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Effects of Platelets Addition on Tension and Elasticity of the Fibrin Glue

Ibrahim A. Mahmood[1] *PhD,* **Fakhir S. Al-Ani**[2] *PhD*

[1]Dept. of Physiology, College of Medicine, Al-Nahrain University, Baghdad, Iraq, [2]Retired

Abstract

Background A biodegradable, biocompatible and natural topical tissue adhesive fibrin glue, help in starting and replicating the last phases of the coagulation cascade. The platelets contain thrombosthenin, actin and myosin filaments, which when activated produces tension and motion that is similar to that produced by skeletal muscle.

Objective To create the fibrin glue both with and without the addition of platelets, and investigate the biomechanical behavior of both formulas in terms of tension and stretching (elasticity).

Methods Creating the "ordinary fibrin glue" by using fibrinogen (from cryoprecipitate) and thrombin. In a different preparation, platelets were added to the cryoprecipitate and thrombin to create "platelets added fibrin glue". Next, the elasticity and tension of the synthetic fibrin glue were assessed—both regular and platelet-added fibrin glue—at various times using displacement and force sensors.

Results When platelets are added to fibrin glue, the elasticity outcomes at one hour and one week are significantly reduced. As well as, there were a significant higher tension results were observed at one-hour and one-week intervals when comparing regular fibrin glue with platelets added.

Conclusion The inclusion of platelets caused the glue's biological behavior to alter, showing as an increase in tension and a decrease in elasticity (stretching). When the applicator has to use this mixture to manage various body parts (such as bone regeneration, graft stabilization, regeneration of periodontal ligament and treating joints arthritis), this modification should be taken into account.

Keywords Fibrin glue, Platelets, tension, elasticity

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List of abbreviations: C:T = Cryoprecipitate: Thrombin , C:T:P = Cryoprecipitate: Thrombin: Platelets

Introduction

Fibrin glue is a natural, biocompatible and biodegradable topical tissue adhesive help in starting and replicating the last phases of the coagulation cascade [(1-2)]. When employing surgical suture, this adhesive helps prevent and stop the development of some complications. It works with both classic threads and contemporary mechanical staplers. Many complications can arise from the "classic" approach of wound repair, including significant inflammation, fistulae, extensive fibrosis and tissue ischemia, which can impede tissue recovery. However, by utilizing such glue, the likelihood of these consequences can be reduced [(3-5)].

In light of this, it is stated that the ideal scenario would involve wound healing without the need for sutures, the ability to withstand a certain amount of mechanical stretching, and the creation of optimum circumstances to ensure that the injured area heals quickly and

is free of extraneous objects [6]. So, the use of human fibrin glue in various surgical procedures has grown rather widespread.

Fibrin glue has been evaluated for safety and effectiveness in conjunctival autograft fixation in primary pterygium [7]. As well as, it is commonly used in urologic surgery as a tissue adhesive, hemostatic agent, and/or urinary tract sealant. Furthermore, when the fibrin glue is applied directly from the syringe or on a carrier, the success rate in halting bleeding during the 5-minute bleeding period after coronary artery bypass grafting is 92.6%, but the success rate with traditional topical treatments is only 12.4% [8].

Two primary components are used to polymerize fibrin glue: the first is often concentrated human fibrinogen, and the second is typically bovine thrombin and calcium chloride [9-10]. These two components are mixed together at the moment of application either concomitantly or successively [11]. Two syringes with tips that form a common port or two routes for excellent application can be used to accomplish this technique. By injecting the two components together at the site of delivery, blood coagulation's intrinsic and extrinsic mechanisms are avoided but the physiological final stages of coagulation cascade are faithfully replicated. So, Clots formed by these agents are similar to normal clots, which, later on, broken down by fibrinolysis and reabsorbed naturally over the course of several days [12].

