

## ORIGINAL CONTRIBUTION

# Fibrin Glue Eliminates the Need For Packing After Complex Liver Injuries

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*Hemostasis after traumatic liver injury can be extremely difficult to obtain, particularly in coagulopathic patients who have suffered extensive liver damage. We determined the ability of a fibrin glue preparation (FG) to terminate ongoing bleeding using a new, clinically relevant porcine model of complex hepatic injury. Anesthetized swine (n = 6, 18 to 19 kg) received an external blast to the right upper abdomen and were immediately anticoagulated with intravenous heparin (200 u/kg). Uncontrolled hemorrhage from blast continued from time of injury (t = 0 minutes) to t = 15 minutes. Lactated Ringer's solution was infused to keep mean arterial pressure (MAP) > 80 mm Hg until the end of experiment (t = 90 minutes). Animals underwent routine surgical techniques to control bleeding, and FG was employed in the event these measures failed. Estimated blood loss and fluid resuscitation volume were measured. Serial MAP, arterial base excess, and temperature were recorded. Animals were severely injured with significant blood loss prior to laparotomy (26 ± 6 cc/kg) and during routine surgical efforts to arrest hemorrhage (11 ± 2 cc/kg). Bleeding could not be controlled with standard techniques in any animal. FG rapidly controlled hemorrhage and eliminated the need for packing. Re-bleeding was noted in only one animal (portal vein injury). FG can control severe hepatic hemorrhage when surgical techniques fail. Further work in the clinical arena is warranted to determine the potential benefits of FG in arresting hemorrhage in hemodynamically unstable coagulopathic patients with complex hepatic injuries.*

## INTRODUCTION

Liver injuries have an associated mortality of approximately 25 percent with exsanguination being the primary cause of death [1, 2]. Hemostasis in these patients is hindered by the development of coagulopathy, which results from hypothermia, dilution of clotting factors, and thrombo-

cytopenia. Control of bleeding in severe liver injuries frequently requires packing, with definitive repair delayed for hours while the coagulopathy is corrected [3]. New methods for obtaining rapid hemostasis in this setting would obviate the need for second-look procedures and represent a significant addition to the surgeon's

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<sup>b</sup> Abbreviations: DFSD, dry fibrin sealant dressing; FG, fibrin glue; Lactated Ringers, LR; MAP, mean arterial pressure; p, pulse; t, time.

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armamentarium. Fibrin glue (FG)<sup>b</sup> has been effective in arresting hemorrhage in animal models of hepatic injury [4-7]. Unfortunately, current recipes for FG preparations have required either the use of pooled coagulation factors carrying the associated risk for viral disease transmission, or involve time consuming autologous extraction techniques which are not easily applicable to the trauma setting [8]. Purified FGs, which are free of viral contamination, are now available and can be stored in a refrigerator in the operating room and rapidly reconstituted. This technical advance represents a potential milestone for the use of FG in trauma victims. We hypothesized that the use of a new, viral-free FG would eliminate the need for packing in the setting of uncontrollable bleeding in a porcine liver injury model.

## METHODS

The experimental protocol was approved by the University of Miami, School of Medicine, Animal Care and Use Committee and met the NIH guidelines for animal use.

Male Yorkshire random-bred male pigs (n = 6, 18 to 19 kg) were sedated with intramuscular ketamine (10 mg/kg) and anesthetized with intravenous pentobarbital (10 mg/kg). Animals were orotracheally intubated and ventilated (Ventilator Harvard Apparatus, South Natick, Massachusetts); FiO<sub>2</sub> 1.0; tidal volume 10 cc/kg; respiratory rate was adjusted to maintain arterial pCO<sub>2</sub> at 40 ± 5 torr. Femoral arterial and venous catheters were placed for hemodynamic monitoring, fluid resuscitation, and anesthetic infusion. Mean systemic arterial pressure (MAP) and pulse were continuously recorded by an amplifier-monitor (Datascope 2000A, Paramus, New Jersey). Arterial blood gases (Ciba Corning 238 pH/Blood Gas Analyzer, Medfield, Massachusetts) and arterial hemoglobin

saturation (SaO<sub>2</sub>) (Palco Pulsoximeter model 340, Santa Cruz, California) were measured. After completion of surgical procedures, general anesthesia was maintained with a continuous infusion of sodium pentobarbital (17 mg/kg/hr). Rectal temperature was serially measured. Heating pads were turned off at the moment of injury.

