Determination of Efficacy of New Hemostatic Dressings in a Model of Extremity Arterial Hemorrhage in Swine

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Background: The HemCon (HC) bandage and QuickClot have been used over the past 6 years for treating external compressible hemorrhage in combat casualties. Previously, we tested three new hemostatic agents in granular/powder forms that were superior to these products. In this study, four new dressings (preselected) that are more suitable for battlefield application were evaluated. The efficacy and acute safety of the dressings were tested in our standard arterial hemorrhage model.

Methods: Anesthetized pigs (n = 38, 37 kg) were instrumented, and arterial blood was collected for hematological and coagulation assays. After splenectomy, the right femoral artery was isolated, injured (6 mm arteriotomy), and unrestricted bleeding allowed for 45 seconds. A hemostatic dressing (HC RTS [n = 6], Celox-D [CXb, n = 6], TraumaStat [TS, n = 10], Combat Gauze [CG, n = 10], or placebo gauze [PG, n = 6]) was then applied over the wound randomly and compressed for 2 minutes. Fluid resuscitation was administered and titrated to maintain a mean arterial pressure of 65 mm Hg. Animals were observed for 180 minutes or until death. Computed tomography angiography was performed on survivors and tissues were collected for histology.

Results: No differences were found in baseline blood measures, pretreatment blood loss or fluid infusion among groups. HCs and CXb testing discontinued after six unsuccessful tests, and the data were excluded. Stable hemostasis was achieved in two PG, two TS, and eight CG pigs in remaining groups resulting in stabilized mean arterial pressure and significantly different survival rates (20 – 80%, p < 0.05) but not different from PG (57.9 ± 36.2 minutes). The average survival time of CG-treated animals (167.3 ± 5.9 minutes) was also significantly longer (p < 0.05) than that of TS- (90.0 ± 15.3 minutes) or PG-treated (121 ± 19.3 minutes) pigs. Posttreatment blood loss was less in CG (37.4 ± 17.3 mL/kg) than that of the other two groups (TS = 79.8 ± 13.8 mL/kg and PG = 75.5 ± 23.8 mL/kg), but this difference was not significant. No significant rise in wound temperature (≥1°C) was recorded after treatment with dressings and computed tomography images showed no flow through the vessels. Histologic observations showed mild to moderate changes in treated vessels with no difference between CG and PG. In vitro analysis of blood treated with CG or PG (lesser extent) showed increased clotting rate and clot strength. TS treatment had no effect on blood clotting activity.

Conclusion: CG was the most effective dressing tested in this arterial hemorrhage model. The hemostatic property of CG is attributed to its raw material (nonwoven Rayon and polyester blend), kaolin coating, and the large surface area (3 inch × 4 yd) of this absorbent sponge. CG is now recommended as the first line of treatment for life-threatening hemorrhage on the battlefield, replacing HC.

Key Words: Combat gauze, TraumaStat, Celox D, HemCon, Hemorrhage control, Side effect, Swine.

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Uncontrolled hemorrhage is the leading cause of death (50%) among combat casualties and is the second major cause of death in civilian trauma patients.1–4 Massive bleeding and trauma are major risk factors leading to the lethal triad of life-threatening coagulopathy, which include persistent hypothermia, metabolic acidosis, and inability to form clot and establish hemostasis.5,6 Hemorrhage also plays a significant role in late morbidity and mortality because of multiple organ failure that may be caused by prolonged hypotension, sepsis, and massive red cell and plasma product transfusion.7,8

A review of autopsies of 982 combat deaths in the current conflict by an expert panel showed that nearly 24% of the deaths could have potentially been prevented with prompt and effective treatment.9 Of these 24% victims, majority (85%) died of potentially preventable hemorrhage with one of three being compressible and two of three being noncompressible wounds. Although there is no hemostatic modality to treat noncompressible (internal) hemorrhage in the prehospital phase, since 2003 two new hemostatic products, QuickClot and HemCon (HC) bandage, have become available for treating compressible (external) hemorrhage in the battlefield in addition to tourniquets. Despite these advancements, some of the compressible hemorrhages could not be controlled promptly and eventually led to the death of soldiers. Thus, hemorrhage control and the search for more effective hemostatic modalities continue to have a high priority in the US Army Combat Casualty Care Research program.

As part of these efforts, we have recently identified three new hemostatic agents in granular/powder forms that were significantly more effective than the current hemostatic products used on the battlefield.10 These included WoundStat...
(WS), a smectite mineral granules with potent hemostatic activity that forms highly adhesive clay material when mixed with blood; Super Quick Relief (SQR), a mineral-based hemostatic powder with which blood forms an artificial scab on the wound and seals injuries; and Celox (CX), a chitosan-based powder that seals bleeding sites by chemical and mechanical linkage with red blood cells in wounds. The hemostatic efficacy and acute safety of these products were tested against a groin arterial hemorrhage in swine that could not be controlled by standard gauze, QuikClot, or HC bandages (100% mortality). WS was found to be the most efficacious agent, followed by SQR and CX with 100%, 70%, and 60% survival rates, respectively. However, histologic examination of treated tissues (femoral artery and nerve) revealed significant thermal damage by SQR and presence of WS microscopic residues inside the treated arteries.10

The use of granular/powder agents are advantageous for treating complex irregular wounds because they can be spread and tightly packed in the wound, covering all the bleeding sites that may be missed when a small size inflexible dressings are used. On the other hand, handling and precise application of granular or powder agents to deep penetrating wounds with profuse bleeding are generally more difficult and inefficient than dressing, particularly in the field under extreme cold, insufficient light or windy conditions. For these reasons, we searched to find flexible and efficacious dressings that could have the advantages of granular agents but easily applied without the chance of spillage in any circumstances. We identified two new flexible hemostatic dressings namely Combat Gauze (CG) and TraumaStat (TS) plus Celox-D (CXb), dressing like packages of chitosan particles similar to regular CX powder but enclosed in four small transparent bags, which dissolve rapidly (<10 seconds) when they come in contact with blood. Unlike CX powder, the CXb were easy to apply to a wound with profuse bleeding and stayed in place without the risk of being washed away by blood loss.

