Study Report **Dec 5, 2016**

Efficacy Assessment of a chitosan-based dressing (HEMO-bandage™) to Control Arterial Hemorrhage in Normal and Coagulopathic Swine

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Introduction: Hemorrhage remains the greatest threat to survival in the first 24 hours after traumatic injury (1). It accounts for nearly 50% of death on the battlefield and 39% of civilian trauma deaths (2-4). The majority of these fatalities occur before the wounded soldiers or trauma patients are brought to the hospital (4-6). Improvement in personal protective gear and body armor has led to increased numbers of causalities with multiple extremity injuries, significant tissue loss, and amputations. These extremity injuries have increased the need for better tourniquets and more effective hemostatic dressings (7).
Presently none of the FDA approved/cleared hemostatic dressings meet all requirements of an ideal dressing for battlefield application. The standard tactical dressing (Combat Gauze) requires 3 minutes compression and often does not provide hemostasis immediately after application. It is also much less effective in controlling hemorrhage in casualties who may develop traumatic coagulopathy in the field.

The USAISR has established a research program focusing on improving hemorrhage control therapies and evaluating new hemostatic products that may be more effective against exsanguinating hemorrhage on the battlefield. Our effort is directed partly to screen new hemostatic products/agents developed by academia and industries, and to identify and support improvement of those that are more effective and safe against severe hemorrhage.

HEMO-bandage is a new chitosan-based dressing made by Core Leader Biotech in Taipei, Taiwan. It has recently received FDA clearance for clinical application in the United States. This non-absorbable dressing is indicated for topical application and for temporary control of moderate to severe external bleeding that results from traumatic or surgical injuries. The primary hemostatic mechanism of chitosan dressings is tissue adhesion (producing physical barrier), therefore this dressing may be effective in controlling hemorrhage in patients with traumatic coagulopathy. The efficacy of HEMO-Bandage was demonstrated to be greater than the standard Army dressing (Combat Gauze) for control of arterial hemorrhage in normal pigs (a company sponsored study, unpublished work).

Objectives: The purpose of this preliminary study was to examine the efficacy of HEMO-bandage to control arterial hemorrhage (trauma application) in our standard arterial injury model in swine. The initial two experiments will test the dressings in pigs with normal blood clotting function. If these experiments are successful, the next two experiments will test the product in pigs with moderate coagulopathy. The coagulopathy will be induced by moderate hypothermia (core temperature 34°-35° C) and 25% isovolemic hemodilution prior to inflicting arterial injury and hemorrhage.

Test material: The HEMO- bandage is made of chitosan fibers woven into a long soft bandage (3.1 in x 39 in, Z-folded) appropriate for treatment of traumatic wounds. Unlike other chitosan dressing, HEMO-bandage is quite flexible and pliable for easy packing of complex open and deep penetrating wounds. It contains 8.5 to 9.0 grams of chitosan in in each pack which is more and works faster than any similar chitosan dressing. The chitosan fibers are stable and woven into a sturdy bandage that poses no risk of leaching out and causing embolism in patients. The HEMO-bandage has a net-like structure that allows blood to penetrate into the dressing hence creating more surface area for red cells and platelets to interact with the fibers and provides better adherence to damaged tissues. For control of hemorrhage, only one dressing was packed in each wound. A bleeding wound was treated for the second time using a second dressing if hemorrhage was not controlled after the first treatment (two treatments only).

Methods: This study was approved by the Institutional Animal Care and Use Committee of the U.S. Army Institute of Surgical Research and conducted in compliance with the Animal Welfare Act and the implementing Animal Welfare Regulations. All animals received care and were used in accordance with the principles of the *Guide for the Care and Use of Laboratory Animals*.

Our standard arterial (9) model as described in the approved Type Protocol (A-04-011-TP) was used for testing HEMO-bandage. Briefly, anesthetized, mechanically ventilated pigs were instrumented to monitor vital signs (blood pressures and heart rate), administer fluid, and collect arterial blood samples for laboratory analysis. A midline laparotomy was then performed followed by splenectomy and fluid replacement. Following splenectomy, the abdomen was closed with sutures and the skin stapled closed. Next, an approximately 10 cm incision was made on the left groin and the femoral artery was isolated and prepared for the injury. The standard arterial injury was made (6-mm hole) with an aortic punch and unrestricted (free) bleeding was allowed for 30 seconds. The wound was then packed with one HEMO-bandage, covered with a laparotomy sponge and compressed manually for 3 minutes. Compression was then released and hemostasis observed for 5 minutes. Fluid resuscitation (500 ml Hextend + 2 liter LR) was administered intravenously at 50 ml/min as needed to raise and maintain mean

arterial pressure (MAP) between 60-65 mmHg. If bleeding occurred within 5 minutes after compression, the treatment was repeated once more using a new HEMO-bandage. Hemostasis was observed and the animal monitored for 2hrs or until death (MAP<20 mmHg and $etCO_2$ <15 mmHg). Shed blood during this period was collected and measured to determine post-treatment blood loss. At the 2 hour post-treatment mark or earlier, the animals were humanely euthanized.

