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# JOURNAL *of* SPECIAL OPERATIONS MEDICINE™

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# Salmon Thrombin-Fibrinogen Dressing Allows Greater Survival and Preserves Distal Blood Flow Compared to Standard Kaolin Gauze in Coagulopathic Swine with a Standardized Lethal Femoral Artery Injury

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## ABSTRACT

We have previously shown that lyophilized salmon thrombin and fibrinogen (STF) embedded in a dissolvable dextran dressing is as efficacious as Combat Gauze™ (CG) with regard to controlling hemorrhage and survival in non-coagulopathic swine with femoral artery lacerations. A major limitation of currently available advanced field dressings is the inability to control hemorrhage in coagulopathic casualties because of the exhaustion of host coagulation proteins. We tested the hypothesis that the STF dressing would be better able to control hemorrhage and prolong survival in coagulopathic swine compared to CG. Survival rate was 50% in CG-treated animals versus 90% in STF-treated animals. Survival time was significantly greater in STF-treated animals. Clots formed over the arterial injury in 100% of STF-treated animals compared to 0% in CG-treated animals ($p < 0.001$). STF-treated animals consumed less host coagulation factors, including platelets ($p = 0.03$). Survival after limb manipulation that simulated casualty evacuation was significantly higher with the STF dressing ($p < 0.005$). Angiographic observation of distal blood flow was seen twice as often with the STF dressing as with CG. The STF dressing allows a high survival rate, significantly greater survival time, and a significantly more stable dressing than CG in coagulopathic swine. The clot formed by the STF dressing also enables restoration of distal blood flow to the limb potentially resulting in higher limb salvage.

## Introduction

Exsanguination, whether on the battlefield[1] or at Echelon II and III facilities,[2] remains the leading cause of death among combat casualties with potentially survivable injuries. Hemorrhagic shock also carries a high mortality in civilian trauma centers.[3] The search continues for more efficacious field dressings that will improve survival.

Other than the standard Army field dressing, only mineral- and shellfish-based dressings have been cleared by the U. S. Food and Drug Administration (FDA) for use in the tactical environment. These dressings act by both compression of the injured vessel and initiation of the intrinsic coagulation pathway. Many researchers feel that plasma-derived coagulation factors such as fibrinogen will comprise the next generation of advanced field dressings because they will not rely on an intact host coagulation system (often compromised in combat casualties) and they can form a seal over the injury site.

Dissolvable dextran dressings embedded with lyophilized salmon thrombin and fibrinogen (STF) are as effective as Combat Gauze™ in controlling hemorrhage and survival in non-coagulopathic swine with lethal arterial injuries.[4] In addition, the STF dressing enables distal blood flow in the injured vessel by sealing, but not occluding flow, at the arteriotomy site.

Given that STF can form a fibrin clot in the absence of host coagulation proteins, we undertook this experiment to test the hypothesis that the STF dressing can seal a lethal arterial injury to control hemorrhage and allow survival under the conditions of coagulopathy and hypothermia. We used CG as our control dressing. The study protocol was modified from the United States Army Institute for Surgical Research (USAISR).[5]

## Materials and Methods

### *Materials*

Thirty-one adult, female, domestic, breed indifferent, purpose-bred swine were obtained from Genetiporc, Hancock, MN. This study was performed at The Integra Group, Brooklyn Park, MN. The protocol was approved by The Integra Group Institutional Animal Care and Use Committee, and was in compliance with *The Guide for the Care and Use of Laboratory Animals* (National Research Council, 1996). The animals were maintained and observed for a minimum of three days to allow acclimatization and observation by facility veterinarians. The animals had *ad libitum* access to food and water. They were denied access to food for 12–18 hours prior to surgery.

The control dressings, Combat Gauze™ (CG), were obtained directly from the manufacturer (Z-Medica Corp., Wallingford, CT). Combat Gauze™ was chosen because it is the only advanced dressing recommended by the Committee for Tactical Combat Casualty Care (CoTCCC)[6], and it is the standard against which other dressings must be compared in order to be considered by the CoTCCC. It is a z-folded 3 x 144 in gauze dressing coated with kaolin. The salmon thrombin-fibrinogen dressings (STF) were obtained directly from St. Teresa Medical, Inc. (7448 W 78th Street, Bloomington, MN). The prototype dressings consisted of a mixture of thrombin and fibrinogen isolated from plasma of Atlantic salmon (*Salmo salar*) and embedded into a matrix of electrospun dextran. The dextran dissolves immediately upon contact with water (blood), allowing the proteins to hydrate and for the thrombin to hydrolyze fibrinogen into fibrin. The active dressing was adhered to a backing of non-woven cotton gauze (3 x 144 in) to allow application similar to CG.

Four animals were used in a pilot study to determine hemodynamic and laboratory inclusion criteria and to refine the surgical technique in this challenging hemorrhage model.

*Surgical Preparation*

Anesthesia was induced using intramuscular Telazol® (4.4 mg/kg) based on body weight measurements taken that morning. After intubation anesthesia was maintained using isoflurane inhalant anesthetic (0.5 to 5%). The left carotid artery and internal jugular vein were exposed surgically. The artery was cannulated for continuous measurement of mean arterial pressure (MAP), as well as for withdrawal of blood for laboratory analysis and blood gas measurements. The internal jugular vein also was cannulated for administration of resuscitation fluids. Adjunctive atropine (0.05mg.kg IV) was administered if indicated by observed cardiac rate changes in order to maintain the heart rate above 80 beats per minute (bpm). A rectal thermometer was inserted.