### *Liver injury methodology*

After a 15-minute equilibration period, a liver injury was induced. A nail driver (Ramset/Red Head, Low Velocity Piston Type Fastening Tool, Model 4170, Wood Dale, Illinois) with a 22 caliber charge (Pneutek, Inc., A22ND-2 low velocity, power load, Hudson, New Hampshire) was fired onto a solid aluminum disk (5 cm diameter, 1 cm thick). It was taped halfway between the xiphoid process and the anterior axillary line below the costal margin. This technique was adapted from one previously described [9]. At full inspiration, the endotracheal tube was clamped prior to inducing the external blast to the abdominal wall that generated the Grade III liver laceration, which resembles the clinical scenario (t = 0 minutes). Injuries were reproducible, inducing an area of deep contusion, 4 to 6 cm in diameter, stellate in shape, between segments IV and V of liver. Immediately after the contusion, the animals were anticoagulated with intravenous bovine heparin (200 units/kg). After a 5 minute period, resuscitation was begun using Lactated Ringer's (LR) solution at 20 cc/kg infused over 10 minutes (t = 5 to t = 15 minutes). At t = 15 minutes, laparotomy was performed to control hemorrhage. Free intraperitoneal blood was determined by change in the weight of lap pads. Estimated blood loss was determined using the following formula: cc of blood = (wet-dry lap pads) gm/1.043. The degree of liver injury was assessed. Surgeons used standard techniques for

hepatic repair including Pringle maneuver for no more than 10 minutes when utilized, deep liver suture, electrocautery, local tamponade, and hemostatic agents (SURGICEL™, Johnson and Johnson, Arlington, Texas). Blood loss during repair using these standard techniques was measured. Ten minutes after the start of laparotomy ( $t = 25$  minutes) if the surgeon determined that bleeding was not controlled, FG was made available. The FG, consisting of human topical fibrinogen complex, human thrombin, and calcium chloride, was constituted just prior to celiotomy. Kits containing the FG (Two-Component Fibrin Sealant Kit Vapor Heated, Immuno-US Inc, Rochester, Michigan) were removed from refrigerator at 4°C and warmed to 37°C. The fibrinogen complex (human sealer protein concentrate) and fibrinolysis inhibitor were mixed and drawn into a 2 cc syringe. The human thrombin was reconstituted using calcium chloride and drawn into a second 2 cc syringe. The two syringes were loaded into a y-shaped single-port injecting device for application. The product was readied in 10 to 15 minutes prior to application, and each application totaled approximately 3 ml, with a maximum of three kits utilized per pig. The glue was applied to the edge of the liver and brought into proximity as it was compressed until there was no actual hemorrhage and then sealed. If there was active hemorrhage after the full application, the surgeon would then pack all bleeding areas with

lap pads. The abdomen was closed 15 minutes after initiating FG repair ( $t = 40$  minutes). After laparotomy, maintenance fluids (LR) were continued until the end of the experiment to maintain MAP greater than 80 mm Hg. Ninety minutes after injury, re-laparotomy was performed to determine evidence of rebleeding and the animals euthanized with intravenous euthanasia solution (sodium pentobarbital, sodium phenytoin), 10mg/kg.

### Statistical analysis

Statistical analysis was performed using a statistical software package (Statistica, Statsoft Inc., Tulsa, Oklahoma). Groups were compared by analysis of variance (ANOVA) with post hoc comparisons (Tukey's HSD test). Differences were considered statistically significant when  $p < .05$

## RESULTS

Mean animal weight for six swine was 18.6 kg (range 18 to 19 kg). There were no differences noted in heart rate in the group at baseline compared to end of experiment. The liver injury did result in a significant decrease in MAP in the group following laparotomy with attempted surgical hemostasis. A significant decrease in body temperature occurred ( $38 \pm .4$  vs.  $34 \pm .3^\circ\text{C}$ ) between  $t = 0$  and the end of the experiment. A significant decrease in arterial base deficit was also noted after injury (See Table 1.).

