Hemostatic Agents for Control of Intracavitary Non-Compressible Hemorrhage: An Overview of Current Results[1]

Bijan Kheirabadi, PhD, Harold G. Klemcke, PhD
US Army Institute of Surgical Research
3400 Rawley E. Chambers Avenue
Fort Sam Houston, TX 78234-6315

Email: bijan.kheirabadi@amedd.army.mil

ABSTRACT

The majority (~80%) of hemorrhagic deaths on the battlefield are due to intracavitary hemorrhage that is not accessible for direct compression and cannot be treated with externally applied hemostatic agents. In an attempt to address this issue, a project was initiated to evaluate the efficacy of different hemostatic products when introduced into a closed hemorrhaging body cavity. Two thrombin-based hemostatic agents have been tested thus far in rat and rabbit models. In the initial phase, these agents were tested by direct and immediate application over severe parenchymal injury without compression in open-abdomen models. In the second phase of the project, the hemostatic agents were infused 5 minutes after a liver injury in closed-abdomen injury models. In the phase 1 open abdomen studies, both hemostatic agents decreased blood loss when compared with placebo-treated control animals. This decreased blood loss corresponded to an increase in survival rates that was not, however, statistically significant. In the phase 2 closed abdomen study neither hemostatic agent was able to produce a significant change in blood loss or survival rates when compared to control animals. The hemostatic properties of both hemostatic agents involve binding with injured tissues. Such characteristics require contact of these agents with damaged, bleeding tissues. In the closed abdomen model, contact is made difficult by ongoing hemorrhage and pooled blood. The failure of both products to demonstrate efficacy may also have been due to model design. In the severe liver injury model, bleeding is most severe in the first few minutes after the injury that unless treated promptly the consequences cannot be reversed by later interventions. Additional studies in more appropriate models with alternative hemostatic agents will further evaluate the potential for intracavitary approach to treat the noncompressible hemorrhage.

1.0 INTRODUCTION

Hemorrhage is the greatest threat to survival in the first 24 hours following traumatic injury [1]. It accounts for nearly 50% of all deaths on the battlefield and 39% of civilian trauma deaths [2-5], most of which occur before patients reach the hospital [6-7]. Traditional methods available to pre-hospital personnel for controlling hemorrhage, such as applying tourniquets or pressure dressings, or clamping, are practical for extremity or superficial truncal wounds. However, these types of wounds account for only 10% of hemorrhage-related combat deaths and even fewer hemorrhage-related civilian deaths [8-9]. The majority of hemorrhagic deaths

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on the battlefield (up to 90% in Vietnam war) are due to intracavitary hemorrhage that is not accessible to direct pressure and cannot be controlled by these traditional methods [8]. This has left first responders with no means to treat truncal hemorrhage other than fluid resuscitation . Paradoxically, the administration of intravenous fluids, by diluting coagulation factors and platelets, can further exaggerate bleeding. Even after arrival in a hospital, patients with acute bleeding from truncal injuries can present significant challenges to surgeons who possess multiple surgical techniques, sophisticated equipment, and variety hemostatic materials [10]. Currently, surgical interventions are the only available methods for controlling noncompressible hemorrhage and preventing death. Developing methods to control or reduce intracavitary hemorrhage within the first critical hours following injury, before patients reach the hospital could have a significant impact on morbidity and mortality rates for trauma patients.

1.1 Intracavitary Intervention

A novel concept for treating intracavitary hemorrhage was first introduced by Holcomb and colleagues [11]. They proposed the creation of a hemostatic agent for direct infusion into a bleeding body cavity. The hemostatic agent would then self-expand throughout the closed cavity and interact with bleeding sites to stop hemorrhage. In a penetrating injury the hemostatic agent would be introduced through the hole left by the wounding mechanism. To implement this concept, the hemostatic agent would have to: 1) reduce blood loss and increase survival when applied directly on an active bleeding site without manual compression; 2) spread efficiently and reach the internal injuries in the closed cavity (e.g. abdomen); 3) remain active during and after indirect application and interact with bleeding sources to reduce overall blood loss and related fatalities. To put this concept to the test two hemostatic products, fibrin sealant (FS) foam and FloSeal were considered. **FS foam contained human fibrinogen, human thrombin, and calcium chloride ($CaCl_2$), and was reconstituted in saline and transformed into foam with the aid of a chemical reaction. FloSeal was obtained from commercial sources and contained bovine-derived thrombin and collagen particles.** Both hemostatic agents were evaluated in modified rat or rabbit liver injury models.