In a microliter of blood, there are typically 150,000 to 400,000 platelets, also known as thrombocytes, which are tiny discs with a diameter of 1-4 µl. Platelets have a short lifespan of 8 to 9 days [13-14]. When an artery is broken, the collagen-containing connective tissue beneath is revealed. This causes platelets to be drawn to the area and stick to each other, as well as, the negatively charged connective tissue, accumulating a cluster of platelets to seal the break. The platelets contain thrombosthenin, actin and myosin filaments, which when activated platelets produces tension and motion that is similar to that produced by skeletal muscle [15].

The objective of the study was to synthesize the fibrin glue (with and without platelets addition) and to explore the effect of platelets addition on the fibrin glue elasticity and tension.

Methods

From October 2011 to June 2012, this experiment was carried out at the Laboratories of Department of Physiology, at College of Medicine, Al-Nahrain University. The materials used for synthesis of the fibrin glue were:

1. Cryoprecipitate: Obtained from the national blood bank.
2. Platelets: Obtained from the national blood bank.
3. Thrombin: from BIOLABO CO., these thrombin vials were lyophilized, bovine source.
4. Calcium Chloride (CaCl2): Calcium Chloride Dihydrate (BDH chemicals LTD pool, England).

Synthesis of the fibrin glue

By using an incubator (vortexTM), the experiment's fibrin glue preparation and storage were maintained at a constant temperature of 37°C and full humidified state. Before usage, the cryoprecipitate was thawed to 37°C. A vial of thrombin was dissolved by adding 4 ml of 50 mM $CaCl_2$ [16]. One hunderd µl of thrombin were employed in all fibrin glue production tests.

Procedure

Two mixtures were prepared, the first consisted of 800 µl of cryoprecipitate with 100 µL of thrombin (C:T), while the second consisted of 800 µl of cryoprecipitate with 800 µL, then 100 µl of thrombin was added (C:T:P).

The elasticity tests

Measuring the experimental glue's elongation by varying the weights used.

Procedure

By applying equilibrium weight on the opposite side, the displacement transducer's lever was kept horizontal while the clot was clipped between it and the micromanipulator. This weight was only used to maintain the lever's horizontal position, it was only used for calibration. The weights were progressively increased by adding known weights one after the other, lengthening the clot.

The displacement transducer, which was drawn on chart paper by a polygraph (Harvard apparatus limited, Universal Oscillograph, USA, 1979), registered the elongation of the clot. Elasticity tests were repeated ten times throughout the 1 hour, 7 days, and 14 days of the glue synthesis. Statistical analysis was conducted using the mean values.

The tension tests

This test was to measure the tension that established in the fibrin glue by pulling of the clot using the micromanipulator.

Procedure

After the clot was clipped between the force transducer and the micromanipulator, the latter was adjusted so that it would gradually draw the fibrin glue lower, increasing the displacement by one mm at a time. Throughout that time, a polygraph was used to record the rising clot tension and the force transducer measured it. (lctromed limited, multitrace 2, USA, 1979), which, on chart paper, write this signal. Ten repetitions of the tension test were conducted after 1 hour, 7 days, and 14 days of glue synthesis. Statistical analysis was conducted using the mean values.

Statistical analysis

The obtained data were presented as mean ± standard deviation of mean. In graphic presentation, the means of the data were used alone (i.e., without standard deviation). Student t-test (paired and unpaired) was used for comparison between two groups for different ratios and models. P value less than 0.05 was considered significant.

Results

The elasticity tests

Results showed that there was an increased length of the glue with an increased weights for both formulae at 1 hour, 7 days and 14 days durations of glue synthesis. At 1 hour duration of synthesis, the C:T:P, glue could resist up to 40 g weight with elongation not more than 0.5 mm, after which it was torn, while C:T glue can resist up to 37.5 g with increasing length of the glue up to 12 mm. At 7 days duration, C:T:P could resist up to 40 g weight with 2.5 mm lengthening, but the C:T glue could resist -only- up to 22.5 g, after which it was torn with increased length till 3 mm. At 14 days duration, the C:T:P can resist only up to 18.75 g weight, after which it was torn.

Statistical analysis showed a significant higher elasticity C:T in comparison to that of C:T:P ($P = 0.0003$).