**Table 1.**

Time	T = 0	T = 5	T = 15	T = 25	T = 40	T = 60	T = 75	T = 90
P (beats/min)	160 ± 5	190 ± 10	158 ± 8	161 ± 8	150 ± 7	148 ± 5	150 ± 7	152 ± 7
MAP (mm/Hg)	95 ± 7	69 ± 6	78 ± 3	54 ± 5*	70 ± 3*	69 ± 3*	69 ± 3*	63 ± 3*
Time (°C)	38 ± .4	38 ± .4	38 ± .3	37 ± .2	36 ± .3*	35 ± .3*	35 ± .4*	34 ± .3*
ABE (mEq/L)	-4 ± 1	-5 ± 2	-6 ± 1	-7 ± .9	-10 ± 1	-11 ± 1	-11 ± 1	-11 ± 2

Table 1 lists results of hemorrhage study. P, pulse; MAP, mean arterial pressure, ABE, arterial base excess. \* denotes statistical significance.

The animals were severely injured in this model with significant blood loss prior to laparotomy ( $26 \pm 6$  cc/kg) and during routine surgical techniques from  $t = 15$  to  $t = 25$  minutes ( $11 \pm 2$  cc/kg) with significant fluid required during this initial resuscitation,  $t = 5$  to  $t = 15$  minutes (LR:  $33 \pm 9$  cc/kg). Bleeding could not be controlled with routine hemostatic techniques in any animal. FG rapidly controlled hemorrhage and no animals needed to be packed. Re-bleeding was noted in one animal. (This was due to an unrecognized portal vein injury, which appeared to be controlled initially with FG application.)

## DISCUSSION

The principal findings of this study were: 1) FG terminated hemorrhage which was not controllable with conventional surgical techniques. 2) FG-treated animals did not require packing for their complex hepatic injuries in our model.

At the turn of the century, surgeons first experimented with hemostatic materials to arrest hemorrhage from vital organs. In 1911, Cushing used fragments of raw muscle and solidified blood clots for hemostasis without adverse effects [10]. At Cushing's suggestion, Grey performed experiments with fibrin extracted from sheep's blood to control bleeding from skin and cerebral lacerations in cats and dogs [11]. Harvey converted bovine fibrin into a paper-like hemostatic material, which he used in wound repair and intestinal anastomoses [12]. FG was first used by plastic surgeons in nerve anastomoses

more than 50 years ago [13]. In 1944, Cronkite reported the use of thrombin and fibrinogen together as skin grafting agents in a manner similar to the present day [14]. FG was "re-discovered" in the 1970s with the development of microsurgical techniques. During the past two decades, FG has been used increasingly as a sealant or hemostatic agent in many areas of surgery [8].

The mechanism of action of FG involves the stimulation of natural clotting factors (Figure 1). FG is formed by multiple components such as fibrinogen, factor XIIIa, thrombin, calcium chloride and aprotinin. Fibrinogen is converted to fibrin by thrombin; and fibrin molecules polymerize in the presence of calcium and factor XIII to form a stable fibrin clot. FG is eventually metabolized without residue [6].

Experimental work has supported the notion that FG is beneficial in arresting hemorrhage from visceral injury. Jakob and his group were among the first to show fibrin sealant to be beneficial in arresting hemorrhage during hepatic injury [5]. In anticoagulated rats with liver damage, they demonstrated an improvement in survival when conventional suture repair techniques were compared with fibrin impregnated collagen. The fibrin sealant was completely absorbed without foreign body reaction or fibrosis at 28 days [5]. Kram and colleagues evaluated the efficacy of FG in superficial and deep liver lacerations [6]. Twelve adult dogs were anesthetized and underwent laparotomy followed by formation of a significant hepatic injury or



Figure 1. Clotting Mechanism.

lobar resection. After noting brisk bleeding, FG was applied. Effective hemostasis and good local and systemic compatibility were observed. Histologic examination of the liver performed at 12 and 24 hours and at two, three, six, and eight weeks revealed no gross evidence of hepatic disruption, hematoma formation or recurrent bleeding. By four to six weeks, the FG was absorbed and the underlying hepatic parenchyma appeared well regenerated. Dulchavsky et al. found fibrin sealant to be protective in a murine model of contaminated hepatic injury [4]. Anesthetized rats received a liver laceration via laparotomy, and the peritoneum was inoculated with *Bacillus fragilis*. Animals treated with FG had decreased abscess formation and fewer adhesions when compared to routine hepatorrhaphy. In earlier work, we showed that another FG product (Baxter Pharmaceuticals, Hyland Division, Los Angeles, California), statistically significantly reduced the operative blood loss associated with a severe liver injury when a group of animals receiving FG were compared to those receiving standard surgical methods only [7]. Pigs receiving standard methods required packing in six of seven animals, while none of FG treated animals (seven of seven) required packing. Estimated blood loss was  $875 \pm 265$  cc in the control group and decreased significantly to  $300 \pm 59$  cc in the FG treated animals.