3. CXb, a product of SAM Medical (Portland, OR), is composed of small chitosan particles similar to regular CX powder but enclosed in four small transparent bags, which dissolve rapidly (<10 seconds) when they come in contact with blood. Unlike CX powder, the CXb were easy to apply to a wound with profuse bleeding and stayed in place without the risk of being washed away by blood loss.

4. HC RTS (HCs) dressing is an advanced version of chitosan dressing made by HemCon Inc. (Portland, OR), which is presofteren, more flexible, and thinner than the original HC dressing.

5. PG, used as a control material, was identical to CG but was not coated with kaolin clotting agent. These surgical gauze rolls were also obtained from Z-Medica Corporation along with CG.

In Vivo Methods

Yorkshire cross-bred pigs (castrated males only) weighing 34 kg to 42 kg were purchased from Midwest Research Swine and used in this study. The original intent was to test each material in 10 pigs but for reasons described below, some materials were tested in only six animals. Before the surgery date, venous blood samples were collected from pigs and complete blood count (CBC) and standard coagulation parameters (prothrombin time, activated partial thromboplastin time, fibrinogen) were measured to ensure these values are within normal range before proceeding with experiments. Pigs were fasted for 12 hours to 18 hours before the surgery with free access to water. On the day of surgery, pigs were premedicated with buprenorphine (0.025 mg/kg, intramuscular [i.m.]) for analgesia and glycopyr罗late (0.01 mg/kg, i.m.) to reduce saliva secretion and block vagally mediated bradycardia during the surgical procedure. Animals were induced with an injection of tiletamine-zolazepam (Telazol, 4–6 mg/kg, i.m.) and anesthetized initially with 5% isoflurane in oxygen via face mask. They were then intubated and mechanically ventilated with 100% oxygen. The tidal volume and ventilation rate were adjusted to maintain an end tidal Pco₂ of 40 mm Hg ± 2 mm Hg.
Anesthesia was maintained with 1% to 2% isoflurane added to oxygen gas by the ventilator. Maintenance fluid, lactated Ringer’s (LR), was administered at 5 mL · kg⁻¹ · hr⁻¹ through a venous line placed in an ear vein.

Surgical Procedures

The right carotid artery was cannulated for blood draws and direct recording of blood pressure (systolic, diastolic, and mean) and heart rate throughout the experiment. A 9-mL blood sample was collected from the arterial line and mixed with 1 mL of Na citrate (3.2%) as anticoagulant and used for thrombelastography (TEG) assays, as described later. The right jugular vein was also catheterized for administering resuscitation fluid. A midline laparotomy was then performed, followed by a splenectomy to minimize blood changes that may occur as result of autotransfusion from the pig’s contractile spleen. The blood loss from splenectomy was replaced by infusing LR at three times the weight of the spleen. A cystotomy was also performed to aid in the drainage of urine. The abdomen was then closed with suturing, and the skin was stapled. Preinjury (baseline) blood samples were collected from the arterial line for CBC, coagulation, and blood gas analysis.

To create a severe hemorrhage in the groin area, ~5 cm of femoral artery was dissected free from surrounding tissues, and the overlying abductor muscle was removed. Injury to the adjacent femoral vein and nerve was avoided. The vessel was then bathed with a few milliliters of 2% lidocaine to relax vasospasm and dilate the artery to its normal size. To measure wound temperature, a microelectrode was sutured to the muscle adjacent to the vessel but at least 1 inch away from the arteriotomy site, so that it would not interfere with the hemostatic treatment. Next, a 10-minute stabilization period was allowed (no manipulation) and baseline data including mean arterial pressure (MAP) and body temperature were recorded. A stable MAP of 60 mm Hg or higher was required before any intervention. After 30 seconds compression to occlude the artery and stop the bleeding, a sponge and press were placed on the wound. If rebleeding occurred during this period, the laparotomy gauze was removed and the failed agent taken out and replaced with fresh material. The 2-minute compression was then repeated with a new laparotomy gauze. Wounds were treated at most twice with each product regardless of hemostatic outcome. Hemostasis was then observed for the next 3 hours with laparotomy gauze left in place. Any shed blood during this period was measured and recorded as posttreatment blood loss.

After the infusion of Hextend, fluid administration was continued with LR (100 mL/min, maximum of 10 L) as needed to raise and maintain the MAP at 65 mm Hg throughout the experiment. The MAP of 65 mm Hg approximates systolic pressure of 90 mm Hg, which is in agreement with the level of permissive hypotensive resuscitation regimen. Animals were monitored up to 3 hours or until death as determined by end tidal Pco₂ <15 mm Hg and MAP <20 mm Hg. Final blood samples (arterial) were collected for hematological measurements before euthanizing the animals.

Surviving animals were CT scanned and images of arterial blood flow and vascular structures in their legs were obtained. Next, the treated legs of surviving pigs were flexed and stretched five times simulating walking condition to test the stability of the hemostasis provided by the test agents. At the conclusion of experiments, the product was removed from the wound to check the status of injury and the fate of the vessel. Animals were then killed and tissue samples including the injured artery, adjacent femoral vein, femoral nerve, and muscle tissues were collected for histologic examination. Histologic slides were prepared according to a standard procedure and stained with hematoxylin and eosin. The slides were coded and examined by a board certified veterinarian pathologist who was blinded to the treatment group. Once the examination of individual slides was completed, the codes were broken, and the results were categorized under each specific product. Control tissue samples (for histologic comparisons only) were collected from the contralateral leg of a few surviving pigs. The control arteries were isolated in the same manner, perforated with 6-mm puncher and bled for 45 seconds before harvesting for histology along with other tissues.