2.0 OPEN-ABDOMEN TREATMENT OF PARENCHYMAL BLEEDING (DIRECT APPLICATION)

FS foam and FloSeal were first tested in open abdomen models that allowed easy access to the wounds and direct application of hemostatic agents on the injuries. A positive outcome in this initial phase of the study was a prerequisite for further evaluation of the agents.

2.1 FS Foams

2.1.1 First Formulation

In the initial proof-of-principle study [11], a formulation of fibrin sealant foam with high thrombin (36 IU/ml) and low fibrinogen (1.9 mg/ml) concentration was developed and tested. The foam was rapidly prepared and sprayed directly on the bleeding surfaces of rat liver tissues immediately following sharp resection of 60% of the median hepatic lobe. Subsequently, animals were resuscitated (3.3 ml/kg) to maintain their blood pressure at pre-injury levels and observed for 30 minutes or until death. The FS foam treatment decreased blood loss (~50%) compared with untreated or placebo foam (IgG replaced fibrinogen) treated injuries (P<0.01). However, this reduced blood loss did not influence the survival of the treated group. A tight binding between fibrin clots and injured hepatic tissue was observed which might have been responsible for the overall reduction in blood loss. But when the FS foam was introduced into a closed-body cavity at a site distal to the

liver injury, no change in blood loss or survival was observed among the treated animals (unpublished data). The high thrombin activity and rapid clotting may have prohibited this formulation of FS foam from dispersing sufficiently throughout the cavity to reach the bleeding targets. Hence, the first formulation was considered inappropriate for intracavitary treatment purposes.

2.1.2 Second Formulation

A new formulation for FS foam was designed and tested extensively in the laboratory. The concentration of individual components of this foam were optimized based on an *in-vitro* tissue adhesion test developed in our laboratory. The new FS foam formula contained high concentrations of human fibrinogen (20 mg/ml) and albumin (10 mg/ml) with low levels of thrombin activity (3.3 IU/ml) and $CaCl_2$ (0.9 mg/ml) and polymerized more slowly than the previous formula. This foam attached firmly to rabbit liver slices even if the tissues were covered with fresh blood (not anticoagulated).

2.1.3 Rabbit Model

To evaluate the hemostatic efficacy of this foam *in-vivo*, a new liver hemorrhage model was developed in rabbits. This species offered several advantages over rats: 1) the larger circulating blood volume (200-250 ml) in mature animals (3 kg) permitted blood sampling during operation without affecting the blood pressure; 2) higher bleeding volumes in rabbits minimized adverse effects of small variations in final blood/blood clot collections and blood loss measurements; 3) less efficient blood clotting ability and lower survivability of rabbits with hemorrhage provided better conditions for testing hemostatic agents [12].

2.1.4 Anticoagulants

During the course of model development different anticoagulant regimens were tested. The parenchymal injury consisted of sharp resection of 1/3 of the middle and left lobes and 1/4 of the right lobe of the liver without damaging the gall bladder and bile ducts. Subsequently, the abdominal incision was closed and animals were resuscitated with lactated Ringer's (LR) solution at a constant rate (1 ml/kg/min) to maintain mean arterial pressure at 80% of baseline value (pre-injury level). This relatively consistent liver injury and resuscitation protocol nonetheless produced wide variations in blood loss and resulted in only 30% mortality among rabbits. Resecting larger portions of the liver frequently resulted in rapid exsanguination and no opportunity for hemostatic treatment.

Therefore, in order to produce persistent liver bleeding with relatively large and reproducible blood loss in rabbits, anticoagulant treatment was incorporated into the protocol. Initially, heparin (200 IU/kg) was used as an anticoagulant. The above-noted liver injury in heparinized animals caused persistent bleeding with 100% mortality within a 60-minute observation period. When FS foam was tested in the heparinized model, no hemostatic benefit and no interaction between the fibrin clot and bleeding tissues was observed. It became evident that the presence of heparinized blood in the wound may have inhibited the activity of the thrombin in the foam and prevented cross-linking of fibrin to tissues. Therefore, a new anticoagulant, heparinoid, which has little or no effect on thrombin activity, was selected. The anticoagulant activity of heparinoid is predominately due to the inhibition of Factor Xa activity in blood.

