

## TENSILE STRENGTH AND FIBRINOGEN YIELD IN FIBRIN GLUE PREPARATIVES WITH AND WITHOUT FREEZE-DRYING METHOD

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### ABSTRACT

Fibrin glue is a useful biological product to stop bleeding, tissue adhesive, and to accelerate wound healing. Preparation of Fibrin glue requires fibrinogen and thrombin components. The conventional cryoprecipitation method performed at the Blood Bank can be used to improve the quality of the fibrinogen component. The freeze-drying process can increase the retention time of plasma products at room temperature. Fibrinogen yield and tensile strength are quantitative and qualitative parameters for the preparation quality of fibrin glue. This study focused on finding differences between tensile strength and fibrinogen yield in fibrin glue preparative by cryoprecipitate with and without freeze-drying methods. This study was an in-vitro laboratory experiment design by comparing the fibrinogen yield and tensile strength of fibrin glue preparation from cryoprecipitate plasma, with and without a freeze-drying process. The results were analyzed comparatively using a paired T-test. The plasma fibrinogen content of the sample was  $237.66 \pm 67.10$  mg/dL. The fibrinogen content of the cryoprecipitate component without freeze-drying process was  $327.74 \pm 103.42$  mg/dL with a fibrinogen yield of  $1.38 \pm 0.25$ . The fibrinogen content of the cryoprecipitate component with freeze-drying process was  $251.20 \pm 103.91$  mg/dL with a fibrinogen yield  $1.04 \pm 0.25$ . Tensile strength of fibrin glue from cryoprecipitate without freeze-drying process was showed an average  $0.52 \pm 0.18$ . Tensile strength of fibrin glue from cryoprecipitate with freeze drying process showed an average of  $0.33 \pm 0.12$ . There was a significant difference between fibrinogen yield and tensile strength of fibrin glue preparation by cryoprecipitation method with and without freeze-drying process. There was a significant difference on fibrinogen yield and tensile strength in the preparation of fibrin glue by the freeze-drying process which was probably due to changes in the structure and function of fibrinogen proteins.

**Key words:** Cryoprecipitate, freeze-drying, fibrin glue, fibrinogen yield, tensile strength

### INTRODUCTION

Fibrin glue is an essential biological product and widely used in surgery to improve hemostasis. Fibrin Glue is also known as a tissue adhesive and a bioactive substance, such as growth factors and antibiotics. Thus, fibrin glue is often used by almost all surgeons specialists to improve wound healing process, reduce sutures, and accelerate tissue growth.<sup>1</sup>

Moreover, fibrin glue preparation can be commercialized or individually used.<sup>2</sup> The advantages of commercial fibrin glue preparations are stickier, easily to be used, and can be stored for a long time at room temperature. However, the disadvantages of commercial fibrin glue preparations are expensive and have a high risk of rejection due to allograft and xenograft materials. On the other hand, fibrin glue preparations individually are usually prepared at the time of request for use due to their lower price and no risk of rejection since they come from the patient's own self.<sup>1</sup>

Fibrin glue preparatives, furthermore, requires fibrinogen and thrombin components.<sup>3</sup> The fibrinogen components used for fibrin glue preparatives can be obtained from the spin centrifuge and blood plasma cryoprecipitate therapies. The cryoprecipitate therapy is routinely used and mostly carried out at the blood bank to obtain antihemophilic factor VIII products.<sup>4</sup> The fibrinogen component obtained in the cryoprecipitate therapy is actually higher than that obtained by the spin centrifuge method.<sup>5</sup> Based on the data from the Indonesian Red Cross (PMI) of Surabaya city in 2016 - 2017, the demand for cryoprecipitate products was reduced by 76% from 1,066 to 254 bags. This can be due to the presence of a purer, safer and more effective recombinant factor VIII concentrate product for hemophilia treatment. Request of autograft fibrin glue for patients who will undergo elective surgery can be routinely performed in the cryoprecipitate therapy to improve the quality of fibrinogen components in fibrin glue preparation.