Wound Treatment and Resuscitation

The surgeons were blinded to the identity of test materials until the time of agent application. To the extent that was possible, the products were applied according to the manufacturers’ instruction. After the 45-second free bleed, while bleeding continued, a package of each product was opened, and the material was packed in the wound. The material was covered immediately with a folded laparotomy sponge and pressed against the wound with sufficient and equal pressure to occlude the artery and stop the bleeding. The arterial injuries, dressing applications, and compressions were done by the same investigator (B.K.) for all the experiments to minimize variability. After 30 seconds compression, fluid resuscitation was started by infusing 500 mL of Hextend (6% hetastarch in balanced electrolytes plus glucose) to compensate for pretreatment blood loss. The colloid fluid was administered at 100 mL/min intravenously, and targeted to raise the MAP to 65 mm Hg, the average baseline blood pressure of anesthetized pigs. Compression was stopped after 2 minutes and hemostasis observed for 3 minutes without removing the laparotomy gauze. If rebleeding occurred during this period, the laparotomy gauze was removed and the failed agent taken out and replaced with fresh material. The 2-minute compression was then repeated with a new laparotomy gauze. Wounds were treated at most twice with each product regardless of hemostatic outcome. Hemostasis was then observed for the next 3 hours with laparotomy gauze left in place. Any shed blood during this period was measured and collected as posttreatment blood loss.

In Vitro Methods

TEG method was used to examine the hemostatic property of each test agent in vitro. The TEG machines (TEG Hemostasis Analyzer 5,000, Hemoscope, Niles, IL) were calibrated before use with quality control standards obtained from Hemoscope. To prepare the samples, 2-mm diameter circular pieces were cut out from each dressing using a punch biopsy instrument. Ten milligrams of each dressing pieces were placed in a small plastic vial and 2 mL of citrated blood, freshly collected from the arterial line, was added and capped. The vials were gently inverted eight times and 340-μL blood samples were taken and placed in TEG cups for analysis.
Calcium chloride (20 μL of 0.2 mol/L) was added to the cups before adding blood samples to overcome the anticoagulant effect. The coagulation effects of the new dressings were compared with standard kaolin vial, a known activator of the contact (intrinsic) clotting pathway. A recalcified blood sample with no treatment was also tested as control. Samples were tested in duplicate and tracing continued until 30 minutes after the clot reached maximum strength. The following variables were measured for each sample at 37°C: reaction time (R, minutes, the time that the initial fibrin formation is detected); clotting time (K, minutes, the speed of clot formation and is the time from the R time until a clot with a fixed firmness is formed); angle (α, degree, the kinetics of clot development); and maximum amplitude (MA, millimeter, the maximum strength or firmness of the developed clot). The velocity of clot formation was also calculated as the first derivative of the TEG tracings and maximum clotting velocity (Vmax, millimeter/minute) was determined.

Data Analysis

Data are expressed as mean ± standard of error of the mean (SEM) and analyzed by analysis of variance, Fisher’s exact, and Log rank for statistical comparisons. p values were adjusted according to False Discovery Rate method for bigroup comparison.13 The data with high variance were log transformed for analysis of variance. The nonparametric data were analyzed using Newman-Keuls multiple comparison test, and bigroup comparison was done using Dunnett’s test. A p < 0.05 was considered statistically significant.

RESULTS

In Vivo

No difference was found in baseline physiologic and hematological measurements among the treatment groups (Table 1).

Hemostasis Achievement

The incidence of initial hemostasis achievement (secure hemostasis for at least 3 minutes immediately after treatment) was relatively low ranging from 0% (HCS, CXb) to 30% (CG) with the dressings. In general, two treatments were required to produce hemostasis for all the products except for CG where hemostasis was achieved after the first dressing application in three experiments. The original intent was to test each new dressing in 10 pigs; however, because HCS and CXb failed to produce hemostasis in six consecutive experiments and all treated animals died of bleeding, further testing of these dressings was eliminated, and the related data were excluded from the data set for statistical analysis. The PG was tested only in six pigs because of limited supply.

CG produced immediate hemostasis in three pigs, and this lasted for the entire experiments. In five other animals, it stopped bleeding after 12 minutes to 26 minutes of slow hemorrhage and secured hemostasis for the rest of experiments. The simulated walking condition (movement of the legs) at the conclusion of experiments did not cause rebleeding in surviving animals. The average times that bleeding was controlled by the dressings (hemostasis time) and other hemostatic outcomes are shown in Table 2. The hemostasis time for CG-treated animals was significantly (p < 0.05) longer than TS group.

MAP and Blood Loss

The average MAP of each group, which reflects the hemostatic conditions of the pigs during the experiment, is shown in Figure 1. The baseline pressures (Table 1) and sharp decreases after injury and hemorrhage were not different among the groups. The MAP became significantly different (p < 0.05) 30 minutes after injury for the animals that were treated with CG as compared with those treated with TS. At 60 minutes postinjury, MAP of CG-treated animals was substantially higher than all other groups (p < 0.05).

