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Effects of Tranexamic Acid Addition on Elasticity and Tension of the Fibrin Glue

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Abstract

Background Fibrin glue is a natural, biocompatible and biodegradable topical tissue adhesive that initiates and duplicates the final stages of coagulation cascade. To prevent early fibrinolysis, antifibrinolytic agent may be added to the components of the glue. Tranexamic acid is a synthetic antifibrinolytic lysine analogue that competitively inhibits the activation of plasminogen to plasmin, hence, delays the fibrinolysis activation.

Objective To synthesize of the fibrin glue with and without tranexamic acid addition and explore the biomechanical behavior of both formulae with regard to stretching (elasticity) and tension.

Methods Using thrombin and cryoprecipitate (as a source of fibrinogen) for synthesis of the "ordinary fibrin glue". In another preparation; Tranexamic acid was added to both components (thrombin and cryoprecipitate) for synthesis of the "tranexamic acid added fibrin glue". Then, by using displacement and force transducers we measure elasticity and tension of the synthesized fibrin glue (both ordinary and tranexamic acid added fibrin glue) at different durations.

Results Tranexamic acid addition to the fibrin glue causes significantly higher elasticity results at 1 hour, 1 week durations. Significant lower tension results are witnessed at 1 hour duration, while at 1 week duration, comparison of the tension results of both ordinary and tranexamic acid added fibrin glue show no significant difference.

Conclusion Tranexamic acid addition led to change in biological behavior of the glue presents as increase in its elasticity and decreased tension. This change should be taken into consideration when the applicator needs to use this formula in the management of different areas of human body.

Keywords Fibrin glue, tranexamic acid, elasticity, tension.

Introduction

Fibrin glue is a natural, biocompatible and biodegradable topical tissue adhesive which initiates and duplicates the final stages of coagulation cascade [1-3]. This glue, besides many indications, is helpful to prevent and stop development of some complications that occur when using surgical sutures, both with the traditional threads and with the modern mechanical staplers. In the “classic” method for wound repair, there are many complications; such as major inflammation, fistulae, tissue ischemia, extensive fibrosis and hematomas, which may cause impairment of tissue healing. All these possible complications may be minimized by using fibrin glue [4-7]. Hence, uses of human fibrin glue have become quite common in different types of surgeries [8]. The efficacy and safety of fibrin glue was evaluated in conjunctival autograft fixation in primary pterygium [9-11], also, fibrin glue is frequently used in neurosurgery for dural

sealing, hemostasis, cranial nerve coating, and wrapping of non-clippable cerebral aneurysms [12].

Fibrin glue is polymerized mainly from two main components; first one -typically- contains concentrated human fibrinogen; factor XIII, and the plasma proteins. Second component usually consists of bovine thrombin, calcium chloride, and anti- fibrinolytic agent [2,4]. The two components are mixed together at the moment of application either sequentially or simultaneously [13-16], a mechanism which can be performed by using two syringes with tips forming either a common port or two ways for perfect application. When injected, the two components meet at the point of delivery, both extrinsic and intrinsic mechanisms of blood coagulation are bypassed, but the physiological final stages of coagulation cascade is faithfully replicated [17,18].

After several days, the final fibrin glue is subject to fibrinolysis by both endogenous and exogenous plasmin. Antifibrinolytics such as aprotinin, tranexamic acid, and aminocaproic acid can be added to the mixture to reduce the rate of fibrinolysis and creation of fibrin degradation products [5,16,19,20].

Tranexamic acid [4-(aminomethyl) cyclohexane carboxylic acid is a synthetic lysine analog that competitively inhibits the activation of plasminogen to plasmin - the major fibrinolytic protease- via reversible binding to the lysine binding site on plasminogen. As such, tranexamic acid shows strong antifibrinolytic activity *in vitro* and *in vivo* by preventing the interaction of tissue plasminogen activator, plasminogen and plasmin with lysine residues on the surface of fibrin [12,21-23].

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

Methods

This work was performed at the laboratories of Department of Physiology, College of Medicine, Al-Nahrain University, during the period from November 2011 to May 2012. The materials used for synthesis of the fibrin glue are:

1. Thrombin: It is related to BIOLABO CO., these thrombin vials are lyophilized, bovine source.
2. Cryoprecipitate: Obtained from the national blood bank.
3. Tranexamic acid (EXACYL™): Ampules of tranexamic acid (0.5 g/5 ml, sanofiaventis-France).
4. Calcium Chloride ($CaCl_2$): Calcium Chloride Dihydrate (BDH chemicals LTD pool, England).

Synthesis of the fibrin glue

During the experiment, the fibrin glue preparation and storage was kept under full humidified condition and constant temperature (37^{o}C) by using an incubator (vortex™). Cryoprecipitate was thawed to 37 ^{o}C temperature before used. Thrombin vial was dissolved by addition of 4 ml of 50 mM $CaCl_2$ [16]. In all experiments of the fibrin glue synthesis 100 µl of thrombin and 50 µl of tranexamic acid (= 5 mg) were used.

