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Hemorrhage control is critical to survival of trauma patients. Unfortunately the methods available to stop bleeding have not changed in years (pressure, tourniquets, gauze dressings, and sutures). This suggests that a substantial increase in survival can be effected by a simple and effective method of hemorrhage control. Dry Fibrin Sealant Dressings (DFSD) combine the benefits of liquid fibrin sealants with convenience of a ready to use dry powder dressing composed of pure human thrombin, fibrinogen, and calcium. The delivery of the DFSD is the same as any conventional gauze dressing, simply unwrap the dressing, place on the wound, and apply pressure for one to two minutes. A "fibrin clot" forms almost instantly that stops bleeding from small, medium and large vessels as well as large soft tissue wounds.

We developed a liver injury model that replicates a gunshot wound to the central portion of the liver. In this potentially fatal liver injury model the DFSD instantly stopped bleeding when compared to a blinded control dressing ($p < .05$) and gauze packing ($p < .05$).

The Dry Fibrin Sealant Dressing is simple, fast, lightweight, temperature independent and requires no special training. When fielded in its final form, we see this as a forward projection of advanced hemorrhage control, providing our casualties rapid, lifesaving and definitive control of bleeding.

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FOREWORD

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Dry fibrin sealant dressings (DFSD) have been used successfully with significantly decreased hemorrhage and preserved blood pressure in a ballistic extremity injury model.[1] As this dressing has never been applied to a blunt liver injury, the protocol entitled Dry Fibrin Glue for the Repair of Severe Liver Injury in an Anesthetized Porcine Model was submitted. Before the DFSD could be applied to this type of injury, a realistic and reproducible model needed to be developed. This solid organ injury model allows us to study not only fluid resuscitation but most importantly hemorrhage control. All liver injury models currently in the literature suffer from some or all of the following limitations:

1. Nonrealistic injuries, i.e. sharp excision of portion of lobes, possibly appropriate for study fluid resuscitation but not for effectiveness of hemorrhage control.
2. Nonreproducibility of the injury.
3. Heparin is required to induce and maintain bleeding.
4. Requirement of a controlled pre-bleed for standardization of hemodynamic insult.

Over the past 15 months the grant received from MRMC has allowed our lab to develop and mature a blunt liver injury trauma model that reproducibly causes a grade V liver injury (injury to one or more of the retrohepatic veins).[2] This type of injury has a 60-90% mortality in humans.[3] Dr. Harris has modified the AAST organ injury system to accurately delineate the injury we are creating.

With the recent upgrade of our lab and the American Red Cross's development of the dry fibrin sealant dressing, we have completed a number of studies in this field. The American Red Cross encountered minor difficulties in the development of the dry fibrin sealant dressing, which have since been resolved. While waiting on the development of the dry fibrin sealant dressing, we accomplished several other important milestones in this project.

1. Development of the liver injury model with complete characterization of the injury, and confirmation of reproducibility. Critical to the injury and the success of the model is the specific anatomy of the pig liver and hepatic veins, which is very similar to human anatomy. The injury that we create is not only a parenchymal injury (4cm deep) but typically 1-3 holes in the major hepatic veins, which on dissection are up to 13 mm in diameter and within 2-4 cm of the inferior vena cava. Development and characterization of the injury has proceeded throughout the other studies that I will outline below. At this point we can reliably reproduce a grade V_2 through V_6 injury, which allows us to injure the liver, treat the animal prospectively and then grade the injury retrospectively. This retrospective grading of a prospectively treated injury allows us to correlate the specific injury with the resulting hemodynamic changes.

2. Placement of gauze packs above and below the liver is the standard method for control of major liver injuries in humans.[3] There is no data on this type of treatment in pig liver injuries, therefore this had to be developed, and is now accomplished without difficulty. This is the standard of care portion of the

protocol.