Baseline blood samples drawn for complete blood count (CBC), pro-time (PT), activated partial thromboplastin time (aPTT), international normalized ratio (INR), fibrinogen, chemistries, and arterial blood gas (ABG) to include pH and base excess (BE). Inclusion criteria to proceed with the study included a MAP ≥ 55mm/Hg, hemoglobin ≥ 8g/dL, platelet count ≥ 200,000/mm$^3$, PT ≤ 18 seconds, INR ≤ 2.0, and fibrinogen ≥ 100 mg/dL. All animals met these inclusion criteria, but seven died following induction of coagulopathy before we could produce the arterial injury. Fatal arrhythmias occurred in 25% of coagulopathic animals. Therefore we initiated a prophylactic infusion of 1% Lidocaine at 25 µg/Kg/min prior to coagulation induction and maintained the infusion throughout the duration treatment. No further animals developed fatal arrhythymias.

The right carotid artery was exposed surgically and cannulated for blood-fluid exchange, induction of dilutional coagulopathy, and for terminal angiography. The carotid artery was chosen over the femoral artery to allow for angiographic comparison of the injured femoral artery to the contralateral, uninjured artery.

A urinary catheter was placed through a midline celiotomy for continuous measurement of urine output. This also allowed for some visceral manipulation, but we did not perform a splenectomy. The fascia and skin were closed with continuous 2–0 polyglycolic acid sutures.

Coagulopathy was induced as described previously.[7] Briefly, exchange blood loss comprising 60% of the calculated total blood volume (estimated at 7% of body weight) was withdrawn from the right carotid artery at a rate of 50ml/min using a peristaltic pump. Simultaneously, an equal volume of HextendTM (6% HES in balanced electrolyte solution + glucose) was infused through the jugular vein.

Concurrently, the left inguinal region was exposed surgically and the sartorius muscle was identified in the proximal thigh. It was dissected free of the underlying femoral vessels and removed. A 5cm section of the femoral artery was isolated and the adventitia was dissected away. When present, small muscular branches of the artery were clamped and ligated. The vessel was covered with a small piece of gauze saturated with 2% lidocaine to dilate the vessel and hydrate the tissues.

A cooling blanket and ice bags were used to reduce the core temperature to a target of 33 + 0.5 °C. Pre-injury measurements of hemodynamic parameters were taken and blood samples were collected.

A stable MAP of at least 55mmHg was necessary before proceeding with the vascular injury and the remainder of the experiment. This level was chosen, rather than 60mmHg, because these young, female animals had a low baseline MAP and 60mmHg would have excluded more than half of our animals. Maintenance fluid was discontinued when this MAP was achieved.

*Vascular Injury and Treatment*[8]

With the MAP above 55mmHg and the core temperature at the target level for hypothermia, two vascular clamps were applied to the femoral artery 2cm apart. The vessel was incised longitudinally with a #11 scalpel through which a 6mm arterial punch was introduced. The injury was created, the clamps were released, and the vessel bled unrestricted for 30 seconds. This blood was collected and weighed as pre-treatment blood loss. During this initial hemorrhage one package of a randomly assigned bandage was selected by the study director and opened for the investigators. The creation of the arteriotomy and the placement

of the bandage also were randomized between the two surgeons (CTF and SWR) in order to minimize bias.

For application of the dressing, we followed the protocol developed by Kheirabadi and colleagues at the USAISR with one modification. Current protocol calls for one dressing application;[9] however, the publication upon which this study was based and to which we sought to compare salmon to human fibrin dressings allowed two applications of dressing.[5] Therefore, in order to allow for consistency with earlier published work, our consensus was to allow either a second dressing application or a second period of compression. (Kheirabadi, personal communication, 21 October 2011).

We packed the CG into the wound over approximately one minute in accordance with the manufacturer's recommendations, covered with a laparotomy sponge and held compression. We applied the STF dressing into the wound, covered the wound with a laparotomy sponge, and held compression. Total treatment time was three minutes for both bandages.

Thirty seconds after application of either dressing, we began fluid resuscitation by infusing 6% Hextend™ 500ml at a rate of 100ml/min. Three minutes after application of either dressing we slowly released the compression and observed the wounds for three minutes. If we observed hemorrhage during this period, we either removed the dressing and applied a second dressing with compression, or simply compressed the original dressing for an additional three minutes. At the conclusion of either one treatment, or when necessary, a second treatment, we gently pulled the skin edges over the sponge and observed the wound for rebleeding.

### *Post-Injury Observation*

We observed the wounds for 2.5 hours following treatment. We collected and weighed the blood lost after injury, but prior to application of the dressing (pre-treatment blood loss), as well as the blood lost after application of the dressing (post-treatment blood loss). Stable hemostasis was defined simply as the point at which the wound stopped bleeding through or around the dressing. The time to hemostasis was recorded as the time period from injury to the time when bleeding stopped. We strived to maintain a minimum MAP of 55mmHg by infusion of LR through the jugular vein.

