

# PREPARATION OF FIBRIN GLUE AS A BIOLOGICAL SEALANT TO CONTROL BLEEDING IN HEART MUSCLE AND BLOOD VESSELS

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## ABSTRACT

Fibrin glue is used for control of bleeding in various surgical procedures. In this work the ability of fibrin glue to seal punctures in the vascular system is demonstrated. Blood samples were taken from rabbits, fibrinogen was separated and fibrin glue was eventually prepared. The rabbits were anesthetized and a mid-line incision was carried out. The heart and abdominal aorta were exposed. Punctures were made in different parts of the vascular system and bleeding was controlled either with (test) or without (control) fibrin glue. Oozing was also tested by scratching the rabbit's ears.

A minimum of 6 rabbits was employed for each experience. The mean bleeding time using fibrin glue was found to be 37 seconds. This average without fibrin glue was more than 3 minutes. This study shows the powerful effect of this biological glue in bleeding control, and its routine use is therefore recommended, especially in major surgery.

*MJIRI, Vol. 17, No. 4, 289-292, 2004.*

## INTRODUCTION

Bleeding control by a kind of sealant following surgical techniques was a major concern for many years. Cyanoacrylate was the first sealant made of a chemical plastic compound.<sup>1-3</sup> This material was found to be histotoxic.<sup>4</sup> Biological sealant was first used by Dr. Spangler in 1976 to control bleeding in cardiac surgery.<sup>5</sup> Since then fibrin glue has been used and found to be of benefit in different surgical procedures.<sup>6,7</sup> Some workers used Gelatin- resorcinol- Formaldehyde/ Glutaraldehyde (GRF) glue or so called "French glue" in surgical technique.<sup>8-12</sup>

Fibrin glue has been most successful in controlling bleeding in cases of removal of urethral stones and in

stabilization of auditory ossicles.<sup>13</sup> This glue is used to control bleeding from ruptured spleen.<sup>14,15</sup> Some researchers used the sealant to prevent air leaks in thoracic procedures.<sup>12,16-18</sup> An important feature of fibrin sealant is its ability to achieve hemostasis at vascular anastomoses; especially in areas that are difficult to approach with sutures or in which suture placement presents excessive risk.<sup>16,19-21</sup> However one should bear in mind that most of the time suture placement is mandatory and cannot be replaced by fibrin glue. Bleeding from needle holes or small arterial tears can usually be sealed by judicious fibrin glue application.<sup>19</sup> Fibrin glue has been especially helpful in obtaining hemostasis in heparinized patients or those with coagulopathy.<sup>19,22</sup> Sealants are widely used in cardiac surgery<sup>18, 23-25</sup> and permit the use of porous

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knitted grafts, even in anticoagulated patients, eliminating bleeding that has prevented the widespread use of those porous grafts in open heart surgery.<sup>19,21,26,27</sup> Beneficial effects of this biologic sealant were evaluated on esophageal perforation by Tasdemir and co-workers.<sup>28</sup>

The use of fibrin glue found much favour in Europe and some other parts of the world. In the United States, because of the fear of possible risk of disease transmission, few reports are available.<sup>17,18</sup> To prevent such a risk the glue can also be prepared from a single donor or preferably autologous blood donations.<sup>29</sup>

The techniques for preparation and application of fibrin glue are described by some researchers. The glue is made by preparation of concentrated fibrinogen from either single donor or autologous blood.<sup>29,31</sup> In Iran a single paper, with the aim of wound healing, has appeared in this field,<sup>32</sup> while the progress of medical services demands for more investigations.

This work was performed with the aim to find the ability of fibrin glue to seal the different ruptures and punctures made in various parts of the rabbit circulatory system.

### MATERIAL AND METHODS

In this work, preparation of fibrin glue was according to Dresdale and co-workers,<sup>30,31</sup> with some minor modifications. Tubes containing 10 mL of blood, taken from rabbits, were centrifuged to separate the fresh frozen plasma (FFP), and kept frozen overnight at  $-18^{\circ}\text{C}$ . The fibrinogen is prepared by thawing the plasma for a few hours at  $4^{\circ}\text{C}$ . The fibrinogen pellet is separated by decanting the supernatant after centrifugation at 3500 rpm for 5 minutes.<sup>33</sup> The fibrinogen pellet can be left in a small amount of FFP at the bottom of the tube. Total volume of the fibrinogen pellet and FFP, obtained from the initial 10 mL of blood volume, is around one mL, which is more than the yield obtained by Dresdale's method. This is because their product was probably less diluted. The process of fibrinogen preparation was performed under a sterile condition. A sample of the final product is also checked for contamination by routine microbiological culture.

In this research concentrated fibrinogen was used fresh, within one hour of the preparation. The product is used after addition of calcium chloride as well as commercial thrombin (bovine thrombin, Enzyme Research Labs, Coa Chrom, A-1230 Wien, Leo Mathauser - Gasse 71, Germany). Lyophilized vials of thrombin with an activity of 500 units per vial are suspended in 0.2 mL distilled water. At the time of operation, 1 mL of fibrinogen is used with 0.2 mL of thrombin.