3. Important not only to this project using dry fibrin sealant dressing, but to all fibrin sealant hemorrhage control type studies, is the article by Dr. Moore's group out of Colorado, delineating the fatal effects of fibrin glue in deep liver injuries.[4] Bovine thrombin, which is in all fibrin glues currently used in the United States was postulated to cause hypotension and death. This study had two human subjects and a single animal. We studied the hemodynamic aspects of bovine thrombin (BT) versus human thrombin (HT) injected intravenously (8 swine). It is apparent from Appendices 2 and 3 that BT is fatal and HT is not (dose = 5000 IU). Analysis of BT and HT by HPLC reveals that bovine is very impure in comparison to human thrombin. Autopsy data showed that BT swine had extensive intravascular clotting, while HT animals exhibited only normal post mortum changes. We feel these differences may not be due to thrombin but to protein impurities in the BT preparation. This is critical to the acceptance of dry fibrin sealant dressing to the trauma community because of the warnings published by Dr. Moore's group in 1991. This information has been presented at a recent Fibrin Sealant Meeting in San Diego, CA. (May 1997) and is currently being collated by Dr. Pusateri for publication.

4. In addition, we studied in 15 animals the effects of standard fluid resuscitation versus no fluid resuscitation in a grade 4 or 5 liver injury. Results show a significant decrease for the non-resuscitated animals, (2997±660ml vs 468±706ml, p=.02) in blood loss over 1 hour. This data was collected while we

were standardizing our liver injury, and will support a separate paper on our liver injury model, currently being written by Dr. Harris. This data also confirms the utility of this model as a realistic type injury (90% fatal in humans) and will allow us to study the type, timing, and amount of resuscitation that maximizes survival.

The American Red Cross, as mentioned previously, has resolved their production problems, allowing us to progress rapidly with testing and evaluation of the DFSD. We have initiated and completed 3 studies and have preliminary data for a seventh. These additional studies all serve to answer crucial questions concerning the utility of DFSD.

The following portion of this report will outline our completed studies and conclusions :

1. The completed liver efficacy study:

Recent reports relate > 50% mortality in Grade V liver injuries from uncontrolled hemorrhage. Current methods for hemorrhage control consist of resection, suture, mesh wrapping, and gauze packing. The latter is fastest, and is used most commonly for these potentially mortal injuries. Packing, however, requires repeat operation to remove the gauze sponge. Definitive hemorrhage control with absorbable dressings could lead to significant advances in the treatment of severe liver injuries. A randomized, blinded study was conducted to evaluate the utility of DFSD for hemorrhage control in severe liver injuries.

A new severe liver swine trauma model, with uniform lacerations of major

lobar veins (with a specially designed clamp) was developed to study the hemostatic properties of the DFSD. Parenchymal wounds measured 4x8x12cm. The fibrin dressing consisted of an absorbable backing with lyophilized fibrinogen (15 mg/cm^2) and thrombin (9 IU/cm^2). Twenty-four swine (40±3kg) were injured and treated in a prospective fashion in one of four groups; (1) DFSD, (2) gauze packing, (3) IgG dressing (inert protein, blinded control), (4) no treatment. All animals were resuscitated with lactated ringers to their pre-injury mean arterial pressure (MAP). Total blood loss (TBL), MAP, temperature, urine output, Hct, platelets, fibrinogen, and PT/PTT were monitored for one hour after injury. Fluid requirement, injury distribution and mortality were recorded. Injuries were reproducible (100% grade V) and uniform among the four groups. Mortality was 0% in the treatment groups (1 and 2) and 58% in the control groups (3 and 4) ($p<.05$). The TBL in group 1 was 544±255ml (mean±sd), group 2 was 1104±645ml, group 3 was 4222±3808ml, and group 4 was 6025±2498ml. TBL in group 1 was less than groups 2, 3, or 4 ($p<.05$). Hct, platelets, and fibrinogen levels fell, while PT/PTT and fluid requirements increased with rising TBL.