In recent studies, Holcomb et al. has shown that a fibrin-based foam could reduce blood loss compared to a placebo foam in a murine model where the median hepatic lobe was excised [15]. This group also studied a porcine model of Grade V liver injury using a clamping technique to induce the lesion. The initial study showed a 51 percent decrease in the amount of blood loss with a dry fibrin sealant dressing (DFSD) over standard packing [16]. These results, however, did not reach statistical significance. A follow-up study

using a similar model showed a one-hour survival of 83 percent in the DFSD group and 0 percent in routine packing and placebo groups [17].

FG may have particular clinical utility in the setting of abdominal trauma [8], but there is limited data on FG utility in liver injury. Kram et al. reported successful repair with FG after hepatic injury in eight coagulopathic patients [18]. Ochsner and coworkers had similar success with hepatic and splenic hemostasis after trauma in eight coagulopathic patients [19]. Recent case reports have attributed hypotensive episodes during intraparenchymal injection in deep liver injuries to FG [20, 21]. These adverse reactions were thought to be secondary to intravascular injection of FG prepared with bovine thrombin, however, these secondary effects were not reported with this new human thrombin product. The studies above represent the sum of animal and clinical research regarding the use of FG in the setting of hepatic hemorrhage.

Our investigation examined the impact of FG upon hemostasis in a model of uncontrolled hemorrhage from liver injury. This model of liver contusion grossly resembles the clinical entity and is similar to one used in our previous study [7]. The use of a porcine model is advantageous because of the similar hepatic anatomy and large animal size that allows use of instruments and techniques from clinical practice. This study differs from the previous one in several ways. It utilizes a newer, FDA approved viral-free FG preparation increasing the clinical relevance of the experiment. In our previous study, control pigs were injured and received only conventional treatment. This resulted in a high mortality during the course of the experiment for the control group. In the current study, animals acted as their own controls.

There are several limitations to the study. Firstly, although our goal was to

simulate a patient's course following a blunt hepatic injury, anesthetizing the animals was necessary to allow for completion of all of the procedures outlined in our protocol. The dose of pentobarbital used in the study was determined from previous experiments [7, 9] and reflects the minimal amount needed to keep the animals sedated throughout the experiment. This may have contributed to the drop in MAP after injury and high resuscitation volumes, but the animals were allowed to equilibrate under anesthesia without any manipulation for 15 minutes before injury. Second, in order to replicate the coagulopathy that individuals experience as a result of remaining in the field for extended periods of time, all of the pigs received a large dose of intravenous heparin. Heparin was given to increase blood loss as well as make the animals less likely to respond to either packing or FG. The supra-therapeutic dose of heparin induces a severe coagulopathy in excess of that necessary for clinical anticoagulation. Although hypothermia was not severe enough to induce a coagulation dysfunction, there are significant numbers of patients on anticoagulant medication or having an underlying high degree of liver dysfunction that place them in an anticoagulation state. Third, despite our attempts to create a hepatic injury of a specific size and depth, the differences in respective anatomies of the pigs prevented perfectly reproducible contusions between animals. One pig sustained a portal vein injury, which was unrecognized and bled massively following laparotomy. Also, the nature of the injuries was such that they required emergent repair during the laparotomy and precluded any precise measurement of the size of the contused area.

Fourth, comparison of blood loss and resuscitation requirements after injury was limited by the techniques used and variability among animals. The use of lap pads

to measure blood loss, while not precise, allows comparison of initial hemorrhage volumes among the animals. The amount of blood at re-laparotomy in all but one animal was negligible. Our fixed volume resuscitation of 20 cc/kg may have been excessive but reflects an amount routinely used in trauma patients. The decision was made to maintain MAP greater than 80 mmHg in an attempt to replicate the typical clinical scenario.

In a heparinized porcine model of complex hepatic injury, FG controlled hemorrhage after failure of conventional surgical techniques. FG quickly established a hemostatic seal over the bleeding areas. Most importantly, the use of FG permitted safe completion of the abdominal operation without the need for packing of parenchymal injuries. Further work in the clinical arena is warranted to determine the potential benefits of FG in arresting hemorrhage in the coagulopathic trauma patient with complex hepatic injuries.

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