

RESEARCH ARTICLE

# Synthesis of Fibrin Clot from Fibrinogen Platelet Rich Plasma (PRP) for Scaffold Applications

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## Abstract

Platelet-rich plasma (PRP), which contains fibrinogen, is activated by the addition of thrombin and calcium chloride to create fibrin clots. Fibrin is a natural biopolymer that can be applied for medical applications. This study aims to synthesize fibrin clots from fibrinogen platelet-rich plasma for scaffold applications. The optimum amount of fibrin clot can be obtained by varying the ratio of PRP to a mixture of  $CaCl_2$  with the variation of 9:1, 10:1, and 11:1. The highest fibrin clot produced was 5.925 ml with 5.5%  $CaCl_2$ .

**Keywords:** Fibrin, Fibrinogen, PRP, Scaffold

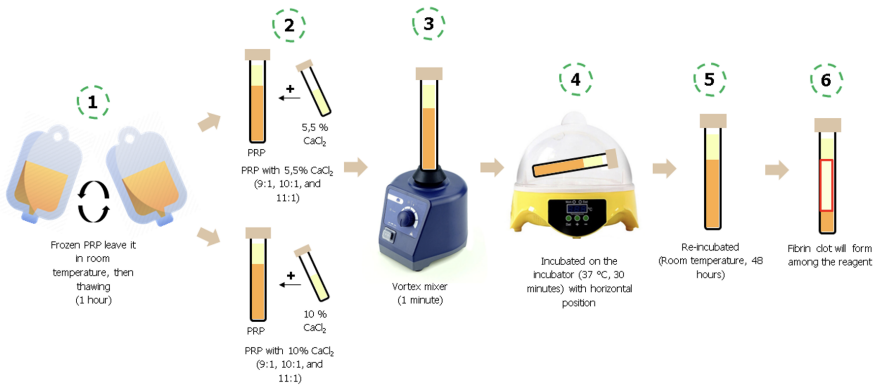
## 1. Introduction

Fibrin is a natural biopolymer formed from platelet-rich plasma (PRP) activation, which contains fibrinogen by adding thrombin and calcium chloride to form fibrin clots [1]. The resulting fibrin clot will be formed into a scaffold that helps tissue regenerate and increase cell attachment, migration, and cell proliferation [2]. The fibrin produced directly contains growth factors contained in PRP. By examining a number of variables, including thrombin concentration, calcium ions, and ionic strength, fibrin clots are formed from fibrinogen. The addition of calcium is a major factor in the formation of fibrin clots because it acts as a cofactor of thrombin, which

modulates fibrin structure, branching, stability, and accelerating the resulting clot formation [3].

Fibrinogen is a large, complex, and important glycoprotein in biological processes such as homeostasis, wound healing, inflammation, and others. Fibrinogen comprises two sets of three polypeptide chains:  $A\alpha$ ,  $B\beta$ , and  $\gamma$  [4]. Fibrin has a function as a barrier and scaffold, which is used to support healing and tissue modeling. The tissue is supported by fibrin by binding proteins and growth factors. These proteins are proteins found in the ECM, namely fibronectin and vitronectin, while for growth factors, namely FGF, VEGF, insulin-like growth factors-1, and the enzyme plasminogen (tPA). The combination of fibrin's binding to proteins and growth factors makes fibrin a major player in increasing interactions between cells [5]. The application of fibrin as a scaffold for tissue engineering can increase cell-cell interactions, adhesion, and proliferation of cells, especially for the induction of osteogenic cells. Formed fibrin can be applied as a substrate and suspension to create the desired scaffold. The aim of this study is to synthesize fibrin clots from fibrinogen platelet-rich plasma for scaffold applications.