Statistical comparison revealed that none of the C:T:P at 1 hour duration could show elongation more than 0.5 mm although the weight used was about 40 g, while the elongation was more than 2.5 mm at 7 days duration. Moreover, the elongation of the glue at 14 days was 3 mm even with <20 g weights were used as seen in figures (1-3).

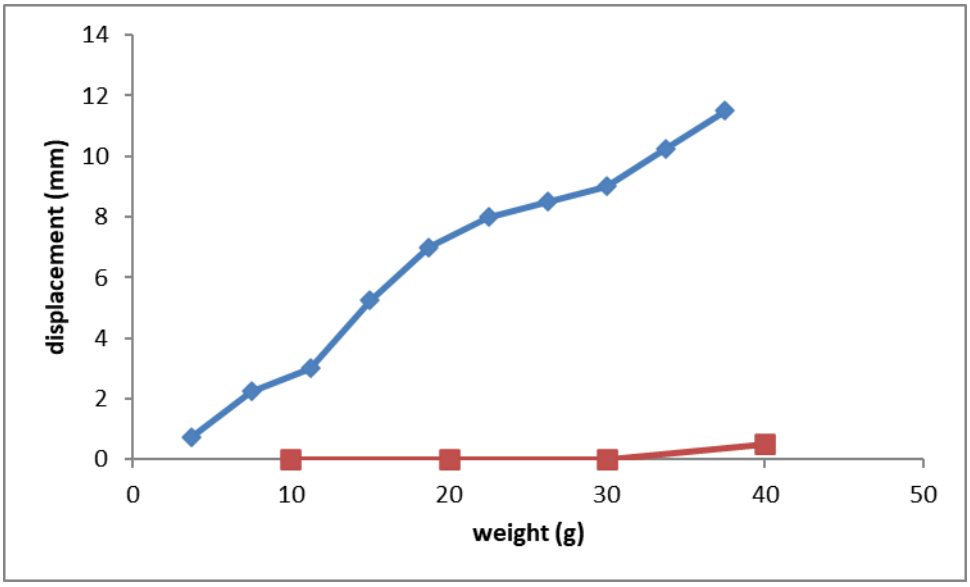

Figure 1. The elasticity results of high concentrations Cryoprecipitate: Thrombin and Cryoprecipitate: Thrombin: Platelets fibrin glue at 1hour duration

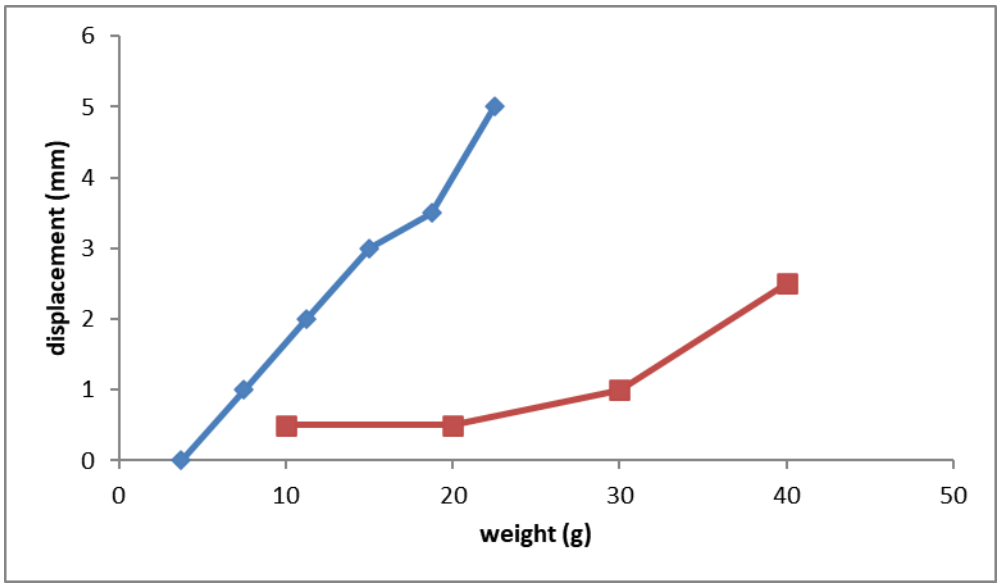

Figure 2. The elasticity results of high concentrations Cryoprecipitate: Thrombin and Cryoprecipitate: Thrombin: Platelets fibrin glue at 7 days duration