Animals that became hypotensive and failed to maintain a minimum MAP of 20mmHg for more than two minutes were considered terminal. The survival time was noted, final blood samples were drawn from the left carotid artery, and the dressing was removed gently from the wound to inspect the arterial injury for clot formation and status of bleeding. The animals then were euthanized with an intravenous barbiturate.

We performed angiography through the cannula in the right carotid in all animals that survived the entire 2.5 hour observation period. We paid particular attention to the injury site, whether or not dye (blood) extravasated from the site, and the presence or absence of blood flow distal to the injury. With respect to distal blood flow, we ascertained whether the blood flowed antegrade, or filled retrograde via collateral circulation.

Following angiography, we fully flexed and extended the entire left hind limb five times to simulate walking and assess the stability of the dressing. Animals that exsanguinated with this test, despite having survived 2.5 hrs, still were considered survivors consistent with USAISR protocol. However, because we considered this to be an important indicator of dressing stability, we also calculated a survival rate that included this test.

Next, we carefully removed the dressing from the wound and observed the injury site. We assessed the presence or absence of clot. In the presence of clot, we ascertained whether or not it was adherent to the injury site, whether it partially or completely sealed the injury, and the quality of the clot. This inspection was performed using 2.5x surgical loupe magnification. In injuries that apparently were sealed we assessed the distal artery for presence of pulsations.

Finally, we euthanized the animals with a lethal intravenous dose of barbiturate. Necropsy was performed on all non-excluded animals for gross and histologic examination, with particular reference to the arterial injury and tissues surrounding it.

## Results

### *Physiologic and Hematologic Parameters*

At baseline we found no difference between the two groups with respect to body weight (34.7 ± 0.4 kg), temperature, MAP, hemoglobin, platelet count, PT, aPTT, INR, fibrinogen, pH, lactate, or base excess (BE). Following exchange blood loss, we saw statistically significant deterioration in most parameters associated with coagulopathy, shock, hypothermia, and metabolic acidosis (temperature, MAP, hemoglobin and platelet concentrations, PT, INR, fibrinogen and lactate). The pre-injury pH and base excess were consistent with a combined metabolic acidosis and compensatory alkalosis in order to maintain homeostasis. These values are summarized in Table 1.

Table 2 shows these same physiologic and hematologic parameters in the two groups just prior to euthanasia in animals that survived the entire 2.5 hours of the study. Comparison to baseline values in Table 1, this table shows that these animals were more coagulopathic, hypothermic

**Table 1** *Physiologic and Hematologic Measurements at Baseline and Pre-Injury*

| Measure | Baseline Combined | Pre-Injury Combined | *p* value* | CG Pre-Injury | WC Pre-Injury | *p* value** |
|---|---|---|---|---|---|---|
| Temperature (°C) | 36.2 ± 0.2 | 33.9 ± 0.1 | < 0.001 | 33.7 ± 0.2 | 34.0 ± 0.1 | 0.14 |
| MAP (mm Hg) | 85.0 ± 5.3 | 63.0 ± 2.9 | < 0.001 | 60.5 ± 2.3 | 65.5 ± 5.4 | 0.41 |
| Hemoglobin (g/dL) | 10.0 ± 0.3 | 5.0 ± 0.5 | < 0.001 | 4.7 ± 0.6 | 5.3 ± 0.8 | 0.57 |
| Platelets (1,000/µL) | 329 ± 28 | 196 ± 25 | < 0.001 | 161 ± 39 | 231 ± 29 | 0.17 |
| PT (sec) | 14.3 ± 0.3 | 17.0 ± 0.6 | < 0.001 | 16.8 ± 0.8 | 17.3 ± 0.9 | 0.68 |
| aPTT (sec) | 15.7 ± 0.5 | 17.2 ± 0.5 | < 0.001 | 16.4 ± 0.7 | 18.1 ± 0.7 | 0.12 |
| INR | 1.5 ± 0.0 | 1.9 ± 0.01 | < 0.001 | 1.8 ± 0.1 | 1.9 ± 0.1 | 0.7 |
| Fibrinogen (mg/dL) | 154.5 ± 12.4 | 79.1 ± 8.7 | < 0.001 | 62.3 ± 8.7 | 95.9 ± 13.3 | 0.05 |
| pH | 7.53 ± 0.02 | 7.54 ± 0.02 | 0.47 | 7.6 ± 0.0 | 7.5 ± 0.0 | 0.4 |
| Lactate (mM) | 421 ± 17 | 235 ± 22 | < 0.001 | 226 ± 29 | 245 ± 35 | 0.69 |
| Base Excess (mM) | 10.0 ± 1.0 | 8.4 ± 1.1 | 0.18 | 8.7 ± 0.8 | 8.0 ± 2.2 | 0.77 |

*Notes:* **p*-values for within-group changes are calculated by paired t-tests
***p*-values for between group differences are calculated by ANOVA
PT and aPTT were determined from whole blood samples; fibrinogen was determined from plasma. N was equal to 10 in all measurements except lactate where n = 8 for CG and n = 7 for STF.