In addition, the quality of fibrin glue product is very dependent not only on the quality of the fibrinogen components used but also on the quantity of the fibrinogen components. Fibrin glue preparations, as a result, require an optimal technique for obtaining fibrinogen quantitatively and qualitatively. Fibrinogen yield is a quantitative indicator, while tensile strength is a qualitative indicator of the strength of fibrin glue products.<sup>6</sup> In other words, to make freeze-drying fibrin glue with a high fibrinogen yield and tensile strength, fibrinogen components used for fibrin glue should not only be good in quality and quantity but also showed a longer shelf life without refrigerators. Hence, this study aimed to compare fibrinogen yield and tensile strength in fibrin glue preparations during cryoprecipitate therapy with and without the freeze-drying method.

## METHODS

This study used a quasi-experimental design conducted in the laboratory by in-vitro on donor plasma samples. The donor plasma samples used were processed using cryoprecipitate therapy procedures with and without freeze-drying method. The procedure of cryoprecipitate therapy was applied with rapid freezing method at 50°C for 55 minutes using a contact shock freezer instrument and slow thawing method at  $\pm 2-6^{\circ}\text{C}$  for 18 hours.

Next, the process of freezing was conducted by freezing plasma samples with a deep freeze instrument at  $- 80^{\circ}\text{C}$  for at least 24 hours, and then freeze-drying process was further carried out by the Lyovac instrument for 24 hours. Afterward, the reconstitution of cryoprecipitates with freeze-drying method was performed using OVB Buffer (Owren's Veronal Buffer) to maintain the pH of the preparation. Examination of fibrinogen levels was then carried out at the beginning before the cryoprecipitate therapy process, after the cryoprecipitate therapy process without the freeze-drying method, and after the cryoprecipitate therapy process with the freeze-drying method using Sysmex CS 2100 with the principle of Clauss method.

Subsequently, fibrinogen field was calculated by measuring the ratio between the fibrinogen content of the cryoprecipitate component without the freeze-drying method or with the freeze-drying method and the initial fibrinogen level before the cryoprecipitate therapy process. Fibrin glue preparation then was prepared by adding

commercial thrombin and  $\text{CaCl}_2$  to both the cryoprecipitate plasma sample group without the freeze-drying method and the cryoprecipitate plasma sample group with the freeze-drying method as depicted in Figure 1. After that, tensile strength examination was performed on the fibrin glue preparation of both the cryoprecipitate plasma sample group without the freeze-drying method and the cryoprecipitate plasma sample group with the freeze-drying method using Shimidzu Autograph AG as illustrated in Figure 2.



**Figure 1.** Mold for making fibrin glue components



**Figure 2.** Tensile strength examination using the Shimidzu Autograph AG instrument

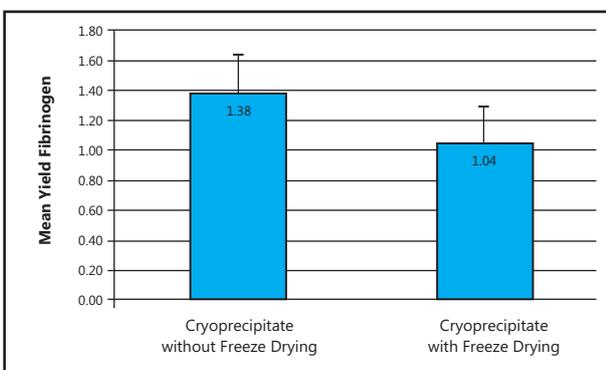
The population of this study consisted of donor plasma samples obtained from the Indonesian Red Cross (PMI) of Surabaya City and then processed into Anti Hemophilic Factor (AHF) cryoprecipitate components. There were some sampling criteria, namely donor plasma from people aged more than

18 years old and weighing more than 46 kg with a systole pressure of 110–160 mmHg, a diastolic pressure of 60–100 mmHg, and Hb level of 12.5-17g/dL. Besides this, those plasma donors should have no infection of negative blood transfusion (HIV, HBsAg, HCV, non-reactive syphilis), and sign an informed consent. Meanwhile, exclusion criteria were donors taking anticoagulant drugs and blood samples of lipemic or icteric donors. Sampling was conducted non-randomly with a consecutive sampling method to select samples that met the inclusion criteria until the minimum number of samples was met. The sample size used in this study amounted to 12 samples. This research had obtained ethical clearance from the Health Research Ethic Committee of the Medical Faculty Airlangga University No. 23/EC/KEPK/FKUA/2018.