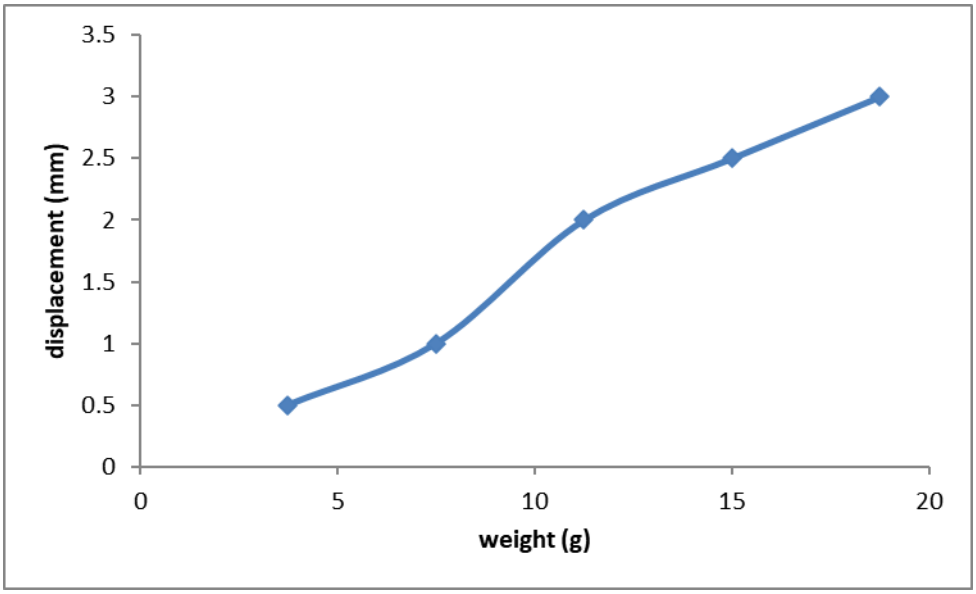

Figure 3. The elasticity results of high concentration Cryoprecipitate: Thrombin: Platelets fibrin glue at 14 days duration

The tension tests

Results of tension tests showed clearly the increased tension of the both formulae with gradual increased displacement at 1 hour, 7 days and 14 days durations, taking in consideration the identical results of C:T at 2-3 mm, 4-6 mm and 8-9 mm, as well as the results of C:T:P were identical at 5-6 mm, 7-8 mm of displacements, both formulae were identical at 1 mm displacement at 1 hour duration (Figure 4). As well as, the tension results of C:T were identical at 1-2 mm, 4-5 mm, and 6-9 mm of displacements at 7 days duration (Figure 5).

Statistical analysis showed a significant higher tension result of C:T:P in comparison to that of C:T at 1 hour, 7 days durations (P = 0.01, 0.002, respectively). On the other hand, C:T:P formula revealed a significant higher tension at 1 hour duration in comparison to the lower results at 14 days duration (P = 0.0004) (Figure 6).