The rabbits were anesthetized with intramuscular injection of ketamine hydrochloride (50 mg/Kg) and

xylozine (5 mg/Kg). An endotracheal tube was inserted via tracheostomy and anesthesia was continued with halothane and oxygen.

A minimum of 6 rabbits was employed for each experiment. A minimum of one and a maximum of three punctures were made on each vessel, to perform the tests as well as the controls. A midline incision was carried out and the abdominal aorta was exposed. Each operation consisted of two separate punctures. The first puncture in which no fibrin glue is applied was carried out as the control and the second puncture in which the bleeding was stopped by fibrin glue was performed, above the control puncture, as the test. Hence avoiding any interaction between the control and the test.

A puncture was made with a green angio-catheter (needle no. 14), such that blood was ejected about 10 to 15 cm. The site of bleeding was compressed by a sterile gauze pad (control puncture). When the bleeding was completely stopped, a second puncture was made using the same needle, the bleeding of which was controlled by fibrin glue with or without using gauze pad (test puncture). To expose the heart and lungs, the incision was extended to mid sternotomy. A control and a test puncture were again made on the ascending aorta, by the same manner. The same was followed for the left ventricle. In all of the experiments control and test punctures were checked for the possibility of bleeding every 20 seconds.

To investigate oozing, two ears of each rabbit were scratched by a surgical blade. The oozing area of both ears was compressed with gauze pad in order to stop bleeding; while on the right ear fibrin glue was added (test), on the left ear no glue was applied (control).

### RESULTS

In our experience, the operations were of three types: punctures made in large blood vessels, capillaries, and heart muscle. The bleeding made on large vessels and aorta were stopped after a minimum of 25 and a maximum of 35 seconds (average 30 seconds), using fibrin glue. The control bleedings were stopped after a minimum of one minute and 30 seconds and a maximum of 2 minutes and 30 seconds, average 2 minutes.

Bleeding from heart muscle was stopped after 50 to 70 seconds, average 61 seconds, by the sealant, while the controls show 110 to 240 seconds, with an average of around 172 seconds.

The capillary oozing test was carried out by scratching the area behind the ears. Bleeding times from oozing sites were less than the above figures, with a minimum of 15 and a maximum of 30 seconds (average 19 seconds), using fibrin sealant. The control performances for oozing show more variable data from 60 to more than 600 seconds, average more than 215 seconds. In one of

**Table I.** Bleeding time obtained from aorta (or large vessels), heart muscle and capillaries.

Rabbit no.	1	2	3	4	5	6	Mean
Aorta Test	27	25	33	35	30	30	30
Control	120	90	100	150	140	120	120
Heart Test	65	60	70	50	60	60	61
Control	120	180	200	180	240	110	172
Oozing Test	20	15	25	30	15	10	19
Control	60	120	90	180	240	>600*	>215

\* In this case bleeding time did not stop after 10 minutes (600 seconds).

the experiments oozing from a rabbit ear did not stop even after 10 minutes, where it eventually stopped after 10 seconds, using fibrin glue. All data are shown in Table I.

## DISCUSSION

Fibrin sealant has been used for years in different surgical techniques. The main reasons for wide consumption of fibrin glue are to reduce the time of operation, control of local bleeding, minimize blood loss after operation and reduce the chance for reoperations for bleeding control. This product is therefore used by different surgeons, to solve many surgical problems mostly in cardiovascular surgery.<sup>5,16,19-21,26,27,34,35</sup> The product is successfully used in wound healing,<sup>36</sup> air fistulas and air leaks in thoracic surgery,<sup>16,19,37</sup> fistulas and leaks in gastrointestinal surgery<sup>38</sup> and leak of cerebrospinal fluid.<sup>29</sup> This glue has also found importance in plastic surgery.<sup>39</sup> Fibrin glue is even useful in patients with coagulation problems or those who undergo heparin therapy.<sup>22,40-42</sup> To prevent any possible risk of disease transmission, the glue can also be prepared from a single donor or preferably autologous blood donations.<sup>29,40</sup>

The data in this work show significant differences between the bleeding time of all procedures in which fibrin glue was used to stop bleeding (test), and procedures which were performed without any glue (control). This finding means fibrin glue has a rapid effect to stop blood loss in different kinds of hemorrhage.

In our work the most serious bleedings in large vessels such as the aorta and heart muscles are artificially made. The mean bleeding time using fibrin glue was found to be 37 seconds, while without fibrin glue, the bleeding time was around 3 minutes. In one of the above experiments which was carried out as the control, oozing on the surface of the ear of rabbit did not stop even after ten minutes. Bleeding in this experiment eventually was controlled af-

ter 10 seconds, using fibrin glue.

## ACKNOWLEDGEMENT

We would like to acknowledge the help of Mr. Kaveh Useffi, BSc, Miss Manijeh Toughi, MSc, Dr Seyed Ali Gaskari, MD, Dr. Shahram Rabbani, DVM and Miss Farideh Farzanfar, BSc, from Imam Khomeini Medical Center, Tehran University of Medical Sciences, Tehran, Iran. We also appreciate the assistance received from Miss Azita Sedigh, BSc, and Mr. Zabihollah Azlegeini, from Razi Institute, Karaj, Iran.

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