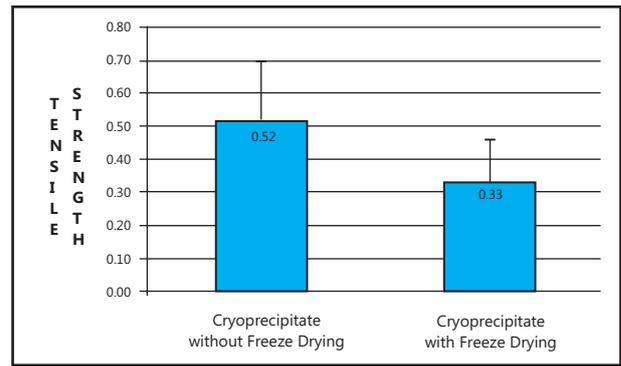
**RESULTS AND DISCUSSION**

Based on the results, the level of plasma fibrinogen samples was 237.66±67.10 mg/dL. The results also showed that the level of fibrinogen contained in cryoprecipitates without freeze-drying method was 327.74±103.42 mg/dL with a fibrinogen yield level of 1.38±0.25. Meanwhile, the level of fibrinogen contained in cryoprecipitates with freeze-drying method was 251.20±103.91 mg/dL with a fibrinogen yield level of 1.04±0.25.

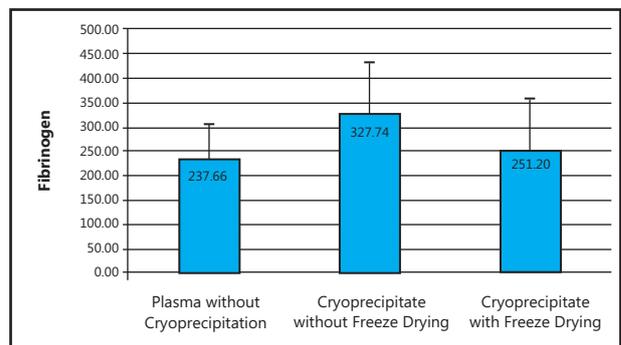
On the other hand, the tensile strength of fibrin glue from cryoprecipitate therapy without freeze-drying method was 0.52 ± 0.18. Meanwhile, the tensile strength of fibrin glue from cryoprecipitate therapy with freeze-drying method was 0.33 ± 0.12. Thus, it could be said that there was a significant difference between fibrinogen yield and tensile strength in fibrin glue preparation during cryoprecipitate therapy with and without freeze-drying methods.



**Figure 4.** Fibrinogen yield values of cryoprecipitates with and without freeze-drying methods



**Figure 5.** Tensile strength values of fibrin glue from cryoprecipitates with and without freeze-drying methods



**Figure 3.** The difference of the initial fibrinogen levels and the fibrinogen levels contained in cryoprecipitates with and without freeze-drying methods

There might be some factors causing the lower mean level of fibrinogen yield in cryoprecipitate components with freeze-drying method than that without freeze-drying method, such as freezing temperature, storage time of the freeze-drying method, and formula for reconstitution of the freeze-drying method.<sup>7</sup> The freezing temperature and the storage time of the freeze-drying process needed to maintain plasma protein levels were based on a rapid freezing procedure using a contact shock freezer. Meanwhile, a special reconstitution formula was needed to prevent protein aggregation or degradation.<sup>8</sup>

On the other hand, possible causes of the smaller mean level of fibrin glue tensile strength on cryoprecipitate components with freeze-drying method than that without freeze-drying method were structural changes and chemical constitution of fibrinogen protein during the freeze-drying process as becoming amorphous so that the surface area, solubility degree, and polymerization process became reduced.<sup>9-11</sup>

## CONCLUSION AND SUGGESTION

In conclusion, there were significant differences in fibrinogen yield and tensile strength in fibrin glue preparations with the freeze-drying method and those without the freeze-drying method due to changes in the structure and function of fibrinogen proteins.

Further research is needed in order to obtain fibrin glue preparation techniques that can maximize fibrinogen yield and tensile strength in cryoprecipitates by the freeze-drying process.

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