<table>
<thead>
<tr>
<th>Measure</th>
<th>HemCon RTS (HCS), n = 6</th>
<th>Celox-D (CXb), n = 6</th>
<th>TraumaStat (TS), n = 10</th>
<th>Placebo Gauze (PG), n = 6</th>
<th>Combat Gauze (CG), n = 10</th>
<th>Overall p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (kg)</td>
<td>38.4 ± 1.1</td>
<td>37.4 ± 1.0</td>
<td>36.4 ± 1.0</td>
<td>34.9 ± 0.9</td>
<td>38.1 ± 0.8</td>
<td>0.08</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>37.7 ± 0.1</td>
<td>37.7 ± 0.1</td>
<td>37.7 ± 0.1</td>
<td>37.6 ± 0.1</td>
<td>37.5 ± 0.1</td>
<td>0.54</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>70.5 ± 2.4</td>
<td>69.0 ± 2.2</td>
<td>74.8 ± 2.2</td>
<td>76.8 ± 2.6</td>
<td>75.2 ± 2.4</td>
<td>0.20</td>
</tr>
<tr>
<td>HGB (g/dL)</td>
<td>10.0 ± 0.04</td>
<td>9.7 ± 0.3</td>
<td>10.1 ± 0.2</td>
<td>9.2 ± 0.2</td>
<td>9.8 ± 0.2</td>
<td>0.24</td>
</tr>
<tr>
<td>HCT (%)</td>
<td>29.4 ± 1.1</td>
<td>28.1 ± 1.0</td>
<td>29.3 ± 0.6</td>
<td>26.7 ± 0.8</td>
<td>28.3 ± 0.5</td>
<td>0.14</td>
</tr>
<tr>
<td>PLT (1,000/μL)</td>
<td>363 ± 42</td>
<td>323 ± 30</td>
<td>410 ± 32</td>
<td>406 ± 69</td>
<td>396 ± 34</td>
<td>0.59</td>
</tr>
<tr>
<td>PT (s)</td>
<td>10.9 ± 0.2</td>
<td>11.5 ± 0.3</td>
<td>11.3 ± 0.3</td>
<td>11.4 ± 0.1</td>
<td>10.9 ± 0.3</td>
<td>0.32</td>
</tr>
<tr>
<td>aPTT (s)</td>
<td>17.2 ± 0.3</td>
<td>16.6 ± 0.3</td>
<td>16.6 ± 0.5</td>
<td>16.9 ± 0.6</td>
<td>17.1 ± 0.5</td>
<td>0.30</td>
</tr>
<tr>
<td>Fibrinogen (mg/dL)</td>
<td>202 ± 10</td>
<td>200 ± 15</td>
<td>205 ± 22</td>
<td>215 ± 11</td>
<td>222 ± 6</td>
<td>0.35</td>
</tr>
<tr>
<td>pH</td>
<td>7.43 ± 0.0</td>
<td>7.4 ± 0.0</td>
<td>7.4 ± 0.0</td>
<td>7.4 ± 0.0</td>
<td>7.4 ± 0.0</td>
<td>0.51</td>
</tr>
<tr>
<td>Lactate (mM)</td>
<td>2.25 ± 0.2</td>
<td>2.3 ± 0.4</td>
<td>2.4 ± 0.3</td>
<td>2.3 ± 0.1</td>
<td>2.1 ± 0.2</td>
<td>0.9</td>
</tr>
<tr>
<td>Base excess (mM)</td>
<td>6.0 ± 0.2</td>
<td>6.4 ± 0.7</td>
<td>4.5 ± 0.7</td>
<td>5.5 ± 0.7</td>
<td>5.3 ± 0.8</td>
<td>0.43</td>
</tr>
</tbody>
</table>

Data expressed as mean ± SEM and analyzed by one-way analysis of variance test. No significant difference was found among groups.

HGB, hemoglobin; PLT, platelets; PT, prothrombin time; aPTT, activated partial prothromboplastin time.

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TABLE 2. Outcomes of Treating a Groin Arterial Hemorrhage With Different Hemostatic Dressings in Swine

<table>
<thead>
<tr>
<th>Outcome</th>
<th>HemCon* RTS (HCs), n = 6</th>
<th>Celox-D* (CXb), n = 6</th>
<th>TraumaStat (TS), n = 10</th>
<th>Placebo Gauze (PG), n = 6</th>
<th>Combat Gauze (CG), n = 10</th>
<th>Overall p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial hemostasis achieved† (no. application)</td>
<td>0/6 (12)</td>
<td>0/6 (12)</td>
<td>1/10 (20)</td>
<td>1/6 (12)</td>
<td>3/10 (17)</td>
<td>NS</td>
</tr>
<tr>
<td>Total time bleeding stopped (min)</td>
<td>3.0 ± 2.2</td>
<td>0.5 ± 0.04</td>
<td>35.7 ± 22.2</td>
<td>57.9 ± 36.2</td>
<td>134.6 ± 22.2</td>
<td>0.02 (one-way ANOVA)</td>
</tr>
<tr>
<td>Pretreatment blood loss (mL/kg)</td>
<td>19.2 ± 1</td>
<td>21.8 ± 1.8</td>
<td>19.3 ± 1.2</td>
<td>19.3 ± 1.6</td>
<td>18.2 ± 0.7</td>
<td>NS</td>
</tr>
<tr>
<td>Posttreatment blood loss (mL/kg)</td>
<td>108.2 ± 7.5</td>
<td>113.8 ± 8.2</td>
<td>79.8 ± 13.8</td>
<td>75.5 ± 23.8</td>
<td>37.4 ± 17.3</td>
<td>NS</td>
</tr>
<tr>
<td>Total resuscitation fluid (mL/kg)</td>
<td>175.3 ± 24.8</td>
<td>189.1 ± 16.2</td>
<td>160.3 ± 14.4</td>
<td>186.2 ± 41.9</td>
<td>123.9 ± 27.2</td>
<td>NS</td>
</tr>
<tr>
<td>Survival rate</td>
<td>0/6</td>
<td>0/6</td>
<td>2/10</td>
<td>2/6</td>
<td>8/10</td>
<td>0.03 (χ²)</td>
</tr>
<tr>
<td>Survival time (min)</td>
<td>74.3 ± 10.5</td>
<td>74.2 ± 5.8</td>
<td>90.0 ± 15.3</td>
<td>121 ± 19.3</td>
<td>167.3 ± 5.8</td>
<td>&lt;0.001 (logrank)</td>
</tr>
<tr>
<td>Peak wound temperature (°C)</td>
<td>36.8 ± 0.2</td>
<td>36.6 ± 0.2</td>
<td>36.6 ± 0.3</td>
<td>37.0 ± 0.1</td>
<td>35.7 ± 0.3</td>
<td>NS</td>
</tr>
</tbody>
</table>

Data expressed as mean ± standard error of the mean; NS = p > 0.1.

* Testing of HemCon bandage and Celox bag was stopped after six unsuccessful experiments. The related data were excluded from the data set for statistical analysis.
† Initial hemostasis was considered to occur when bleeding was stopped for at least 3 minutes after compression.
‡ p < 0.05 versus TS (Newman-Keuls multiple comparison test).
§ p < 0.05 versus PG or TS (logrank test, p values were adjusted with False Discovery Rate method).
ANOVA, analysis of variance; NS, not significant.

Figure 1. The average MAP of each group of pigs (survival and nonsurvival) treated with one type of dressing. The MAP of CG-treated animals increased in response to resuscitation and fluid replacement in each treatment group. In the case of CG, blood gas values (pH, lactate, and base excess) remained closer to baseline levels and along with hemoglobin, were significantly better than TS- or both TS- and PG-treated animals. No significant temperature increase was measured in the wounds as a result of treatment with any of the dressings (Table 2).