For coagulopathic experiments, the right femoral artery was cannulated for withdrawing blood. To induce coagulopathy, 25% of the blood volume of each pig was withdrawn from femoral artery and simultaneously replaced (administered IV) with an equal volume of Hextend solution at 50 ml/min. Hypothermia was also allowed to occur to bring pigs' core temperature to 34°-35°C. Once coagulopathy was induced, the hemostatic experiment (arterial hemorrhage and dressing treatment) was conducted as described above.

Results: HEMO-bandage was properly tested against arterial hemorrhage in two pigs with normal coagulation function, and in another two pigs with pre-existing coagulopathy. Baseline blood pressure and laboratory measurements of blood cell counts, blood gases and coagulation parameters were within the normal range for Yorkshire pigs. These measurements were repeated after splenectomy and coagulopathy induction (pre-injury values) and at the conclusion of experiments (final). These data are listed in Table 1 for experiments in normal and in Table 2 for experiments in coagulopathic pigs.

Hemorrhage treatment in normal pigs: Treatment of arterial bleeding with HEMO-bandage in pigs with normal coagulation function was fully successful. The severity of injury and hemorrhage, as indicated with pre-treatment blood loss, were consistent with previous trials. The bleeding stopped immediately after completion of wound treatment with HEMO-bandage. Blood pressure (MAP) and hemostasis were stable (no rebleeding) during the 2hrs observation time. Only a small volume of Hextend (5 ml/kg) was administered to raise and maintain MAP at target level (60-65 mmHg). Vigorous movement of the treated leg (simulating walking), at the conclusion each experiment, did not cause rebleeding. Taking the bandage out of the wounds at the end of experiment revealed firm attachment of the dressing (last few layers) to the arterial injury site that sealed the defect and prevented further bleeding. Complete removal of the bandage resulted in immediate rebleeding. No chitosan residues, clots or thrombi were found at the injury site.

Hemorrhage treatment in coagulopathic pigs: Packing the groin wounds with HEMO-bandage was unsuccessful in stopping femoral artery hemorrhage in pigs with compromised blood clotting function. Each wound was packed/treated with HEMO-bandage twice but bleeding resumed shortly after manual compression was ended. Bleeding continued and administration

of resuscitation fluid continued throughout the observation periods. Animals exsanguinated and had to be euthanized at 73 and 87 minutes after treatment. Removal of bandages from wound revealed no interaction of the dressing with surrounding tissues or injured artery. The hemostatic outcomes achieved with HEMO-bandage treatment is summarized in Table 3.

Observations and Comments: Obviously, there are not enough data generated here to reach a firm conclusion regarding HEMO-bandage. However, the following comments may be made based on our observations in these few experiments:

1. A severe arterial hemorrhage can be controlled with a HEMO-bandage when the dressing is applied directly on the injury site and compressed briefly. Whether random packing (not targeted to injury site) of a bleeding wound with HEMO-bandage can secure hemostasis is unknown and requires further investigation.
2. Subjects' normal clotting function seems essential for tissue attachment of the HEMO-bandage to seal the vascular defects and stop hemorrhage. It appears that the blood clot that forms between the dressing and blood vessel acts as a glue to bind the chitosan materials to the underlying tissues. Therefore, it is unlikely that this dressing with its current formulation could stop hemorrhage in coagulopathic patients.
3. Unlike other chitosan dressings, HEMO-bandage is made of flexible chitosan fibers woven into a soft and conformable dressing that can easily be packed in complex wounds. It does not shed chitosan particles and leaves no residue in a wound when it is removed.

In summary, the HEMO-bandage is an effective hemostatic dressing that can stop severe traumatic bleeding when applied on the injured vessels. The hemostatic mechanism of this dressing is based on its physical attachment/adhesion to damaged tissues that is mediated by formation of clots at the interface. Further studies are needed to determine the efficacy of this dressing compared with Combat Gauze and other chitosan dressings (Celox gauze and Chitogauze) that are recommend by the Tactical Combat Casualty Committee for use on the battlefield.

Disclaimer: The opinions or assertions expressed herein are the private views of the authors and are not to be construed as official or as reflecting the views of the US Department of Defense.