**Table 2** *Terminal Physiologic and Hematologic Measurements*

| | | CG | | STF | |
|---|---|---|---|---|---|
| Measure | N | Mean ± SD | N | Mean ± SD | *p* Value |
| Body weight (kg) | 10 | 34.4 ± 0.6 | 10 | 35.0 ± 0.4 | 0.42 |
| Temperature (°C) | 10 | 33.4 ± 0.3 | 10 | 33.9 ± 0.2 | 0.30 |
| MAP (mm Hg) | 10 | 40.0 ± 3.8 | 10 | 54.0 ± 6.1 | 0.07 |
| Hemoglobin (g/dL) | 9 | 2.5 ± 0.4 | 10 | 3.1 ± 0.2 | 0.29 |
| Platelets (1,000/µL) | 9 | 106 ± 37 | 10 | 200 ± 20 | 0.03 |
| PT (sec) | 7 | 30.0 ± 5.7 | 10 | 22.0 ± 1.7 | 0.14 |
| aPTT (sec) | 9 | 25.6 ± 2.6 | 10 | 21.6 ± 1.1 | 0.16 |
| INR | 7 | 4.0 ± 1.0 | 10 | 2.6 ± 0.3 | 0.13 |
| Fibrinogen (mg/dL) | 5 | 44.4 ± 4.4 | 7 | 51.1 ± 6.5 | 0.45 |
| pH | 9 | 7.6 ± 0.1 | 10 | 7.5 ± 0.0 | 0.79 |
| Lactate (mM) | 8 | 121 ± 16 | 7 | 147 ± 6 | 0.18 |
| Base Excess (mM) | 9 | 4.2 ± 1.9 | 10 | 6.8 ± 1.8 | 0.33 |

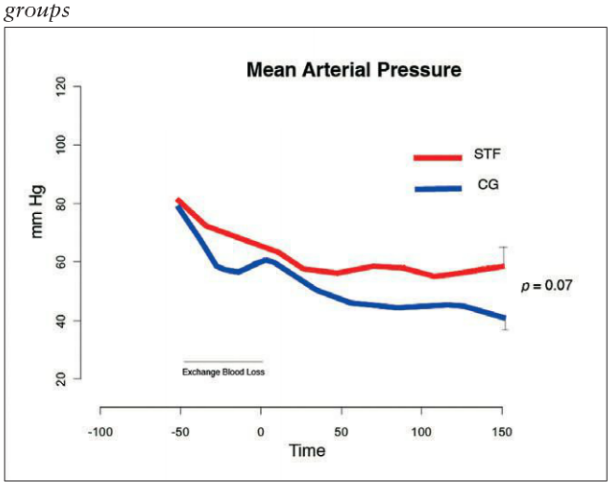
*Note:* Data were analyzed using ANOVA. PT and aPTT were determined from whole blood samples; fibrinogen was determined from plasma. In several cases the values were too low to be measured reliably, so the data were omitted. Blood samples were drawn at time of death regardless of whether or not the animal survived the entire 2.5 hours.

and hemodynamically unstable than at pre-injury state. For the most part these parameters were similar between the two treatment groups; however, the indicators of coagulopathy tended to be greater in the CG-greater animals (PT, aPTT and INR), with platelet concentration achieving a statistically significant difference. The difference in MAP between the two groups approached significance with the STF-treated animals generally maintaining a higher mean pressure, 54mmHg *vs.* 40mmHg (p = 0.7).

### *Vascular Injury and Treatment*

Blood loss was measured at the time of induction of coagulopathy (exchange of blood for Hextend™), following injury before treatment, and from time of treatment to hemostasis. Blood loss with the exchange was similar between the two groups. Both groups of animals lost a similar amount of blood between creation of the injury and application of the dressing, as well as after application of the dressing. The amount of blood lost during exchange far exceeded the amount lost post-injury.

**Figure 1** *Mean arterial pressure (MAP) in the two treatment groups*




Five of the CG-treated animals and six of the STF-treated animals required application of a second dressing, or re-compression of the initial dressing. In one of the STF-treated animals the surgeon misapplied the first dressing such that the proteins were not applied directly to the femoral artery injury. The second dressing application resulted in hemostasis.

Hemostasis was achieved initially in seven of the CG-treated animals and in 9 of the STF-treated animals, although the time to hemostasis was slightly longer for the CG-treated animals. Two of the CG-treated animals remained hemodynamically unstable despite hemostasis. These animals failed to maintain MAP ≥ 20mm Hg and thus were euthanized and counted as non-survivors per USAISR protocol.

### *Survival*

Nearly twice as many animals in the STF-treated group survived 2.5 hours compared to the CG-treated animals, 9 *vs*. 5 ($p$ = 0.14). The STF-treated animals had a significantly higher survival rate than CG-treated animals following the simulated walking test, 9 *vs*. 2 ($p$ = 0.005). The time of survival was significantly greater in the STF-treated animals compared to the CG-treated animals, 146 min *vs*. 120 min ($p$ = 0.05).

### *Wound Inspection, Angiography and Histopathology*

Both of the dressings, in the configurations tested, had the ability to apply and maintain compression, which we observed in all wounds regardless of whether or not they achieved hemostasis. Figure 3 illustrates the difference in associated clot between the two dressings. Clot was present in only two of ten CG-treated wounds and in neither case was the clot either near the arteriotomy or robust. Rather, the two small clots were found in association with a portion of the bandage remote from the injury.