Procedure

800 µL of cryoprecipitate was mixed thoroughly with 100 µL of thrombin (8:1 fibrin glue). 800 µL of cryoprecipitate was mixed thoroughly with 50 µL of tranexamic acid, then 100 µL of thrombin was added (8:1:50 fibrin glue).

The elasticity tests

The test of the elasticity was to measure the elongation of the experimental glue by different weights used.

Procedure

The lever of the displacement transducer was fixed in horizontal position by equilibrium weight applied on the other side, while the clot was clipped between the lever and the micro-manipulator, this weight was just to keep the lever horizontal without increased the length of the glue (just for calibration). The weights were increased gradually by sequential addition of known weights thereby increasing the length of the clot. The elongation of the clot was recorded

by the displacement transducer which was drawn by a polygraph (Harvard apparatus limited, Universal Oscillograph, USA, 1979) on a chart paper. The elasticity test was repeated 10 times at 1 hour, 1 week and 2 weeks duration of the glue synthesis. The mean values were used for statistical analysis.

The tension tests

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

Procedure

The clot clipped between the force transducer and the micromanipulator, then, the latter was moved in such a way that it will pull the fibrin glue downward step by step, each step increased the displacement by one millimeter. During that, the increased tension of the clot was measured by the force transducer and recorded by a polygraph (lctromed limited, multitrace 2, USA, 1979), which draw this signal on a chart paper. The tension test was repeated 10 times at 1 hour, 1 week and 2 weeks duration of glue synthesis. The mean values were used for statistical analysis.

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 show an increased length of the glue with an increased weights used for both formulae at 1 hour, 1 week and 14 days durations of glue synthesis with fibrinolysed 8:1 fibrin glue after 10 days duration. Statistical analysis presents significant higher results of 8:1:50 in comparison to that of 8:1fibrin glue at 1 hour, 1 week durations (*p* values= 0.04 and 0.04, respectively). All clarified significant higher elasticity results of 8:1:50 at 1 hour duration in comparison to lower results at 2 weeks duration (*P* value= 0.03) as seen in fig. 1-3.

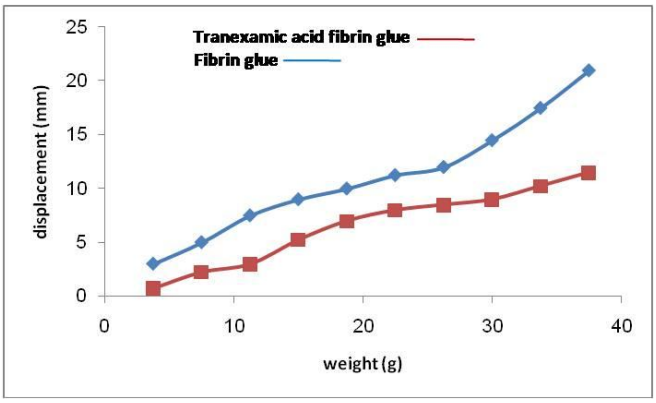

Fig. 1. The elasticity results of the 8:1 and 8:1:50 fibrin glues at 1 hour duration

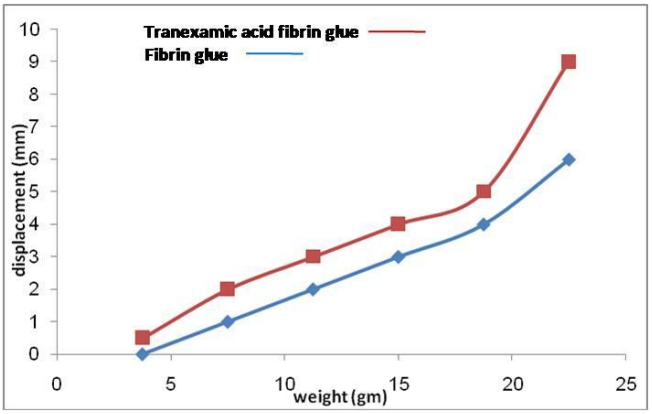

Fig. 2. The elasticity results of 8:1 and 8:1:50 fibrin glue after 1 week of glue synthesis

The tension tests

Results show clearly the increased tension of both formulae with gradual increased displacements at 1 hour, 1 week and 14 days durations. Statistical analysis presents significant higher tension results of 8:1 than that of 8:1:50 at 1 hour duration (*P* = 0.02), while at 1 week duration, tension results of both formulae shows no significant difference (*P* = 0.1). The comparison of the tension results of the 8:1:50 fibrin glue at different durations of times shows that the highest tension results were at 1 hour duration and the lowest tension results were at

14 days duration (*P* = 0.0008) as shown in fig. 4-6.