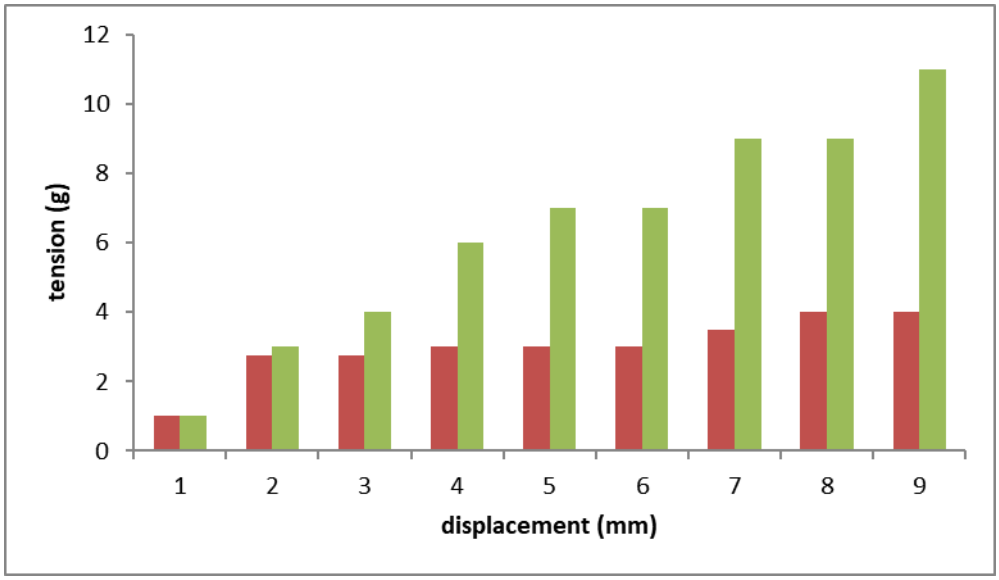

Figure 4. Histogram of the tension results of high concentrations Cryoprecipitate: Thrombin and Cryoprecipitate: Thrombin: Platelets fibrin glue at 1 hour duration

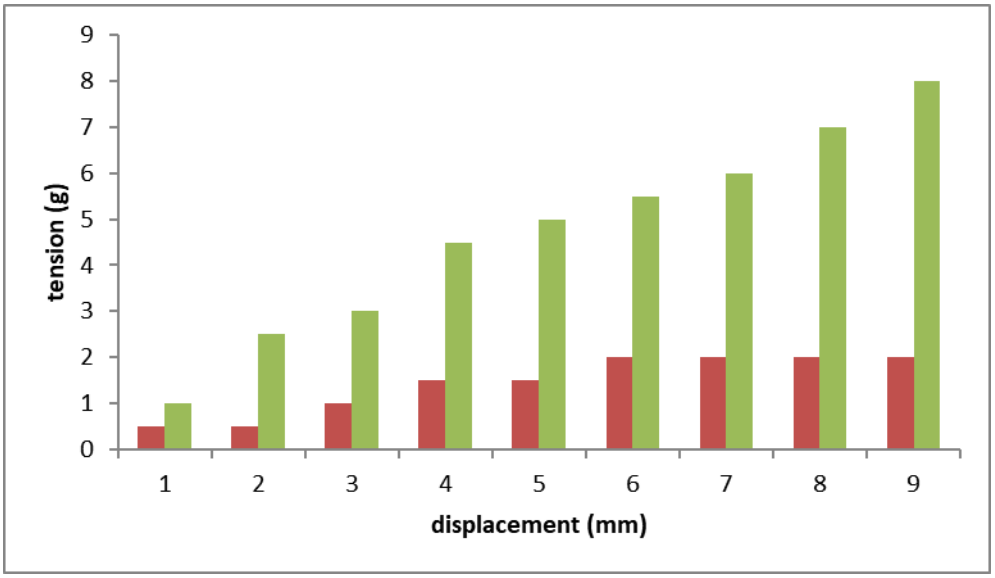

Figure 5. Histogram of the tension results of high concentrations Cryoprecipitate: Thrombin and Cryoprecipitate: Thrombin: Platelets fibrin glue at 7 days duration