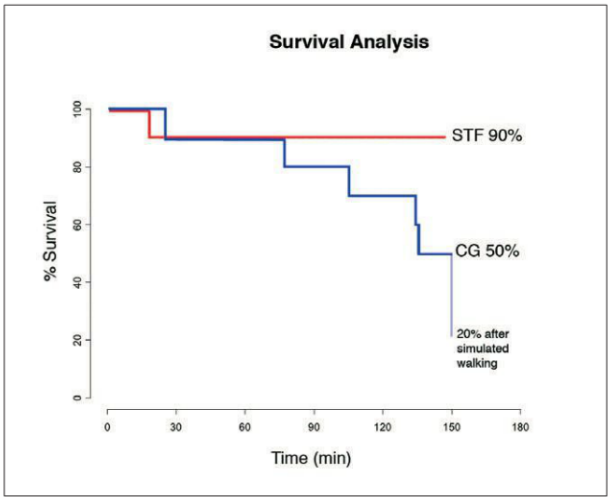
**Table 3** *Endpoints for Treatment of Femoral Arteriotomy with Control vs. Test Dressing*

| Endpoint | CG n = 10 | STF n = 10 | $p$ Value |
|---|---|---|---|
| Animals requiring two dressing applications | 5 | 6 | 0.65 |
| Initial hemostasis achieved | 7 | 9 | 0.58 |
| Time to hemostasis (min) | 40.5 ± 11.2 | 26.2 ± 6.5 | 0.27 |
| Exchange blood loss (ml) | 1435 ± 25 | 1472 ± 17 | 0.23 |
| Pre-treatment blood loss (g) | 155 ± 15 | 165 ± 22 | 0.73 |
| Post-treatment blood loss (g) | 298 ± 31 | 238 ± 37 | 0.23 |
| Total resuscitation fluid (g) | 6549 ± 689 | 5715 ± 646 | 0.39 |
| *Note:* Data were analyzed using ANOVA. | | | |

**Table 4** *Survival*

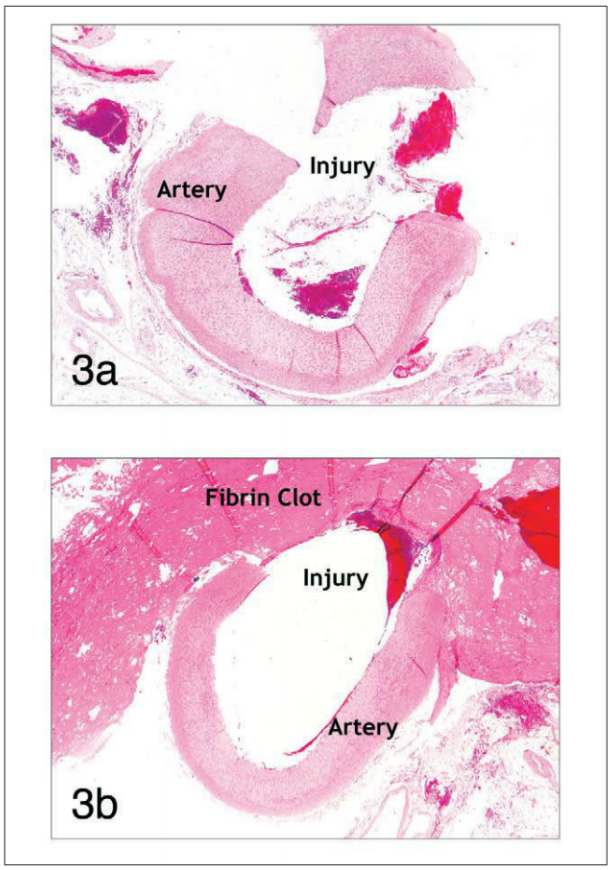
| Measure | CG | STF | $p$ Value |
|---|---|---|---|
| Survival rate, injury to 2.5 hours (%) | 5 (50%) | 9 (90%) | 0.14* |
| Final survival after simulated walking (%) | 2 (20%) | 9 (90%) | 0.005* |
| Survival time (min) | 119.7 ± 14.9 | 145.6 ± 14.2 | 0.05** |
| *Notes:* *Data were analyzed using exact test. **Data were analyzed using ANOVA. | | | |

**Figure 2** *Kaplan-Meier analysis of survival data*




Conversely, clot was observed in all of the STF-treated animals, and in all of these wounds we found a robust clot immediately adjacent and densely adherent to the arterial injury site. Eight of these fibrin clots completely sealed the injury, and one incompletely sealed the injury, allowing 90% of the animals to survive. Only one ineffective clot resulted in exsanguination death of the animal. The STF fibrin clots all remained adherent to

**Figure 3** *Histopathology of typical necropsy findings at femoral artery injury site in animals treated with CG (3a) vs. STF (3b)*




*Note: The lack of clot associated with the injury in 3a compared to the thick clot that is closely apposed to the artery in 3b.*

the tissues during removal of the dressing. Other than the injury site, we found no difference between the two groups with respect to gross necropsy findings or histopathology of the organs.

The STF dressing was significantly more robust than the CG dressing with respect to the simulated walking test. This test was developed to simulate movement of the limb that occurs during extraction and transport of a casualty, a time when re-bleeding is common and difficult to manage. Three of the five surviving CG-treated animals exsanguinated and died immediately following this test, whereas none of the nine surviving STF-treated animals bled and all survived ($p = 0.005$).