The CT images of surviving animals showed complete blockage of blood flow in femoral arteries at the treated site by all the dressings. Flow though collateral arteries, however, was not affected. A representative CT image of a CG-treated pig is shown in Figure 3.

Survival

Eighty percent of CG-, 33% of PG-, and 20% of TS-treated animals lived for the entire experiments (Table 2) with the final MAP of 64 mm Hg ± 2.3 mm Hg. The differences in survival rates among the three groups were significant (p = 0.03), but the difference between CG and TS or PG was not statistically significant (posttest bigroup comparison). The Kaplan-Meier analysis of survival time for all groups is shown in Figure 4. The average survival time of
CG-treated animals (167 minutes) was significantly longer than that of PG- (121 minutes) or TS-treated (90 minutes) pigs (p < 0.05) (Table 2).

Morphologic and Histologic Assessment
At conclusion of experiments, CG, PG, and TS dressings were easily removed from the wounds resulting in the rupture of the hemostatic clot and rebleeding at the injury site in surviving animals. No significant intraluminal clot was found in the distal or proximal ends of treated vessels after dressings were removed. Complete removal of chitosan particles (CXb) from the wound required more effort than other dressings and pieces of the bags (undissolved), and some dry chitosan material were often found in the wound.

The observed histologic changes were moderate injury to endothelial layers of treated arteries, minimal to mild changes in femoral vein and perivenous tissues, minimal to moderate focal necrosis on muscle surfaces, minimal to mild perineural inflammation, and moderate neutrophilic infiltration in all the tissues. Based on the histologic changes, the dressings were ranked by the veterinarian pathologist in the following order: HCs (least change)/TS/CXb/CG/P (most change). The only possibly significant change in any of these tissues was the lack of endothelium in some treated segments of treated arteries (Fig. 5, arrows). The clinical implication of this damage cannot be determined without long-term survival studies. None of the described damages, however, were significant enough to disqualify the use of any of the dressings.

TABLE 3. Final Hematological Measurements in the Operated Pigs

<table>
<thead>
<tr>
<th>Value</th>
<th>HemCon RTS* (HCs, n = 6)</th>
<th>Celox-D* (CXb, n = 6)</th>
<th>TraumaStat (TS), n = 10</th>
<th>Placebo Gauze (PG), n = 6</th>
<th>Combat Gauze (CG), n = 10</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>HGB (g/dL)</td>
<td>1.2 ± 0.2</td>
<td>1.1 ± 0.1</td>
<td>2.1 ± 0.6</td>
<td>2.7 ± 1.2</td>
<td>5.1 ± 0.8*</td>
<td>0.03</td>
</tr>
<tr>
<td>PLT (1,000/μL)</td>
<td>66.8 ± 10.5</td>
<td>63.7 ± 15.0</td>
<td>127 ± 35.9</td>
<td>121 ± 43.8</td>
<td>245 ± 51.4</td>
<td>0.1</td>
</tr>
<tr>
<td>PT (s)</td>
<td>29.5 ± 3.3</td>
<td>28.9 ± 2.6</td>
<td>26.9 ± 5.0</td>
<td>20.5 ± 2.9</td>
<td>17.5 ± 4.7</td>
<td>0.3</td>
</tr>
<tr>
<td>aPTT (s)</td>
<td>40.5 ± 3.9</td>
<td>39.1 ± 1.5</td>
<td>31.2 ± 3.9</td>
<td>32.1 ± 3.7</td>
<td>20.9 ± 2.1</td>
<td>0.05</td>
</tr>
<tr>
<td>Fibrinogen* (mg/dL)</td>
<td>73.5 ± 2.0</td>
<td>120.4 ± 29.1</td>
<td>143 ± 32.4</td>
<td>123 ± 21.9</td>
<td>196.2 ± 10.9</td>
<td>0.2</td>
</tr>
<tr>
<td>pH</td>
<td>7.5 ± 0.04</td>
<td>7.6 ± 0.04</td>
<td>7.6 ± 0.04</td>
<td>7.57 ± 0.04</td>
<td>7.45 ± 0.04†</td>
<td>0.04</td>
</tr>
<tr>
<td>Lactate (mM)</td>
<td>16.1 ± 1.6</td>
<td>14.6 ± 0.4</td>
<td>11.6 ± 1.8</td>
<td>11.9 ± 3.3</td>
<td>2.4 ± 0.3§</td>
<td>0.003</td>
</tr>
<tr>
<td>Base excess (mM)</td>
<td>−2.65 ± −1.8</td>
<td>−2.1 ± 1.8</td>
<td>0.5 ± 1.4</td>
<td>0.1 ± 2.3</td>
<td>4.9 ± 1.6†</td>
<td>0.01</td>
</tr>
</tbody>
</table>

* Testing of HemCon bandage (presoften) and Celox-D was stopped after six unsuccessful experiments. The data related to these products were excluded from the data set for statistical analysis.
† Fibrinogen concentration could not be measured in the final blood samples in 17 animals because of excessive hemodilution. The values represent the average fibrinogen concentration of the samples that were measured successfully in each group.
‡ P < 0.05 versus TS (Newman-Keuls multiple comparison test).
§ P < 0.05 versus PG or TS (Newman-Keuls multiple comparison test).

Figure 3. A typical computed tomography image of the arterial blood flow in the hind legs of a surviving pig treated with CG 3 hours after surgery. Note the blockage of flow in femoral artery where it is compressed by the dressing (arrow). Limited collateral circulation is also seen.

Figure 4. Kaplan-Meier analysis of survival time of pigs treated with each dressing. The CG-treated animals lived significantly longer than PG- or TS-treated (p < 0.05) pigs.
In Vitro

The clotting profiles (TEG tracings) of blood samples treated with hemostatic dressings or kaolin are shown in Figure 6. The traces represent the average measurements of arterial blood samples collected from five pigs before surgery. Treatment of blood with HC or TS dressing, both of which contain chitosan, either had no effect or decreased blood clotting activities as compared with untreated (recalci-fied only) samples. On the other hand, treatment of blood with kaolin alone or kaolin containing gauze (CG) elicited strong response and shifted the clotting curves to the left side indicating faster (shorter R time and bigger angle) and stronger (larger MA) clot formation than untreated blood. To a lesser extent, exposure to regular gauze also stimulated faster and stronger clot formation than controls. The TEG measurements of different clotting parameters (R, K, [α], MA, and Vmax) for each dressing and their analysis are summarized in Table 4.