2.1.5 FS Treatment in Heparinoid-Treated Rabbits

The hemostatic potential of the new formulation of FS foam was reexamined in heparinoid-treated rabbits. Animals were injected intravenously with a dose of anticoagulant (sodium danaparoid, 50 anti FXa

activity/kg) and liver injuries were induced. The bleeding tissues were sprayed with FS foam or placebo foam in which hemostatic components were replaced with an equal quantity of human IgG. The rapid and direct treatment of the injuries with FS foam produced a 37% decrease in blood loss (P=0.07) and a 23% increase in percent survival (Fig. 1a, b). The average survival time in the FS group was 53.3 ± 4.2 min versus 41.3 ± 7.7 min in the placebo-treated group (P= 0.34, Fig. 1c). Although these outcomes were not statistically significant, (mainly because of limited number of animals in each group), the numerical trends provided sufficient positive evidence to justify further testing of the hemostatic capacity of the FS foam in a closed-abdomen model.

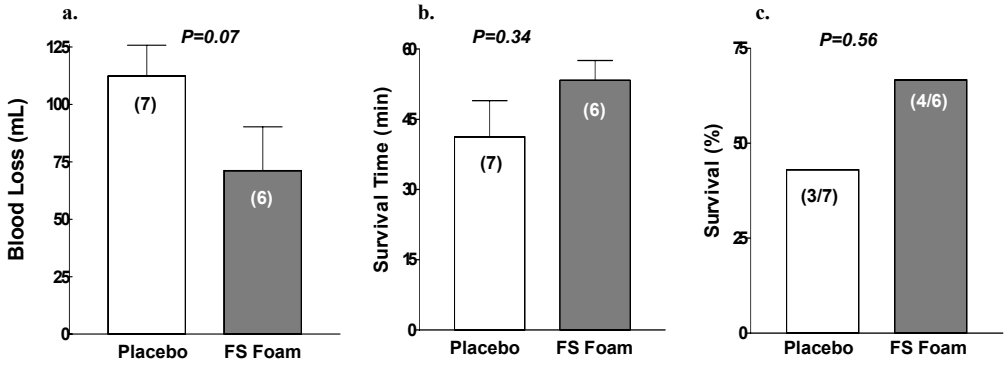

Figure 1: Open-Abdomen Model. Effects of direct and immediate application of placebo or fibrin sealant foam after severe liver injury in rabbits on: a) blood loss into the abdominal cavity; b) survival time after injury; and c) percent survival.

2.2 FloSeal

This FDA-approved hemostatic agent is commercially available (Baxter International Inc). It consists of specially engineered collagen particles and bovine thrombin. Upon exposure to blood, the gelatin (collagen) granules expand ~ 20% to provide some tamponade [13], and thrombin converts endogenous fibrinogen to fibrin that adheres the gelatin granules to the wound. To aid hemostasis, gentle compression of FloSeal mixture over the wounds with moist gauze for 2 to 3 minutes is strongly recommended. This agent has been used in humans to reduce bleeding in a variety of surgical procedures including vascular surgery, cardiac valve replacement and partial nephrectomy [13-15]. It was shown to be more effective in controlling blood loss than a combination of Gelfoam and thrombin in a clinical study involving cardiac surgery [16].

FloSeal was also tested as a hemostatic agent for intracavitary noncompressible hemorrhage using the previously described rat liver injury model (unpublished data). FloSeal paste was prepared according to the manufacturer's instructions by dissolving thrombin in saline and then thoroughly mixing the dissolved

thrombin with Gelatin Matrix. Five ml of the mixture or vehicle solution (saline) were applied directly over the cut liver surfaces immediately after injury without additional compression and the abdominal cavity was closed. Animals were resuscitated (LR, 3.3 ml/min/kg) to maintain their blood pressure at pre-injury levels and they were monitored for 90 minutes or until death. FloSeal treatment significantly reduced the blood loss ($p<0.01$) and numerically increased percent survival (26.7 *vs* 6.3%; $P=0.17$) and average survival time (37.7±9.0 *vs* 20.7±5.2 min; $P=0.12$) among the rats (Fig 2 a, b, c). There was no association between the volume of resuscitation fluid used and blood loss ($P=0.45$). This was the first evidence of the hemostatic benefit of FloSeal for major bleeding when used without the aid of manual compression, an essential requirement for any hemostatic agent under consideration for treatment of noncompressible hemorrhage.