Reestablishment of antegrade blood flow past the injury site and into the affected limb was greater with the STF dressing than with the CG dressing (Figure 4). Seven of the nine surviving STF-treated animals had antegrade distal blood flow demonstrated on angiography, whereas only two of five surviving CG-treated animals reestablished blood flow. Although the $p$ value of 0.07 does not allow us to claim that the difference was statistically significant, we believe that a higher-powered study would demonstrate a difference.

## Discussion

Our results prove the hypothesis that lyophilized salmon thrombin and fibrinogen embedded in a soluble electrospun dextran dressing can control hemorrhage, increase survival time, increase survival rate, and restore distal blood flow compared to the control dressing, Combat Gauze™, in coagulopathic swine with a standardized lethal femoral artery injury.

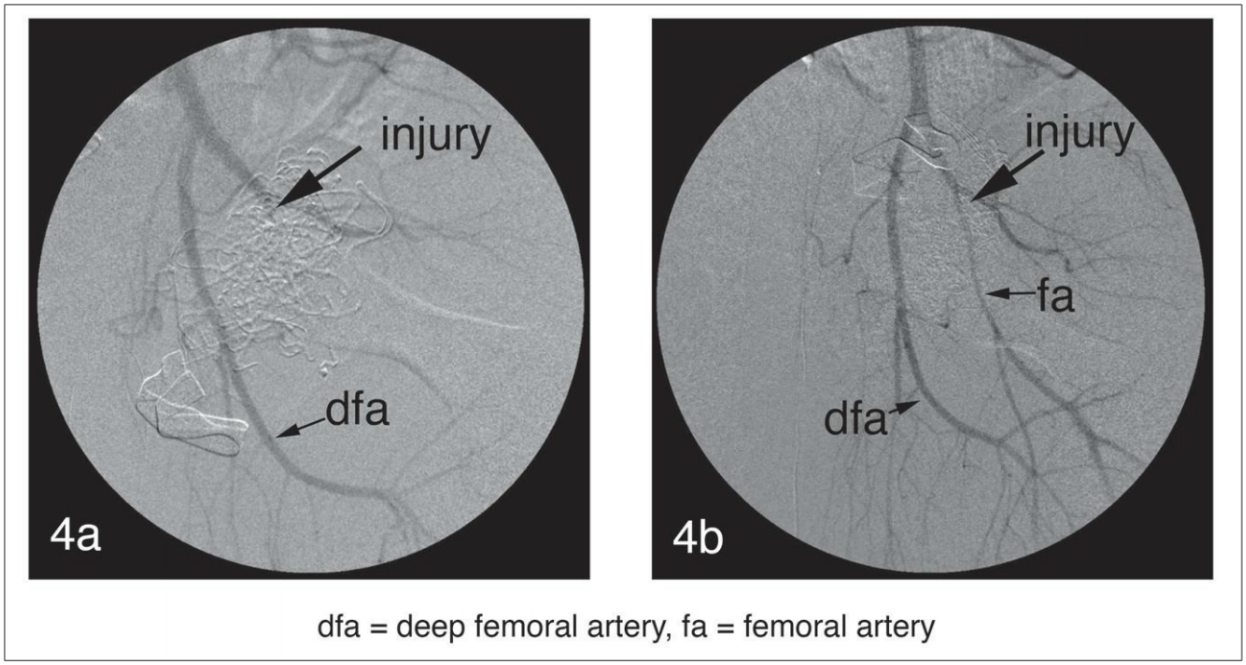
Our previous work demonstrated that the two dressings were equally efficacious with respect to survival and blood loss in this same model without induction of coagulopathy, although we also observed the ability of the STF dressing to restore distal blood flow in that study.

The coagulopathy model is particularly challenging. Acute loss of greater than half of the blood volume often is fatal, and the concomitant insult of hypothermia adds complexity to the model. Of the original 31 animals enrolled in the study, four were used as pilots to test the protocol and seven died before the injury could be created. We calculated 20 animals as the absolute minimum in order to show differences and draw meaningful conclusions. We may have had more difficulty with initial survival than other investigators because our animals tended to be younger and smaller than in most published studies. We modified our protocol to accept a lower pre-injury MAP (55mmHg vs. 60mmHg) and we added a Lidocaine infusion to control arrhythymias. Finally, we included the option of applying a second dressing, a technique published by Kheriabadi,[5] but which he currently does not endorse (Kheirabadi, personal communication, 21 October 2011).

Coagulopathy and hypothermia are common in Special Operations Forces (SOF) combat casualties where immediate treatment may be prolonged by ongoing battle and delayed extractions. Coagulopathy, shock, and hypothermia were induced in this study in order to more rigorously test the dressings in a scenario that simulates actual combat. Induction of coagulopathy in this study amplified the differences between CG and STF and clearly demonstrated the divergence in mechanisms of action of the two dressings. These differences were great enough to reach statistical significance even in this small, underpowered study.

An interesting new observation with this study was that coagulation indices were less perturbed in the STF-treated

**Figure 4** *Angiography of animal treated with CG (4a) vs. STF (4b)*




*Note:* The complete lack of blood flow in the femoral artery in 4a compared to intact flow in 4b.

animals than in the CG-treated animals. Terminal PT, aPTT and INR tended to be more prolonged in the CG group, and platelets were reduced significantly in the CG-treated animals compared to the STF group. This suggests that the STF fibrin clot may inhibit consumption of host coagulation proteins and platelets, perhaps because it supplies coagulation components as part of its construction. These preliminary observations require further investigation.

