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# The effect of anticoagulants on the quality and biological efficacy of platelet-rich plasma

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**Abstract**

**Objectives:** This study investigated the effect of anticoagulants on platelet-rich plasma (PRP) quality to determine the appropriate anticoagulants for PRP production.

**Design and methods:** This study was carried out at the Plastic Surgery Hospital of Peking Union Medical College. The microstructure of platelets collected with heparin, citrate, acid citrate dextrose (ACD) and citrate-theophylline-adenosine-dipyridamole (CTAD) was observed. The extent of spontaneous activation of platelets was detected by measuring sP-selectin in plasma. The amount of TGF-β1 released from PRP and the effect of PRP on cell proliferation were also studied.

**Results:** ACD and CTAD were superior to heparin and citrate in maintaining the integrity of platelet structures and preventing the platelet spontaneous activation. ACD-PRP and CTAD-PRP released more TGF-β1 and significantly enhanced the proliferation of human marrow stromal cells compared to heparin-PRP and citrate-PRP.

**Conclusions:** The PRP quality was closely related to the type of anticoagulants. ACD and CTAD are appropriate anticoagulants for PRP production.


*Keywords:* Platelet-rich plasma; Anticoagulant; Platelet activation; Growth factor; Biological effect

## Introduction

Platelets are derived from the cytoplasm of megakaryocytes. The normal platelets are small, disc-shaped cells without a nucleus, normally measuring 1 to 2 μm in diameter. The resting platelet is divided into three zones. The peripheral zone consists of fluffy glycocalyx coat, cytoskeleton and platelet membrane, responsible for adhesion and aggregation. The sol–gel zone contains the connecting system called the open canalicular system and the dense tubular system, responsible for contraction and support microtubule system. The dense tubular system is very rich in calcium and rich in phospholipase A2, cyclooxygease and thromboxane synthease. The organelle zone contains alpha-granules, dense granules, lysosomal granules, glycogen granules [1]. Alpha-granules contain growth factors (platelet-derived growth factor [PDGF], transforming growth factor-beta1 [TGF-beta1], insulin-like growth factor [IGF] and epidermal growth factor [EGF]), but also other molecules such as beta-thromboglobulin (beta-TG), platelet factor 4 (PF4), low affinity platelet factor 4 (LA-PF4), fibrinogen, fibronectin, thrombospondin, albumin, high-molecular-weight kininogens, coagulation factor V, coagulation factor VIII, plasminogen, histidine-rich glycoprotein, protein S, heparinase, elastase, an inhibitor of collagenase, osteonectin, Vitronectin, etc [2].

Platelet-rich plasma (PRP) is a concentration of platelets in a small volume of plasma. Upon activation, platelets in PRP release the above-mentioned growth factors via exocytosis. Theoretically, the local application of PRP might deliver a higher level of growth factors to specific sites to stimulate tissue healing [3,4]. For example, recent findings have suggested that PRP not only enhances bone regeneration when repairing bone defects and elevating the sinus floor [5–9] but also promotes and accelerates osseointegration when placed in the preparation site for a dental or bone implant [10,11].

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Since the regenerative potency of PRP undoubtedly depends on the level of growth factors released by platelets, the viability of platelets determines the quality of PRP. If platelets are activated during the PRP production process, growth factors in platelets will be released into plasma most of which is discarded during PRP preparation, which consequently decreases the efficacy of PRP when applied to surgical sites. It is therefore obvious that less platelet activation and more growth factor retention in platelets are the keys to improving the potency of PRP.

In hematological studies, the effect of anticoagulants on platelet activation is an area of concern: if anticoagulants cannot prevent, or may even promote, the spontaneous activation of the ex vivo platelets, this would affect the hematologic variables being measured, which in turn could interfere with the clinical evaluation and management of patients at risk from thrombotic and bleeding disorders. Therefore, it is important to seek an appropriate anticoagulant to avoid platelet spontaneous activation in samples used for pathophysiological studies [12–16].

Unfortunately, in both the clinical application and the basic research of PRP, the issue of anticoagulant choice has not been given sufficient attention. Various kinds of anticoagulants are used in PRP preparation [5,8,10,11,17–20], and there have been no reports investigating the association between anticoagulant choice and the biological effects of PRP. Therefore, we conducted a comparative study to investigate the effect of anticoagulants on PRP quality and biological efficacy, to clarify the appropriate anticoagulant choice in PRP production.