## 2. Materials and Methods



**Figure 1.** Synthesis of fibrin clot from PRP.

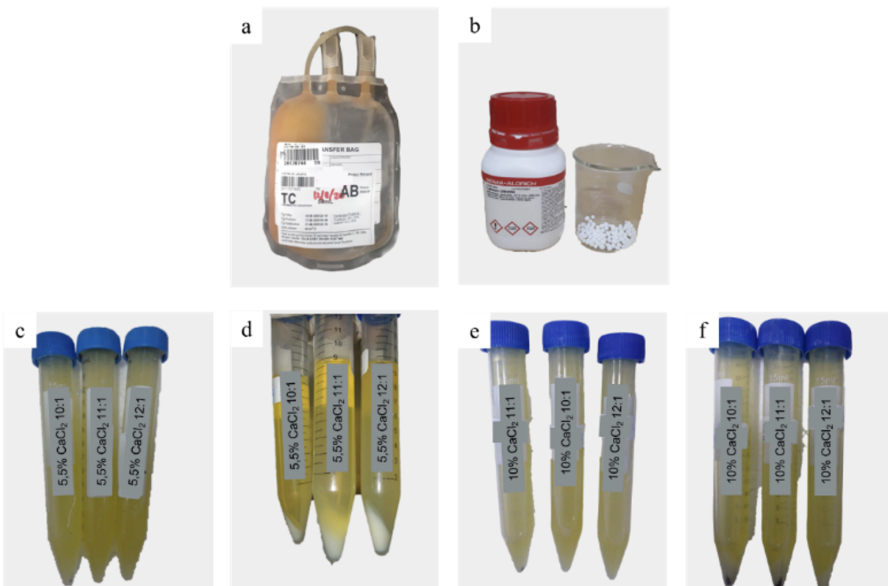
The synthesis of fibrin clots from PRP is shown in Figure 1. PRP stored frozen at  $-20^\circ\text{C}$  was left at room temperature for 1 hour to thaw. A solution of 5.5%  $\text{CaCl}_2$  (0.55 g  $\text{CaCl}_2$  and 10 mL distilled water) or 10%  $\text{CaCl}_2$  (1g  $\text{CaCl}_2$  and 10 mL distilled water) solution was made in a beaker glass, then stirred for 2 minutes at room temperature with a magnetic stirrer. The optimum amount of fibrin clot can be obtained by varying the ratio of PRP to  $\text{CaCl}_2$  (5.5% and 10%) with the variation of 9:1, 10:1, and 11:1. Each variation in the concentration of PRP with  $\text{CaCl}_2$  was pipetted into a 15 mL conical tube for 1 minute. The resulting mixture was then incubated at  $37^\circ\text{C}$  for 30 minutes in a horizontal position to obtain a larger surface area. The mixture was then incubated again for 48 hours at room temperature. Furthermore, the fibrin clot formed between the solutions was taken and stored in a freezer at  $-20^\circ\text{C}$  or directly

used as a material for the fabrication of the scaffold.

### 3. Results and Discussion

PRP is a blood sample with a higher platelet concentration than normal blood plasma [6]. Many studies have shown that PRP can induce colonization, adhesion, proliferation, and cell differentiation because it has growth factors such as PDGF -BB, TGF- $\beta$ 1, and VEGF, which have an important role in the process of bone regeneration and healing [7]. In this study, PRP concentrate obtained from the Indonesian Red Cross was activated by adding  $\text{CaCl}_2$  as an activator agent to form a fibrin clot. The activation process aims to optimize the presence of growth factors in PRP by forming fibrin clots.

In the activation process with  $\text{CaCl}_2$ , there is a blood coagulation process where  $\text{Ca}^{2+}$  ions in  $\text{CaCl}_2$  will activate platelets contained in PRP through activated protease receptors. The active platelets will negate and cause the release of growth factors from  $\alpha$ -granules, which will then convert prothrombin into thrombin and continue to convert fibrinogen into fibrin through polymerization and cross-linking processes. This fibrinogen cleavage will begin the formation of a matrix that allows the formation of a fibrin clot [8, 9].



**Figure 2.** Synthesis of fibrin clot from PRP. (a) A blood bag containing PRP. (b) Preparation of  $\text{CaCl}_2$  solution. (c) A 5.5% solution of  $\text{CaCl}_2$  and PRP before incubation and centrifugation. (d) 5.5%  $\text{CaCl}_2$  and PRP solution after incubation and centrifugation. (e) A 10% solution of  $\text{CaCl}_2$  and PRP before incubation and centrifugation. (f) A 10% solution of  $\text{CaCl}_2$  and PRP after incubation and centrifugation.