In general, the animals treated with CG did not do as well as those treated with the STF dressing. The time between injury and death was significantly shorter in the CG-treated animals, and survival rate was better in animals treated with STF dressings. Mean arterial pressure in CG-treated animals was lower than in STF-treated animals despite the fact that both groups received a similar volume of resuscitation fluid (6.5L in the CG group *vs.* 5.7L in the STF group, NS).

The difference in survival rate was increased following the simulated walking test. This test is included in the USAISR protocols to simulate vibration and motion of the body that occurs during extraction and transportation of wounded casualties. Even vibration from an air evacuation helicopter can cause renewed bleeding. These data are not considered in calculations of survival rate under the USAISR protocol because the maneuver is difficult to standardize, but it is a good test of the hemostatic stability of a given dressing. The CG dressing lost hemostasis in three of the surviving five animals following the simulated walking test, causing these animals to exsanginate. Conversely, all nine STF-treated animals maintained hemostasis and survived until euthanized.

We delayed administration of barbiturate in all animals until after we removed the dressing in order to simulate conditions in a higher echelon surgical facility where early inspection, irrigation, and debridement typically would occur. We observed blood or IV fluid leaking out of the arteriotomy in all CG-treated animals, but in only one STF-treated animal. The STF fibrin clot adhered more tightly to the artery and surrounding tissues than to the gauze backing; the clots continued to provide hemostasis despite removal of the gauze backing. These observations demonstrate the integrity and stability of the fibrin dressing and would suggest that medics who apply this dressing could have some reassurance that renewed bleeding will be less likely during transport of a casualty, and surgeons will be less likely to encounter brisk, uncontrolled bleeding when unpacking and debriding the wounds.

The superior stability of the fibrin dressing compared to CG is due to their different mechanisms of action. With careful observation during limb movement and dressing removal we observed that, in this coagulopathic state, CG achieved hemostasis by direct compression of the injured vessel. Although one proposed mechanism of action of CG is activation of the host intrinsic coagulation

**Table 5** *Clot Formation, Walking Test, and Angiography in Control vs. Test Dressing*

| Endpoint | CG | STF | *p* value |
|---|---|---|---|
| Presence of clot in the wound | 20% | 100% | <0.001 |
| Clot adherent to the arterial injury | 0% | 100% | <0.001 |
| Clot completely sealed arterial injury | 0% | 80% | <0.001 |
| Re-bleed with simulated walking test | 60%<br>3 of 5 | 0%<br>0 of 9 | 0.005 |
| Distal antegrade blood flow re-established | 40%<br>2 of 5 | 78%<br>7 of 9 | 0.07 |
| *Note:* Data were analyzed using exact test. | | | |

cascade, these animals were depleted of clotting factors so that the kaolin did not have a substrate upon which to act. Laboratory data show that these animals continued to consume their coagulation factors after application of the dressing.

This prototype of the STF dressing was designed to mimic the compression effects of CG in order to minimize variables. It used a z-folded gauze of the same dimensions as CG. The differences between the two bandages were the lack of kaolin on the STF dressing and the presence of coagulation proteins. Our observations confirmed our earlier findings that the mechanism of action of the STF dressing primarily was formation of a large, robust clot that sealed over the injury to stop the hemorrhage. Other than during initial application, ongoing compression was not an important mechanism. Indeed, the lack of compression, coupled with the sealing properties of the fibrin clot, allowed formation of a channel for reperfusion of the limb.

While control of hemorrhage, preservation of host coagulation proteins, prolongation of survival time, and improvement in survival rate all are potentially important properties of the STF dressing, the ability of the dressing to enable limb reperfusion could lead to greater limb salvage in modern conflicts where the signature injury is amputation.[4,5,10,11] It is also possible that this property could be useful in organ preservation.

Battlefield treatment of hemorrhage is a life-saving intervention that can save 60% of casualties with potentially survivable injuries.[12] An axiom of tactical combat casualty care (TCCC) is that "the fate of the wounded lays with those who apply the first dressing."[13] Inadequate field treatment of hemorrhage will lead to either death by exsanguination, or hemorrhagic shock and the lethal triad of coagulopathy, metabolic acidosis, and hypothermia[14,15] despite achievement of hemostasis and hemodynamic stability. The search for the ideal field dressing continues.

Fibrin dressings could fulfill most of the criteria of the ideal field dressing (Table 6),[9] but despite decades of research the FDA has not approved human-derived fibrinogen dressings for this application.

Researchers have attempted for more than a century to develop a dressing derived from human fibrinogen.[16] Bergel was the first to use fibrin-soaked gauze to control parenchymal bleeding, but it was not until the advent of plasma protein separation techniques that fibrinogen could be used with thrombin as an adhesive layer in burn casualties during World War II. Transmission of hepatitis halted this practice and led to attempts to use bovine thrombin, but this elicited coagulopathies secondary to development of antibodies to thrombin and Factor V.[17] In 1998, with improved donor screening and viral inactivation processes, the FDA approved cryoprecipitate preparations of human thrombin and fibrinogen.[16] These products are useful in controlling blood loss in elective surgery. Although the risk of disease transmission (hepatitis B and C, human T-cell leukemia virus, HIV, Creutzfeldt-Jakob virus, herpes simplex, prions, etc.[18]) practically has been eliminated, the historical fear of this risk—combined with their cost and storage challenges—has not led to widespread use, especially in the combat environment where deep refrigeration is impractical.