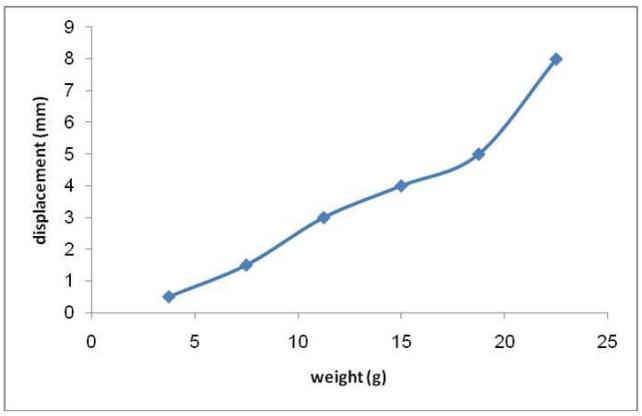

Fig. 3. The elasticity results of tranexamic acid fibrin glue (8:1:50) fibrin glue at 14 days duration.

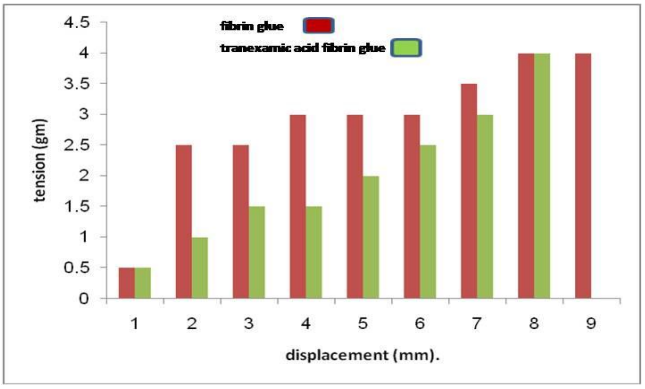

Fig. 4. Histogram of the tension results of 8:1 and 8:1:50 fibrin glue at 1 hour duration

Discussion

The higher fibrin glue elasticity with tranexamic acid addition in comparison to that without tranexamic acid addition may be attributed to change from physiological course to non physiological fine clots, which microscopically are characterized by thinner fibrin fibers [24,25], and so presented as a higher elasticity of tranexamic acid added fibrin glue. Elasticity results of the tranexamic acid added fibrin glue was significantly reduced time dependent. This gradual diminished result may be related to gradual decreased biological activity of the fibrin threads.

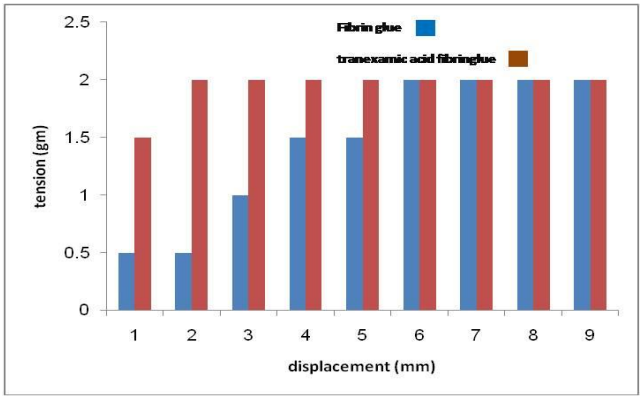

Fig. 5. Histogram of the tension results of 8:1 and 8:1:50 fibrin glues at 1 week duration

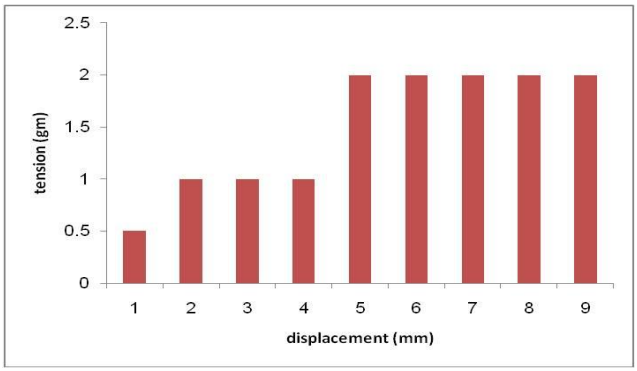

Fig. 6. Histogram of the tension results of tranexamic acid fibrin glue (8:1:50) fibrin glue at 14 days duration.

The lower tension of tranexamic acid added fibrin glue at 1hour may be explain by the change from physiological course to non-physiological fine clots, which microscopically are characterized by thinner fibrin fibers, more branching points, and a reduced pore size. These structural changes may be the reason for reduced tensile strength [24,25].

At 1 week duration, the tranexamic acid added high concentration fibrin glue showed non-significant difference in tension than the high concentration fibrin glue without addition; a result may be attributed to digestion of the fibrin threads by plasmin in non-added tranexamic acid fibrin glue, that caused reduced tension, while the fibrinolysis was delayed in tranexamic acid added fibrin glue [16,21] leading at

last to non-significant difference in tension between both formulae at 1 week duration. Tension results of the tranexamic acid added fibrin glue was significantly reduced time dependent, this gradual diminished result may be related to gradual decreased biological activity of the fibrin threads.

In conclusion, tranexamic acid addition to the components of the fibrin glue is used for delaying or prevents early fibrinolysis of the glue. This work reveals that this addition lead to change in biological behavior of the glue presents as increased elasticity and decreased tension. This change should be taken into consideration when the applicator needs to use this formula in the management of different areas of human body.

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