DISCUSSION

In this study, the efficacies of three promising hemostatic dressings, CG, TS, CXb, and a new formulation of HC bandage (RTS) were examined and compared with a placebo-gauze equivalent to CG but without active agent (kaolin). All the dressings, including PG, were applied to the wound with the aid of a laparotomy sponge that was left in place for the entire experiment.

The results demonstrated that the HC RTS bandage, although thinner and more flexible (conformable), was less effective than the enhanced formulation of this dressing tested in our earlier study.10 The HC RTS bandages initially conformed and sealed the wound, capable of stopping low-pressure arterial hemorrhage, but once fluid was administered and blood pressure returned to baseline level, the adherence of dressing failed and rebleeding started and continued until animals exsanguinated. No secondary hemostasis occurred with these bandages. These results give further evidence that the hemostatic function of chitosan-based products is mediated by tissue adhesive properties and not by the clotting activities of these agents.14 This is also supported by the current TEG analysis of blood samples treated with the chitosan dressing that showed no change or slower clotting function than the untreated samples.

The other chitosan product, Celox-D was also found to be ineffective despite successful results with this product as free powder in our previous study10 and in another investigation.15 The packaging of free powder in small dissolvable bags improved handling and application of the product, but it interfered with the binding of chitosan particles with bleeding tissues. This occurred despite our best effort during application (massage the bags for few seconds) to assure that the bags were dissolved and free powder released in the wound before compression started. Because of six consecutive failures to obtain stable hemostasis with Celox-D or HC RTS, further testing of these products was discontinued.
Treatment with TS produced hemostasis only in two experiments; one developed immediately after application and the other 18 minutes after slow bleeding and both were stable for the duration of experiments. This large and relatively stiff dressing, made of chitosan-coated silica-filled polyethylene fibers, potentially has both hemostatic and adhesive properties. However, TEG analysis of blood samples treated with this dressing showed no increase in clotting activities. Perhaps changes that can increase flexibility and conformity of this dressing may improve the efficacy of this product.

CG was the most efficacious dressing tested in this study resulting in 80% (8 of 10) survival of the animals. The only other dressing that exhibited similar efficacy in this hemorrhage model (66% survival rate) was a prototype fibrin sealant bandage composed of lyophilized human fibrinogen and thrombin on an absorbable backing. However, unlike fibrin sealant or chitosan dressings (adhesive material), CG may not produce hemostasis immediately after application to the wound. In our experiments with CG, immediate hemostasis achieved only in three animals, whereas in five others stable hemostasis developed after a period of slow bleeding. Hemostasis did not occur in two experiments and animals died after massive hemorrhage. Because of this variability, the average blood loss of CG group was not significantly different from the other groups. This may suggest contradictory findings regarding survival benefit of CG if posttreatment blood losses were not different. The average posttreatment blood loss of the eight animals that survived with CG treatment was only 12 ± 4.1 mL/kg and was indeed significantly \( p < 0.05 \) less than

Table 4. Blood Coagulation Measurements With Thrombelastography (TEG) Method After Exposure to Hemostatic Agents

<table>
<thead>
<tr>
<th>TEG Parameter</th>
<th>Untreated Control</th>
<th>HemCon RTS</th>
<th>Kaolin</th>
<th>TraumaStat</th>
<th>Placebo Gauze</th>
<th>Combat Gauze</th>
</tr>
</thead>
<tbody>
<tr>
<td>R-time (min)</td>
<td>12.2 ± 1.3</td>
<td>15.8 ± 1.8</td>
<td>5.1 ± 0.3*</td>
<td>12.6 ± 1.1</td>
<td>8.4 ± 0.5</td>
<td>6.8 ± 0.4*</td>
</tr>
<tr>
<td>K-time (min)</td>
<td>3.7 ± 0.8</td>
<td>6.0 ± 1.1</td>
<td>0.8 ± 0.02*</td>
<td>3.5 ± 0.6</td>
<td>1.6 ± 0.2</td>
<td>1.2 ± 0.1*</td>
</tr>
<tr>
<td>Angle (°)</td>
<td>51.7 ± 4.4</td>
<td>37.1 ± 4.9*</td>
<td>80.2 ± 0.7*</td>
<td>50.3 ± 4.0</td>
<td>67.5 ± 2.2*</td>
<td>73.9 ± 1.8*</td>
</tr>
<tr>
<td>MA (mm)</td>
<td>62.0 ± 1.2</td>
<td>57.2 ± 0.6*</td>
<td>67.2 ± 1.3*</td>
<td>59.3 ± 1.2</td>
<td>67.1 ± 0.9*</td>
<td>68.8 ± 1.3*</td>
</tr>
<tr>
<td>Vmax (mm/min)</td>
<td>11.3 ± 0.8</td>
<td>6.2 ± 1.0</td>
<td>29.8 ± 2.2*</td>
<td>7.5 ± 1.1</td>
<td>15.7 ± 1.6</td>
<td>22.1 ± 1.7*</td>
</tr>
</tbody>
</table>

Data expressed as mean ± standard error of the mean and analyzed by one-way analysis of variance test followed by Dunnett’s test for bigroup comparison.

* Values were significantly \( p < 0.05 \) different than untreated controls.
the other groups. The discrepancy was caused by the large blood loss (139 ± 1.5 mL/kg) of the two animals that died because of unsuccessful treatment.

The hemostatic function of CG appears to be mediated by enhancing blood clotting activities and formation of hemostatic clots in conjunction with the gauze. This was evident at the conclusion of experiments when CG was gently removed from the wound. Hemostatic clots were often seen at the junction of the gauze and injury site which disrupted when the gauze was taken out resulting in immediate profuse bleeding. No intraluminal clot found in the treated vessels.