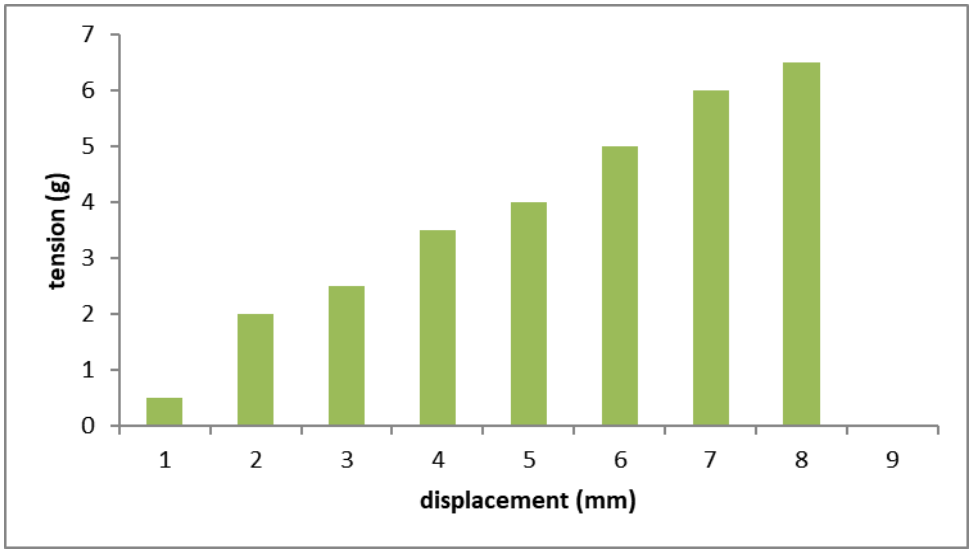

Figure 6. Histogram of the tension results of high concentration Cryoprecipitate: Thrombin: Platelets fibrin glue at 14 days duration

Discussion

The significant increase in length of C:T formula in comparison to that of C:P:T was attributed to the present of fibrin threads, which is cleaved from fibrinogen by the role of thrombin [17]. Fibrin threads. is the most stretchable natural protein, on average fibrin fibers can be passively stretched to 2.8 times their original length and still snap back to their starting length and can be stretched to 4.3 times their original length before they break. This highly elastic property accounts for the extraordinary stretchiness of blood clots [18].

On the other hand, tension tests showed higher significant results of C:P:T formula in relation to that of C:T formula. Numerous chemicals associated to clot formation and solidification are released by the platelets, such as fibrin which released from the alpha granules [19]. The platelets' additional fibrin increased the concentration of fibrin, which causes a clot to form slowly [20]. Moreover; the increased number of cross-linking connections between neighboring fibrin fibers generated by the fibrin-stabilizing factor, which is released from platelets may explain in part the prolongation of the time needed for clot lysis. As well as, the way that actin and myosin in the platelets cause the clot to retract and rip apart fibrin strands that are already joined, resulting in a more compact clot [21].

Accordingly, the lower elasticity of the platelets added fibrin glue was attributed to the contractions of thrombosthenin, actin and myosin in the activated platelets (in a manner similar to that in muscles) that induced clot retraction and pulled of the already attached fibrin strands, and so more compact clot with severe reduced in elasticity of the clot [15,22].

Moreover, the increased tension results of the platelets addition were belonged to the contractions of thrombosthenin, actin and myosin in the activated platelets in a manner similar to that in muscles causing pulling of already attached fibrin strands, which contract, this contraction produced a more compact clot, furthermore, platelets in the clot continued in release fibrin-stabilizing factor, which caused more and more cross-linking bonds between adjacent fibrin fibers. These two factors contributed to the higher tension results of platelets additions at all durations [21].

In conclusion, the addition of platelets to the fibrin glue's constituents strengthens the glue and delays or stops the glue's early fibrinolysis. This research shows that the addition causes a change in the glue's biological behavior, which manifests as more tension and less elasticity. When using this solution to control bleeding in various parts of the human body (such as in bone regeneration, graft stabilization, regeneration of periodontal ligament and

treating joints arthritis), the applicator needs to take this shift into account.

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Author contribution
Both authors had made a substantial, direct, and intellectual contribution to the work, and approved it for publication.

Conflict of interest
The authors declare no conflict of interest.

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Correspondence to Dr. Ibrahim A. Mahmood
E-mail: ibrahemibrahem73@nahrainuniv.edu.iq
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