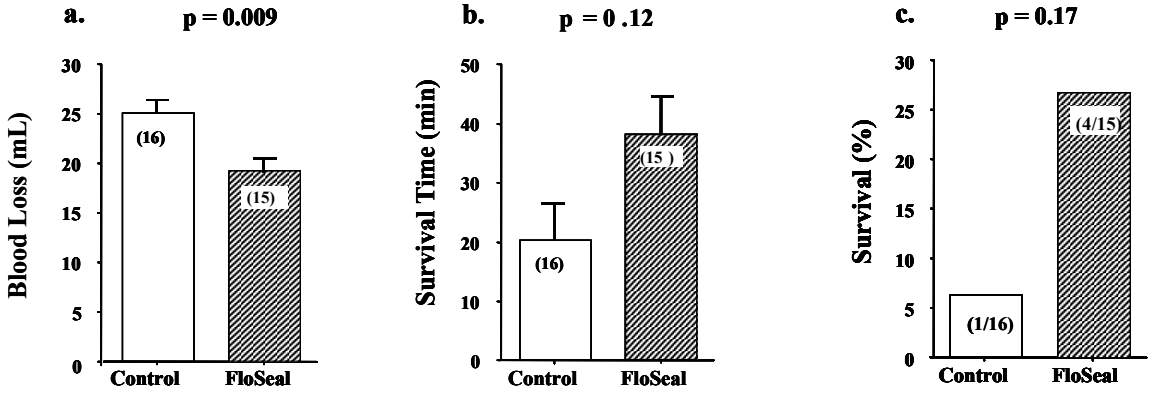

Figure 2: Open-Abdomen Model. Effects of direct and immediate application of saline (control) or FloSeal after severe liver injury in rats on: a) blood loss into the abdominal cavity; b) survival time after injury; and c) percent survival.

3.0 CLOSED-ABDOMEN TREATMENT OF PARENCHYMAL BLEEDING (INDIRECT APPLICATION)

3.1 FS Foam

The efficacy of slow polymerizing FS foam (2nd formulation) was further tested in a closed-abdomen injury model in rabbits. Following heparinoid injection and laparotomy, three frontal lobes of liver were sharply resected as described before and the abdominal incision was closed with suturing. Five minutes following liver injury, the rabbits were either treated with FS foam or left untreated to bleed freely from the injuries (controls). For foam treatment, a plastic tube was inserted into the abdomen (on suture line) and aimed toward the liver. About 100 ml of FS foam was prepared in a large syringe (140 ml) and infused into the cavity through the implanted tube. Animals were resuscitated with LR as in previous experiments (1 ml/min/kg) to maintain their blood pressure at 80% of pre-injury levels and monitored for 60 minutes or until death at which time blood, blood clots and foam clots were collected and weighed to estimate total blood loss (corrected for the weight of the foam).

Infusion of FS foam into the abdomen distal to the bleeding tissues did not reduce blood loss or improve percent survival in the rabbits (Fig. 3a). The only difference was a larger variation in blood loss of individual rabbits in the foam group compared with control rabbits. Inspection of the wounds after completion of the

experiments revealed that in most cases (4/6) foam did not spread well enough to penetrate and cover the resected areas in the left and right lobes of the livers. It reached and attached firmly to the middle lobe where blood had drained away from the tissue.

3.2 FloSeal

The potential intracavitary application of FloSeal was tested in the rat liver injury model. Following sharp resection of 60% of the median hepatic lobe the peritoneal cavity was closed. FloSeal was prepared as for the open abdomen experiments except it was diluted four-fold with saline to reduce FloSeal viscosity and to improve its distribution throughout the abdomen. Five minutes after the liver injury, FloSeal was infused into the closed abdomen ~ 3 cm distal to the bleeding site. Animals were resuscitated and monitored up to 60 min. This procedure did not reduce blood loss (P=0.43) or improve survival (P=0.66) when compared with the saline-treated control rats (Fig. 3b). When the surgery was performed again in a presumably positive control group in which the injured tissue was clamped and the bleeding was abruptly and completely stopped after 5 minutes, there was no improvement in blood loss or survival of animals as compared with saline-treated or untreated groups.