Because the hemostatic function of CG depends on blood coagulation capacity, its efficacy may be affected by the coagulation status of patients and possibly less effective against coagulopathic bleeding than the tissue-sealant products. The TEG analysis of blood treated with this dressing showed the clotting activity of CG to be equivalent to the kaolin agent alone, causing significant increases in the speed and strength of clot formation. The physical characteristics and the type of gauze (50% Rayon and 50% Polyester) used for production of CG also contributed to the hemostatic function of CG. This was evident based on both the in vivo findings with the PG (CG without kaolin) that resulted in 33% survival (two of six) and the in vitro coagulation results (TEG analysis) of blood treated with PG that showed increased clotting rate and clot strength over untreated samples. The flexibility of CG, which is not changed by kaolin coating, and its large size (3.2 inch × 4 yd roll) are additional advantages that allow packing any type of wound with this material and covering the entire damaged tissues. The ease of application and familiarity with gauze material make this dressing a superior product to use in the field for self-application or buddy aid.

The experimental hemorrhage model used for evaluation of dressing was discussed in detail in our previous report. In general, it represents a severe wound in the groin area with partial laceration of femoral artery and high pressure bleeding that cannot be stopped with regular gauze and would be difficult to compress by tourniquet application. To the extent that was possible, all the adjuvant factors, such as retraction and constriction of the injured vessel, hypotension, overlaying tissues, and prolonged compression that will aid spontaneous hemostasis, have been minimized or eliminated to test the true efficacy of each hemostatic product. Although the model provides a reproducible bleeding condition appropriate for laboratory testing, it is limited in that it may not represent a wound that trauma patients or military casualties suffer in the field. It should be recognized that there will be no single animal model that can replicate all types of wounds with different types of bleeding (arterial, venous, or both). Our focus in developing the model was to subject each hemostatic material to the most challenging and difficult bleeding condition which still can be treated with an effective modality. If the material proved to be successful under such condition, albeit may be artificial, it will most likely be effective against a majority of external bleedings.

The successful results with CG dressing were reported to a joint military committee (Tactical Combat Casualty Care Committee) responsible for developing new guidelines for treating wounded soldiers. Based on these results and similar findings by our colleagues at Naval Medical Research Center, the committee has recommended replacing HC bandage with the new dressing. The new Tactical Combat Casualty Care Committee guideline recommends using CG as the first line of treatment for life-threatening hemorrhage on external wounds that is not amendable to tourniquet placement.

In summary, CG was found to be the most effective product among four new dressings tested in this arterial hemorrhage model. This dressing allowed the least amount of hemorrhage (37.4 mL/kg) and resulted in the highest survival rate (80%) in tested animals. The chitosan-based dressings, TS, and CXb were significantly less effective. The efficacy of the CG is attributed to its raw material (special gauze) and the potent clotting agent (kaolin) that coats the dressing. The efficacy of this gauze will depend on normal coagulation function of the patient’s blood and therefore may be less effective in coagulopathic patients. The large size of CG and its flexibility offer additional advantages for packing complex wounds and covering multiple bleeding sites. It had no apparent side effect and did not initiate intravascular clotting during the course of this experiment. Histologic changes caused with this dressing are similar to those produced with regular gauze. This dressing is now recommended as the first line of treatment for life-threatening hemorrhage on combat wounds. We anticipate this dressing to be useful not only for treating combat casualties but also for civilian trauma patients with severe hemorrhage who are brought to hospital swiftly. Although application of CG may not stop the bleeding in these patients immediately, it will certainly reduce their blood loss and possibly the need for blood transfusion afterward.

ACKNOWLEDGMENTS

We thank Ms. Irasema Terrazas and Ashley Cox for their technical assistance in this study. We also acknowledge the staff of our Veterinary Support Division for their support and technical expertise in conducting these experiments.

REFERENCES


**DISCUSSION**

**Dr. Mark Seamon** (Philadelphia, Pennsylvania): Distinguished moderators, members, and guests, I want to thank the authors for the early submission of their manuscript and congratulate them on a very nice presentation. The authors studied the performance of a variety of new hemostatic dressings, including Hemcon, Celox, TraumaStat, Combat Gauze, and Combat Gauze placebo after six millimeter femoral arteriotomies were made in their swine model.

After dressings were applied to thirty-eight pigs and data analyzed, Combat Gauze was found to be superior to the other measured dressings with respect to initial and eventual hemostasis rates, hemostatic time, and survival time. Furthermore, in vitro clotting analysis of the studied dressings revealed improved coagulation parameters in the Combat Gauze treated blood. Still, I have a few questions for the authors.

Please describe in more detail how the surgeons were blinded during these experiments. For example, how did you ensure that an equal amount of pressure was applied to each injured artery using each type of dressing?

Second, why was a goal MAP of sixty-five chosen, when Dr. Mattix has convincingly shown that pre-definitive surgical control resuscitation should be limited in our penetrating trauma victims?

Third, how do you explain some seemingly contradictory results? For example, how can there be a survival benefit in the Combat Gauze group without a difference detected in the post-treatment blood loss volume? I would have expected these two measured endpoints to be directly related.

Lastly, are your results applicable to civilian penetrating trauma? Initial hemostasis was achieved in three of ten pigs treated with Combat Gauze and eventual hemostasis was achieved in twelve to twenty-six minutes in five others. Furthermore, it took thirty to sixty minutes to detect a difference in either mean arterial pressure or survival time between Combat Gauze and the other measured dressings. How applicable is this to my city, where pre-hospital times average fifteen to twenty minutes for penetrating trauma victims?

I would like to congratulate the authors on yet another well-designed study that comes with a several-year history of related reports. Indeed, the authors contributions to this topic are significant, both for academic and practical reasons, as they continue to refine pre-hospital care for our troops injured on the battlefield. Lastly, I would like to thank the Eastern Association for the Surgery of Trauma for the privilege of discussing this important manuscript.