In this model DFSD provided hemorrhage control and decreased TBL in Grade V liver injuries compared to gauze packing and a blinded control. Further studies are required to delineate efficacy in cold, coagulopathic and survival models. Dry fibrin sealant dressings may provide simple, rapid and definitive control of life threatening liver injuries without need for reoperation.

A completed study of the effects of varying fibrinogen concentration on hemostasis in the liver model:

Dry Fibrin Sealant Dressings (DFSD) are composed of purified human thrombin, purified human fibrinogen, and calcium which are freeze dried on to an absorbable mesh backing. The strength of the clot formed by the DFSD is dependent on the concentration of fibrinogen. In a previous study, a DFSD containing 15 mg/cm^2 of fibrinogen effectively stopped hemorrhage after Grade V liver injury in swine. This study was conducted to determine the effect of fibrinogen dose on blood loss. Twenty-four pigs were randomly and equally assigned to receive one of four treatments: 1) DFSD containing 15 mg fibrinogen/cm^2; 2) DFSD containing 8 mg fibrinogen/cm^2; 3) DFSD containing 4 mg fibrinogen/cm^2; or 4) DFSD containing 15 mg human IgG/cm^2 and 0 mg fibrinogen/ cm^2, as a control. Each DFSD measured 10.2 x 10.2 cm. Investigators were blinded to treatment. Under general anesthesia, each pig underwent laparotomy and splenectomy. Following a 15 min stabilization period, a Grade V liver injury was induced using a specially designed instrument. After 30 sec, three to four of the appropriate DFSD were applied to the injury. Resuscitation with lactated Ringer's was simultaneously initiated and animals were resuscitated to pre-injury MAP. The abdominal incision was then closed and the pig monitored for 60 minutes. Animals were euthanized at 60 minutes. Blood samples were collected at pre-injury and at 30 and 60 minutes post-injury. CBC, fibrinogen, PT, and PTT were determined at each time point. At 60

minutes, total fluid use and the volume of blood present in the abdominal cavity (total blood loss) were determined. Additionally, the liver was excised and the injury was scored to confirm a grade V injury. In 3 pigs in each group, the clot formed by the DFSD was subjectively evaluated and classified as follows: 1) Not adhered to the injury; 2) Minimally adhered; 3) Adhered; and 4) Well adhered. Survival to 60 min was also determined. One animal was dropped from the study because the injury was Grade IV. Injuries were similar across treatment groups. Blood loss was significantly affected by treatment group. Blood loss in the control group was 2819 $\pm$ 629 ml (mean $\pm$ SEM), which was significantly greater than any other group ($p < .05$). Blood losses in the remaining groups did not differ significantly and were 588 $\pm$ 629 ml, 632 $\pm$ 703, and 758 $\pm$ 629 ml in the 15, 8, and 4 mg fibrinogen/cm^2 groups, respectively. Total fluid use was 2757 $\pm$ 565 ml, 1448 $\pm$ 505 ml, 1775 $\pm$ 505 ml, and 2208 $\pm$ 565 ml for the control, 15 mg, 8 mg, and 4 mg fibrinogen/cm^2 groups, respectively. Fibrinogen and all CBC components decreased as blood loss increased. PT and PTT increased. Of the pigs in which the clot formed by the DFSD was examined, 3/3 control DFSD were not adhered. In the 15 mg group, 3/3 DFSD were well adhered. In the 8 mg group, 3/3 were adhered and in the 4 mg group, 3/3 were slightly adhered. One pig died prior to 60 minutes in each of the control, 4 mg, and 8 mg groups, while all animals survived to 60 minutes in the 15 mg group. This study confirms the previous finding that DFSD decreases blood loss following Grade V liver injury in swine. However, fibrinogen dose

above 0 did not affect blood loss in this study. The data suggest that fibrinogen levels from 4 mg to 15 mg/cm^2 are effective for hemorrhage control. Based on assessments of the clot quality, it appears that dose is related to quality, with the higher dose associated with more firm and tightly adhered clots. While lower doses are effective for hemorrhage control during the initial 60 min, longer term survival will likely depend on clot quality. For future survival studies, we feel that the 15 mg DFSD should be used.