Figure 2 shows several variations to obtain the most optimum *fibrin clot*. Figures 2 (a) and (b) are the material preparation stages by *thawing* to dilute PRP and prepare a solution of  $\text{CaCl}_2$ , which will be used as an activator compound. Variations in the

concentration of  $CaCl_2$  were carried out by making a solution of 10%  $CaCl_2$  and 5.5%  $CaCl_2$ . Then, the ratio between the PRP and the  $CaCl_2$  solution was carried out with the variation of 10: 1, 11: 1, and 12: 1. The mixture for each variation was put into a 15 mL conical tube, and then a vortex mixer is carried out for 1 minute to mix as shown in Figures 2 (c) and (e). The mixture was then incubated in a 37°C incubator for 30 minutes to accelerate the formation of fibrin clots.

Furthermore, the mixture was then centrifuged at a speed of 3000 rpm for 20 minutes to precipitate the fibrin clot to the base of the tube so that it is easier to separate from the liquid, as in Figures 2 (d) and (f). The fibrin clots produced at each variation are presented in Tables 1 and 2. In Table 1, the most optimal amount of fibrin clot is produced at a  $CaCl_2$  concentration of 5.5% and a ratio between PRP and  $CaCl_2$  12:1.

**Table 1.** The amount of fibrin clot produced from PRP and  $CaCl_2$  (5.5 or 10%) after incubating in 37°C incubator for 30 min.

<b>CaCl<sub>2</sub> concentration</b>	<b>PRP Ratio: CaCl<sub>2</sub></b>	<b>PRP (mL)</b>	<b>CaCl<sub>2</sub> (mL)</b>	<b>Fibrin clot (µl)</b>
<b>5.5%</b>	10:1	9.1	0.9	884
	11:1	9.2	0.8	392
	12:1	9.3	0.7	889
<b>10%</b>	10:1	9.1	0.9	766
	11:1	9.2	0.8	723
	12:1	9.3	0.7	836

The rest of the solution from each different  $CaCl_2$  concentration was then collected, and each was again incubated at room temperature for 48 hours. It aims to see the effect of incubation time on the formation of fibrin clots. The resulting fibrin clot produced can be seen in Table 2.

**Table 2.** The amount of fibrin clot produced  $CaCl_2$  5.5% and 10% after incubating at room temperature for 48 hours.

<b>CaCl<sub>2</sub> concentration</b>	<b>The mixture of PRP and CaCl<sub>2</sub> (mL)</b>	<b>Fibrin clot (mL)</b>
<b>5.5%</b>	27.835	5.925
<b>10%</b>	27.675	4.127

Fibrin clots contain growth factors, unique structure, good stability, barrier formation, and proteins in the extracellular matrix and have excellent regeneration functions, making fibrin clots an important role in cell interactions. This is proven by research by Helgerson et al. regarding fibrin clots and their function in tissue

engineering to increase the adhesion, proliferation, and induction of osteogenic cells [5, 10]. The process of obtaining maximum results is due to certain techniques in obtaining a large number of fibrin clots. The technique uses  $37^{\circ}\text{C}$  temperature and a 5%  $\text{CO}_2$  incubator and places the centrifuge tube containing the PRP and  $\text{CaCl}_2$  mixture horizontally during the incubation process. This is related to the surface area of the reaction between PRP and  $\text{CaCl}_2$ . With a large surface area, the resulting fibrin clots will increase. This study is consistent with that conducted by Escobar *et al.*, using  $\text{CaCl}_2$  as a fibrinogen activation agent in PRP to form fibrin clots with a concentration of 10%  $\text{CaCl}_2$  in a ratio of (9:1) to provide the maximum number of fibrin clots [11]. However, the fibrin-activating agent from PRP fibrinogen will produce better formation and have optimal interaction capabilities if combined with additional thrombin enzymes to the mixture of PRP and  $\text{CaCl}_2$  using a thrombin concentration of 10% [1].

#### 4. Conclusions

Fibrin clots from fibrinogen platelet-rich plasma for scaffold applications were synthesized successfully. Fibrin was extracted from PRP with  $\text{CaCl}_2$ . The optimum condition for extraction of the fibrin clot is the results for the ratio 12:1 with the concentration 5.5%  $\text{CaCl}_2$ . Besides, the usage of an incubator with 5%  $\text{CO}_2$  and placing the centrifuge tube containing the PRP and  $\text{CaCl}_2$  mixture become the keys to successful fibrin clot synthesis.

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