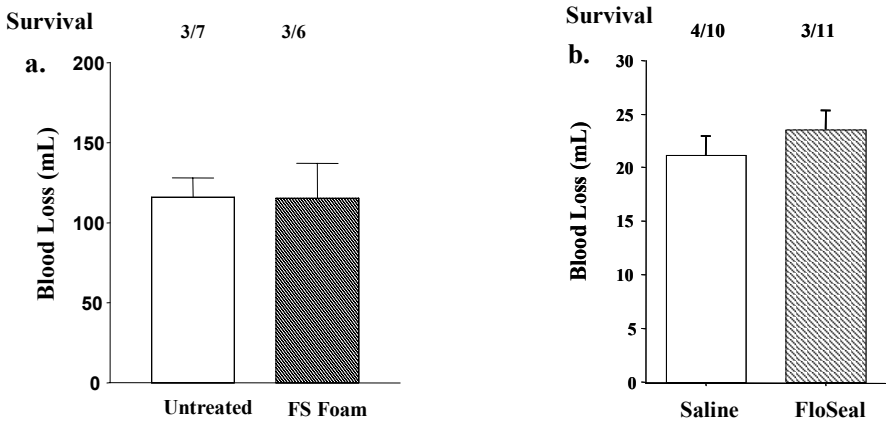

Figure 3: Closed-Abdomen Model. a) Effects of indirect and delayed (5 min) application of fibrin sealant foam after liver injury in rabbits on internal blood loss and survival. b) Effects of indirect and delayed (5 min) application of saline or FloSeal after severe liver injury in rats on blood loss into the abdominal cavity and percent survival.

4.0 DISCUSSION

Despite positive results of FS foam and FloSeal when applied directly without manual compression in an open-abdomen model, these agents were ineffective in preliminary evaluations of indirect application in the closed-abdomen models. The reasons for hemostatic failure may be similar for both products. For FS, the interaction and binding of fibrin clot with the injured tissues is an essential step for achieving hemostasis. This interaction may also be a component of FloSeal's hemostatic ability. The poor distribution, weak and incomplete binding of FS foam with injury sites appeared to be the main cause of its ineffectiveness.

There were several factors that may have impeded the distribution of the foam and FloSeal in the closed abdomen. First, the five-minute delay in application of the hemostatic agents was implemented in both intracavitary studies to more closely reflect a realistic battlefield situation in which no medical care would be available to soldiers immediately after wounding. Such a delay, however, might have resulted in significant blood pooling around the liver, rendering the injury sites less accessible for treatment. In addition, significant blood loss during this crucial period may have made the later intervention essentially futile. This was clearly demonstrated in the control group of FloSeal study in which complete cessation of bleeding five-minutes after injury had no effect on the final measurements. Second, the flowing blood from the tissues could have also impeded the penetration and interaction of the agents with the bleeding sites. Third, in rabbits, the ineffectiveness of the foam may have been related to the anatomical location of the liver wound. The close proximity of the peritoneal wall to the right and left lobes and the natural drainage pathway of the blood on the lateral sides of the abdomen might have prevented the dispersion and contact of the foam with those tissues. Although foam covered the middle lobe, and perhaps stopped the bleeding in this region blood flow from the middle lobe might have shifted to the lateral lobes and bleeding continued with the same intensity from these lobes that were not covered by the hemostatic agent. The four-fold dilution of FloSeal necessary for closed-abdomen usage might have also weakened the interaction of this agent with damaged tissue and reduced its hemostatic efficacy.

Theoretically, intracavitary injection of hemostatic agents could be a successful method for treating noncompressible hemorrhage, but the preliminary testing with FS foam and FloSeal in the animal models described above did not show any potential benefit. Although the models seemed appropriate for the initial hemostatic testing of the agents with direct application, they did not meet some bleeding characteristics necessary for indirect and delayed application of the products. The following options and changes will be considered in future studies to further investigate intracavitary treatment of noncompressible hemorrhage.

- Development of a new noncompressible hemorrhage model with a slow but persistent bleeding profile in rabbits that would be responsive to delayed treatment with hemostatic agents.
- Addition of a high-pressure liquified gas propellant to hemostatic products to facilitate introduction and distribution of the agents into the cavities. The propellant gas will have to be vented rapidly from the abdominal cavity to prevent sustained high insufflation pressure and its potentially harmful effects (e.g., abdominal compartment syndrome).
- Intracavitary infusion of gel/foam preparations of true hemostatic agents (e.g. thrombin, reptilase). Unlike fibrin sealant products, these agents promote hemostasis by stimulating the patient's own blood to clot and seal injuries to stop the bleeding. With the use of these agents, the blood pooling around the injuries could actually be beneficial and promote hemostasis instead of acting as a physical barrier.
- Development of an integrated approach that combines intravascular and intracavitary treatments. For example, the essential coagulation precursors such as fibrinogen and phospholipid membranes may be

injected intravascularly as additives to fluid resuscitation procedures, and hemostatic enzymes infused into cavities to induce coagulation and control bleeding.