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Table 1. Laboratory measurements of the normal pigs during hemorrhage experiments (n=2)

Values (MEAN ± STDEV)	FEMORAL INJURY BASELINE HEMO-Bandage Normal n=2	FEMORAL INJURY PRE-INJURY HEMO-Bandage Normal n=2	FEMORAL INJURY FINAL HEMO-Bandage n=2
Body Weight	41.6 ± 0.85	.	.
Temp. (°C)	37.8 ± 0	37.5 ± 0.4	38 ± 0.2
MAP (mmHg)	73.5 ± 17.7	78.5 ± 7.8	67.5 ± 3.5
HGB (g/dL)	9.4 ± 0.4	9.5 ± 0.3	8.7 ± 0.3
HCT (%)	28.2 ± 1.34	28.9 ± 1.34	26.2 ± 0.78
PLT (1000/µL)	435.5 ± 15	321.5 ± 63	318 ± 55
PT (sec)	11.5 ± 0.3	11.4 ± 0.1	11.5 ± 0.1
aPTT (sec)	15.3 ± 0.71	15.4 ± 0.85	15.4 ± 0.85
Fibrinogen (mg/dL)	222.1 ± 0.9	210.2 ± 7.4	193.7 ± 11.4
pHt	7.5 ± 0.08	7.5 ± 0.02	7.5 ± 0.01
Lac (mM)	2.1 ± 0.2	1.9 ± 0.01	1.2 ± 0.06
BE (mM)	6.4 ± 1.2	9.1 ± 0.1	11 ± 1.1

MAP: mean arterial pressure; HGB: hemoglobin; HCT: hematocrit; PLT: platelet; PT: Prothrombin Time; aPTT: activated Partial Thromboplastin Time; Lac: Lactate level; BE: Base excess. Final time was 2 hr after HEMO-bandage application.

Table 2. Laboratory measurements of the coagulopathic pigs during hemorrhage experiments (n=2)

Values (MEAN ± STDEV)	FEMORAL INJURY BASELINE HEMO-Bandage 25% Hemodilution n=2	FEMORAL INJURY PRE-INJURY HEMO-Bandage 25% Hemodilution n=2	FEMORAL INJURY FINAL HEMO-Bandage 25% Hemodilution n=2
Body Weight	39.3 ± 0.42	.	.
Temp. (°C)	37.5 ± 0.35	34.8 ± 0.07	34.6 ± 0.07
MAP (mmHg)	68.5 ± 3.54	66.5 ± 0.71	17.5 ± 0.71
HGB (g/dL)	9.1 ± 0.21	5.5 ± 0.14	3.3 ± 0.49
HCT (%)	27.5 ± 0.99	16.8 ± 0.28	10.4 ± 1.48
PLT (1000/µL)	245.5 ± 14.85	136 ± 11.31	76.5 ± 7.78
PT (sec)	11.6 ± 1.41	12 ± 1.41	24.1 ± 2.05
aPTT (sec)	14.9 ± 0.07	15.2 ± 0.64	25.8 ± 1.2
Fibrinogen (mg/dL)	225.7 ± 32.81	172.6 ± 36.63	.
pHt	7.5 ± 0.02	7.5 ± 0.01	7.7 ± 0.19
Lac (mM)	2 ± 0.55	2.1 ± 0.52	11 ± 3.98
BE (mM)	5.1 ± 0.99	7.3 ± 0.92	-2.1 ± 4.45

MAP: mean arterial pressure; HGB: hemoglobin; HCT: hematocrit, PLT: platelet; PT: Prothrombin Time; aPTT: activated Partial Thromboplastin Time; Lac: Lactate level; BE: Base excess. Final time noted in the text.

Table 3. Hemostatic outcomes of treating arterial hemorrhage with HEMO-bandage in normal and coagulopathic pigs

Values (MEAN ± STDEV)	FINAL Normal Pigs n=2	FINAL Coagulopathic Pigs n=2
Hemostasis Achieved (Initial)	2/2	0/2
Hemostasis Achieved (FINAL)	2/2	0/2
Pre-Treatment Blood Loss (mL/kg)	8.7 ± 0.44	7.2 ± 2.39
Post-Treatment Blood Loss (mL/kg)	0.8 ± 0.13	69.7 ± 10.59
Total Fluid Resuscitation Given (mL/kg)	5 ± 0.68	89.6 ± 2.04
Hemostasis Maintained (min)	120 ± 0	1.6 ± 0.35
Survival Time (min)	120	80.2 ± 9.6
Survival Rate	2/2	0/2