**Dr. Bijan S. Kheirabadi** (San Antonio, Texas): Thank you, Dr. Seamon, and thank you for the keen and insightful questions and I will try to answer them one by one. Regarding the randomization of the product, essentially the surgeon and assistant to the surgeon were kept blinded about what product was going to be tested on each animal, up to the point that the product was actually handed in. I was the one who placed all the products.

That randomization was done outside of the OR, ahead of the experiment, before even the study was started by an investigator not directly involved with the study. So I could not be biased in terms of the injury or isolation of the vessels and so forth. However, once the product was handed to me and I knew what it was the identity of product was released. In terms of how I make sure we apply equal pressure on each product from one animal to another, in the initial studies we actually used a Doppler to check whether we have blood flow on the distal part and the pressure was adjusted in such a way that the blood flow to the distal part of the injury was completely obstructed and vessel was occluded. There was no bleeding and no blood flow down stream.

After doing many of these experiments, we have now got enough experience that we exert almost equal pressure from one animal to another, essentially enough to stop the bleeding and occlude the blood vessels. Therefore, we’re not being biased in terms of how much pressure we put from one product to another.

Regarding a MAP of sixty-five mmHg, the sixty-five mmHg correspond to about ninety mmHg systolic pressure. On the battlefield, for the patients who are in shock, the recommendation is to give enough fluid to raise the blood pressure to such a degree that you can palpate radial pulse. Having a radial pulse corresponds to about ninety mmHg systolic pressure. The mean arterial pressure that we have selected corresponds to the pressure of the combat casualties that might be resuscitated on the field.

In addition, some casualties may have even higher than ninety mmHg systolic pressure with bleeding requiring hemostatic treatment. If we test the products below this pressure, we will have better success, but the point is that we want to make sure that the product works at a normal or slightly below normal systolic pressure. Testing at significantly below sixty five mmHg would not be appropriate for actual use later on in the soldiers that may have blood pressure substantially higher than sixty five mmHg.

Regarding the contradiction between the survival benefit and insignificant decrease of blood loss with Combat Gauze, there were ten animals in that group and in two
animals, Combat Gauze failed to produce hemostasis. In the other eight that the animals survived, the average blood loss was only twelve mL per kilogram. These data were significantly less than the blood losses seen with other products. Therefore, if you subtract the blood loss of two dead animals from the rest of the data (survival animals), you will find a strong correlation between the post-treatment blood loss and the survival rate. The reason it did not become significance was because of the death of two animals added significantly larger blood losses to the data base and increased the variability of the data set.

Finally, whether this will have any impact in civilian trauma, that’s true that we only obtained three immediate hemostasis and five hemostasis developed after twelve to twenty minutes after Combat Gauze applied. The point is that if we did not use the Combat Gauze in these situations to control bleeding, or even if you used regular gauze, the animals would have lost significantly more blood and most likely would have died. For that matter, using a Combat Gauze, which may cost only twenty dollars or so for the patient, may save him from losing significant amounts of blood and maybe save him from getting an additional transfusion when he gets to the hospital and maintain his blood pressure at a higher level. The fact that Combat Gauze did not produce hemostasis doesn’t mean that the animals were bleeding profusely. There were oozing and so there was no complete hemostasis, but in all of them, the bleeding was significantly reduced.

**Dr. Lawrence Lottenberg** (Gainesville, Florida): I’m somewhat troubled by the fact that about a year or two years ago, the Army issued a statement that Hemcon was the product to be used and now, after what seems like a single swine study another product is being endorsed.

The problem that I see in my community is with the police and fire rescue groups. They pick up this information and they run with it. I would really appreciate it if there would be some cautioning about the use of this. What are the human data in the use of Combat Gauze from the war zone that would complement the animal study?

It’s very concerning, because the example that I can give you is we had a guy who had a stab wound to the epigastrium and it turned out to be a cardiac injury that was bleeding out and the guy came in with about a pound of Chitosan powder on his abdomen and chest. All of this is very concerning with such a preliminary study.

**Dr. Bijan S. Kheirabadi** (San Antonio, Texas): First of all, the reason of changing the tactical combat casualty from Chitosan to this new product, it wasn’t just based on this study. There was a concurrent study done by Navy scientist that reached to a similar conclusion. Again, that also was an animal study.

As you know, all of these products are recognized as device. They get FDA approval without going through large clinical trials and the fact that Chitosan dressing brought on and was recommended five years ago was that, at the time, that was no better product that we could find and chitosan dressing was found to be significantly better than gauze.

What we have seen in the past five years are significant improvement of the old product and new products that have come along. Do we have data that supports how these work in soldiers? In the few cases that they have been tested, the results were very positive. Combat Gauze is currently being sent to the field and so we really don’t have much data to comment on.

Based on the experimental data that I presented and the concurrent study with the Navy and other groups that are currently going on, everybody seems to be very happy with Combat Gauze, because it’s easy to use and it seems to be safe and more effective than what we have had in the past.

**Dr. Andrew Kerwin** (Jacksonville, Florida): Do you have any data about using it in a liver injury, packing it and leaving it?

**Dr. Bijan S. Kheirabadi** (San Antonio, Texas): This product, as well as all the other hemostatic products, are FDA approved as a device for external wound treatment. They don’t have indications for putting internally. If someone is using it for wrapping a liver, it’s off-label use and so therefore, this is not the indication that they got from the FDA.

**Dr. Andrew Kerwin** (Jacksonville, Florida): Do you have any clinical data then about using it in the abdomen like that, packing a liver?

**Dr. Bijan S. Kheirabadi** (San Antonio, Texas): No, we have not. We have no clinical or experimental data at this point.

**Dr. Patrick Reilly** (Philadelphia, Pennsylvania): One final question. Dr. Kheirabadi, again, just from a disclosure standpoint, any industry support in doing these studies?

**Dr. Bijan S. Kheirabadi** (San Antonio, Texas): Thanks for reminding me of this. No, there has been no funding from industry. The only thing we have asked from the industries is to donate their materials. Some materials were purchased, others were not commercially available and were donated by the companies, and in that way they participated in the studies. But in terms of funding, this study was solely funded by the U.S. Army Medical Research and Material Command. I would like to express my gratitude to the Eastern Association for the Surgery of Trauma for the privilege of presenting this manuscript.