5.0 SUMMARY

The intracavitary administration of hemostatic agents offers a novel concept for treatment of noncompressible hemorrhage. However, the preliminary testing of this concept with two hemostatic agents failed to show measurable benefit in reducing parenchymal bleeding. The reasons for this failure appeared to be more related to the hemorrhage models, treatment conditions, and the agent formulas (designed for direct application) used for evaluating this hypothesis rather than the concept itself, but clearly, there are several technical problems associated with testing of such a novel approach that have yet to be overcome. Future studies with alternative hemostatic agents will focus to resolve these issues and further evaluate the potential benefit of intracavitary treatment of noncompressible hemorrhage in clinically relevant and potentially treatable models.

6.0 REFERENCES

1. Hoyt DB, Bulger EM, Knudson MM, et al. Death in the operating room: an analysis of a multi-center experience. *J Trauma*. 1994;37:426-432.

2. Bellamy RF. Causes of death in conventional warfare, *Mil Med*. 1984;149:55-62.

3. Acosta JA, Yang JC, Winchell RJ, Simons RK, Fortlage DA, Hollingsworth-Fridlund P, and Hoyt DA. Lethal injuries and time to death in a level one trauma center. *J Am Coll Surg*. 1998;186:528-533.

4. Sauaia A, Moore FA, Moore EE, Moser KS, Brennan R, Read RA, Pons PT. Epidemiology of trauma deaths: a reassessment. *J Trauma*. 1995;38:185-193.

5. Shackford SR, Mackersie RC, Holbrook TL, Davis JW, Hollingsworth-Fridlund P, Hoyt DB and Wolf PL. The epidemiology of traumatic death: a population-based analysis. *Arch Surg*. 1993;128:571-575.

6. Zimmerman L, Veith I. *Great ideas in the history of surgery*. San Francisco, CA: Norman publishing; 1993.

7. Gabriel R, Metz K. *A History of Military Medicine.* Westport, CT:Geenwood Press; 1992

8. Carey ME. Analysis of wounds incurred by U.S. Army Seventh Corps personnel treated in Corps hospital during Operation Desert Storm, Feb 20 to Mar 10, 1991. *J Trauma*. 1996:40(suppl):S165-S169.

9. American College of Surgeons. *Advanced Trauma Life Support*. Chicago, IL: American College of Surgeons; 1997.

10. Chen RJ, Fang JF, Lin BC, et al. Factors determining operative mortality of grade V blunt hepatic trauma. *J Trauma*. 2000;49(5):886-91.

11. Holcomb JB, McClain JM, Pusateri AE, et al. Fibrin sealant foam sprayed directly on liver injuries decreases blood loss in resuscitated rats. *J Trauma*. 2000; 49:246-250.

12. Barroso-Aranda J, Chavez-Chavez RH, Schmid-Schonbein GW. Spontaneous neutrophil activation and the outcome of hemorrhagic shock in rabbits. *Circ Shock*. 1992; 36:185-190.

13. Reuthebuch O, Lachat ML, Vogt P, Schurr U, Turina M. FloSeal: a new hemostyptic agent in peripheral vascular surgery.
Vasa. 2000; 29(3):204-6.

14. Weaver FA, Hood DB, Zatina M, Messina L, Badduke B. Gelatin-thrombin-based hemostatic sealant for intraoperative bleeding in vascular surgery.
Ann Vasc Surg. 2002;16(3):286-93.

15. Richter F, Schnorr D, Deger S, Trk I, Roigas J, Wille A, Loening SA. Improvement of hemostasis in open and laparoscopically performed partial nephrectomy using a gelatin matrix-thrombin tissue sealant (FloSeal).
Urology. 2003 Jan;61(1):73-7.

16. Oz MC, Cosgrove DM 3rd, Badduke BR, Hill JD, Flannery MR, Palumbo R, Topic N. Controlled clinical trial of a novel hemostatic agent in cardiac surgery. The Fusion Matrix Study Group.
Ann Thorac Surg. 2000 May;69(5):1376-82.