A completed study of the effect intraabdominal placement of DFSD has on adhesion formation in the rat. This question is important because we envision the DFSD being left in all body cavities as a hemostatic adjunct.

Thirty-six rats were used in an objective model evaluating the strength and extent of adhesions formed following laparotomy in control rats and rats treated with the dry fibrin sealant dressing (DFSD) . Animals were assigned randomly and equally to receive either the control or DFSD treatments and to be sacrificed at either seven or 42 days, for a total of four treatment-time combinations. All animals underwent laparotomy on day 0. In each animal, a standard cecal/peritoneal wall defect and four peritoneal wall ischemic buttons were produced. Next, a DFSD was placed in apposition to the induced lesions in each rat assigned to the DFSD group. In the control group rats, no treatment was given. Incisions were then closed and the rats were allowed to recover. Animals were then euthanized at either seven or 42 days post-treatment and tissues were collected. A 1 kg mechanical force gauge was used to measure the

force in grams required to separate the cecal/peritoneal wall defect adhesions. This represented an objective measure of the type (strength) of adhesions formed. The number of adhered ischemic buttons was determined visually in each rat. This represented an objective measurement of the extent of adhesions. Each rat was classified as positive for cecal/peritoneal wall adhesions if the force required to separate the adhesion was greater than 0 g. Each rat was classified as positive for ischemic button adhesions if at least 1 ischemic button adhesion was observed.

No effect of treatment on the force required to separate cecal/peritoneal wall adhesions was observed. Additionally, no treatment by time interaction was observed. Force required to separate cecal/peritoneal wall adhesions in DFSD rats after 7 days was 448 $\pm$ 74 g (mean $\pm$ SEM), while the required force after 42 days was 818 $\pm$ 74 g. Mean forces observed in control rats were 344 $\pm$ 74 g and 691 $\pm$ 74 g after 7 and 42 days, respectively. Time post-treatment affected adhesion strength ($p < .01$), regardless of treatment. Values at 42 days were significantly greater than those observed at days 7 ($p < .01$). Treatment did not affect the number of ischemic button adhesions observed and no treatment by time interaction was observed. Additionally, no effect of time was observed. The number of ischemic button adhesions observed in DFSD rats after 7 days was 3.3 $\pm$ 0.6, while the number observed after 42 days was 2.4 $\pm$ 0.6. The number of buttons in control rats were 2.2 $\pm$ 0.6 and 1.7 $\pm$ 0.6 after 7 and 42 days, respectively. Proportions of rats exhibiting cecal/peritoneal wall adhesions

or ischemic button adhesions was not affected by treatment at either 7 or 42 days. After 7 days 9/9 and 8/9 rats in the DFSD and control groups displayed cecal/peritoneal wall adhesions. All 9 rats observed in each group displayed cecal/peritoneal wall adhesions at 42 days. Ischemic button adhesions were observed in 9/9 and 6/9 DFSD and control rats at 7 days. After 42 days, 6/9 and 5/9 rats exhibited these adhesions in the DFSD and control groups respectively. These data show that both neither strength nor extent of post-surgical adhesions was affected by DFSD, as compared to controls. Although increased post-surgical time increased the strength of adhesions, this increase was similar in the DFSD and control groups. Based on these data, it does not appear that the DFSD either increases or decreases the formation of adhesions following abdominal surgery.

We have initiated a pilot survival study (n=4) utilizing the liver model as described. Initial results are promising with animals returning to normal activity in 12-24 hours. This study will not be complete prior to this report deadline.

In summary we have completed initial efficacy and survival testing of the DFSD in a near fatal liver injury model. Additionally we have looked at and answered initial questions concerning the safety of using human vs. bovine thrombin to control parenchymal bleeding. These efforts have been successful and we plan to expand on the survival studies and efficacy in cold coagulopathic animals.

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