophilia.

Thrombin is the most potent platelet activator. After thrombin activation, platelets aggregate to form plugs, becoming activation sites for the coagulation cascade and produce various growth factors. PDGF and TGF are the two most abundant components contained within alpha-granules of platelets. PDGF-AB and PDGF-BB have the highest affinity to the PDGF receptor. Activation of PDGF receptors subsequently regulates various expressions responsible for physiological responses, such as cell proliferation, cytoskeleton rearrangements and, fibroblast chemotaxis. TGF-β1 is the predominant form of the TGF-β family. TGF-β induces chondoblasts and mesenchymal cell proliferation and produces extracellular matrix.[12-14]

The addition of platelets to fibrin glue in the current study resulted in releasing more growth factors, similar to a related study.[15] PGFs stimulate and recruit more platelets to aggregate as a meshwork that strengthen the fibrin clot. PGFs are able to stimulate cell proliferation, matrix remodeling and angiogenesis to enhance wound healing.[9] The levels of growth factors in PRP, used as control, were significantly lower than those of the squeezed-out contents of fibrin glue with platelet except for TGF-β1. The storage of PRP at -80°C before the growth factor determination and the process of platelet preparation could stimulate the platelets.[16] However, the sample size of the current study was rather small. The specific cause of the elevated levels of TGF-β1 could not be definitely identified.

After the fibrinogen solution with and without platelets was mixed with thrombin solution, both glues with and without platelets were well-formed within five minutes. Their appearances were similar except for the more yellowish color of fibrin glue with platelets due to the presence of plasma. Fibrin glue with platelets showed less reduced mass, reflecting better stability, compared with those without platelets in all human fluids.

The cost of the commercial fibrin glue containing PRP and dilution of thrombin with calcium solution

decreased by 50%. The concentration of thrombin at 250 IU/mL was sufficient to induce fibrin crosslinking and also activate platelets to aggregation and degranulation leading to the release of various growth factors necessary for wound repair. Only 0.5 to 1 mL of PRP was needed for the fibrin glue preparations of 2 mL and 4 mL, respectively. Therefore, autologous platelets can be used to reduce the risk of transfusion-transmitted diseases, except for those presenting platelet dysfunction. We acknowledged that platelet numbers in the test system could have affected the results. Further study using titration of PRP amount is needed. The limitation of this study was the small sample size. Further clinical trials are needed to emphasize its application.

In conclusion, adding PRP to fibrin glue released PGF and enhanced the stability of the fibrin glues in human body fluids. The cost of commercial fibrin glue could be decreased by 50%.

Declaration of Interest

All authors declare having no conflicts of interest.

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