Lyophilization of human coagulation proteins renders possible their deployment in the tactical environment. Human dry fibrin sealant dressings (DFSD) have shown great efficacy compared to other advanced dressings in controlling hemorrhage in both femoral artery injury and liver laceration models, under both normal and coagulopathic conditions.[5,7,10,11,19-24] A fibrin sealant dressing was tested in an FDA approved trial in the Global War on

**Table 6** *Ideal Hemostatic Dressing for Tactical Application*[9]

| Property |
|---|
| Is approved/cleared by the U. S. FDA |
| Stops severe arterial and/or venous bleeding in < 2 min |
| Has no toxicity or side effects |
| Causes no pain or thermal injury |
| Poses no risk to medics |
| Is ready to use and requires little or no training |
| Is durable and lightweight |
| Flexible to fit complex wounds and easily removed without residue |
| Is stable and functional at extreme temperatures for > 2 wks |
| Is practical and easy to use under austere conditions |
| Is effective on junctional wounds not amenable to tourniquet |
| Has a long shelf life, > 2 yrs |
| Is inexpensive and cost effective |

Terror where it successfully was used on a Special Forces casualty.[11] Despite this limited success, cost of reagents, fear of contagions, and the complex FDA regulatory process remain obstacles to their adoption.

Coagulation proteins from other non-human sources can circumvent some of these barriers. Proteins from other species are abundant and unlikely to transmit pathogens, however mammalian allogenic proteins can elicit immune responses that can adversely affect the coagulation cascade.[25]

Salmon thrombin and fibrinogen offer several advantages over human and mammalian proteins.[26] They are abundant, inexpensive to purify, and stable to sterilization with gamma irradiation.[27] Salmon thrombin hydrolyzes salmon fibrinogen in *in vivo* mammalian hemorrhage experiments,[4,28] activates human fibrinogen to form fibrin clots that are structurally and rheologically identical to human fibrin clots, and activates human platelet aggregation.[29] That salmon coagulation proteins normally are active at low ambient temperatures makes them particularly attractive for hypothermic, coagulopathic casualties.

The immunogenicity of salmon thrombin and fibrinogen has been studied in rats and rabbits[27] and in swine.[30,31] All three species produced antibodies to salmon proteins, but none demonstrated cross-reactivity to the host coagulation proteins. Further, the development of antibodies had no effect on coagulations parameters, including PT, aPTT, INR, thrombin time, or fibrinogen levels. Normal histopathological healing was observed in wounds of swine treated with a salmon thrombin-fibrinogen dressing that had an intraperitoneal exposure to the proteins three months earlier.[31] These studies point to the safety of salmon coagulation proteins in mammals.

## Conclusions

Our earlier work demonstrated that the STF dressing is more effective than standard gauze[28] and as effective as Combat Gauze™ (CG)[4] with respect to hemostasis and survival in non-coagulopathic swine with lethal arterial injuries. The present study showed clear superiority of the STF dressing over control dressing with respect to survival rate, survival time, consumption of clotting factors, and dressing stability during simulated transport and "second look" surgery. The mechanism of action of the dressing enables reperfusion of the limb past the injury site, creating the possibility of higher limb salvage in combat casualties. The dressing is safe, portable, inexpensive, lightweight, stable, easy to apply, and efficacious. We believe it is a good candidate for the next generation of advanced field dressings.

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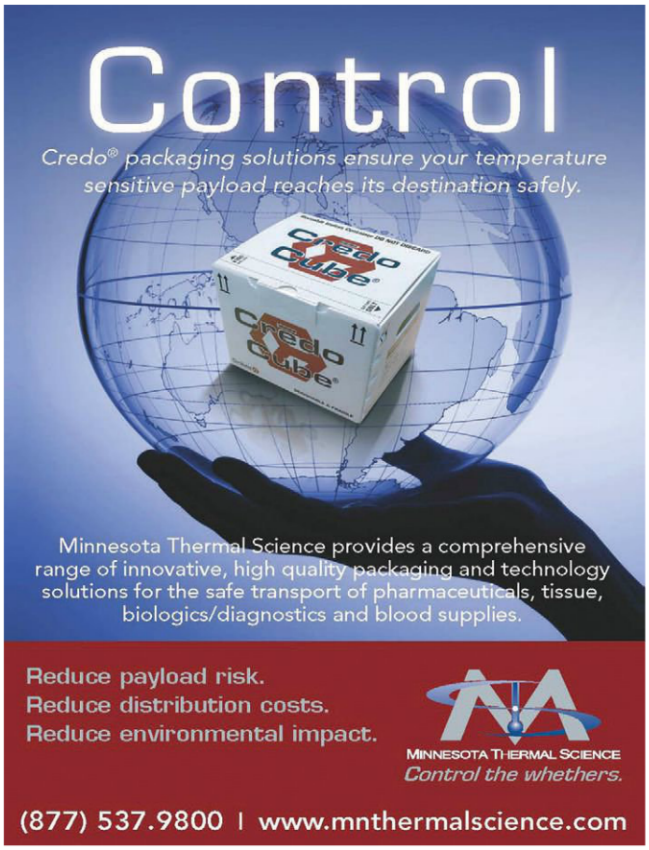
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