We studied anticoagulants commonly used in PRP preparation or hematology, including heparin, citrate, acid citrate dextrose and citrate-theophylline-adenosine-dipyridamole. The quality of PRP undoubtedly depends on platelet status, so changes in platelet morphology and the extent of spontaneous activation of platelets collected using different anticoagulants were investigated. In addition, the amount of growth factor released from activated PRP and the biological effect of PRP on cell proliferation were studied.

This study was carried out at the research center of Plastic Surgery Hospital of Peking Union Medical College. 30 volunteer donors provided their whole blood and 3 volunteer donors provided their bone marrows for our study.

## Materials and methods

### *Special reagents and apparatus*

Sodium citrate (SC) 0.129M; heparin sodium (HS) 1000 U/mL; ACD, composed of 0.48% (w/v) citric acid, 1.32% (w/v) sodium citrate and 1.47% (w/v) glucose; CTAD, composed of 0.11 mM/L citrate, 15 mM/L theophylline, 3.7 mM/L adenosine and 0.198 mM/L dipyridamole; and activating solution, consisting of 10,000 U of human thrombin (Sigma, China) dissolved in 1 mL 10% $CaCl_2$. Unless otherwise stated, the chemicals were obtained from Sigma (Steinheim, Germany). The instruments used were a laboratory-standard centrifuge (KUBOTA 5500, Japan), transmission electronic microscope (TEM) (EM 301, Phillips, Eindhoven, Netherlands), microcentrifuge (Hitachi CT15E, Japan) and microplate spectrophotometer (SpectraMax 190, Sunnyvale,USA).

### *Collection of blood and experimental protocol*

The study was conducted with the informed consent of volunteer subjects and approval from the Ethics Committee of the Peking University Health Science Center, Beijing, China. The 30 volunteer donors, 10 male and 20 female, aged between 18 and 41 with an average of 26-years-old, were enrolled in this experiment. All donors were on no medications, including aspirin and other non-steroidal anti-inflammatory drugs, during the preceding 2 weeks. The experimental protocol is summarized in Fig. 1. The first 1–2 mL of blood was discarded to avoid the effects of traces of thrombin generated during venipuncture.

The 16 mL whole blood anticoagulated by SC was drawn from each donor and centrifuged at 302 *g* for 15 min at 20 °C. The plasmas of three donors in each experiment were mixed and centrifuged at 1209 *g* for 15 min at 20 °C. The upper, platelet-poor plasma (PPP) was named SC-PPP and used to resuspend the platelet pellets.

To study platelet spontaneous activation and growth factor (TGF-β1) release from activated PRP, 28 mL whole blood samples from each donor were drawn into four 10 mL syringes which contained 1 mL ACD, CTAD, SC and HS, respectively, with 7 mL of blood going into each syringe. The whole blood in the 12 syringes from 3 donors was transferred to 12 conical bottom centrifuge tubes (15 mL, Corning, USA) and centrifuged at 302 *g* for 10 min at 20 °C. Yellow plasma from 3 tubes of each anticoagulant group was pooled in one 15 mL centrifuge tube. Following manual platelet counting, the pooled plasma volume from each group was adjusted to ensure that the total platelet count of each of the four groups was equal. The adjusted pooled plasma of each group was quartered and aliquots were centrifuged at 1209 *g* for 10 min at 20 °C at 2 h, 6 h, 12 h and 20 h post-venipuncture. All samples were stored at room temperature before centrifugation. After all of the upper PPP was transferred to another tube, the platelet pellet and a few of the red cells at the bottom of tubes were resuspended by 1.0 mL SC-PPP; the resulting mixture was platelet-rich plasma (PRP). The PPP and PRP samples were coagulated by adding 30 μL activation solution, permitted to clot overnight at 4 °C, stirred by pipette and centrifuged at 2150 *g* for 10 min at 4 °C. The clear supernatants were named PPP-serum and PRP-serum and were transferred to microcentrifuge tubes (Eppendorf, China) and stored at −70 °C until used.

For TEM observation of platelets, the pooled plasma of each anticoagulant group was quartered without counting the platelets or making volume adjustments. Aliquots were centrifuged at 1209 *g* for 10 min at 20 °C at 2 h, 6 h, 12 h and 20 h post-venipuncture. The upper PPP was discarded, while the platelet pellet was processed for TEM analysis. All samples were stored at room temperature before centrifugation.

For the preparation of PRP culture medium, the volume of pooled plasma from each of the four groups was adjusted to produces equal total amounts of platelets. The adjusted, pooled plasma was not quartered and was directly centrifuged at 1209 *g*