North Pacific Surgical Association

Chitosan based advanced hemostatic dressing is associated with decreased blood loss in a swine uncontrolled hemorrhage model


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KEYWORDS:
Hemostatic dressing;
Chitosan;
Kaolin;
Hemorrhage control;
Care under fire;
Combat casualty care

Abstract

BACKGROUND: The purpose of this study was to compare standard gauze (SG) and advanced hemostatic dressings in use by military personnel in a no-hold model.

METHODS: A randomized, controlled trial was conducted using 36 swine. Animals underwent femoral arteriotomy, followed by 60 seconds of uncontrolled hemorrhage. After hemorrhage, packing with 1 of 3 dressings—SG, Combat Gauze (CG), or Celox Rapid gauze (XG)—and a 500-mL bolus of Hextend were initiated. Pressure was not held after packing, and animals were followed for 120 minutes. Physiologic parameters were monitored continuously, and electrolyte and hematologic laboratory assessments were performed before injury and 30 and 120 minutes after injury. Dressing failure was determined if bleeding occurred outside the wound.

RESULTS: All animals survived to study end. Baseline characteristics were similar between groups. No statistical difference was seen in initial blood loss or dressing success rate (SG, 10 of 12; CG, 10 of 12; and XG, 12 of 12). Secondary blood loss was significantly less with XG (median, 12.8 mL; interquartile range, 8.8 to 39.7 mL) compared with SG (median, 44.7 mL; interquartile range, 17.8 to 85.3 mL; P < .02) and CG (median, 31.9 mL; interquartile range, 18.6 to 69.1 mL; P = .05). Packing time was significantly shorter with XG (mean, 37.1 ± 6.2 seconds) compared with SG (mean, 45.2 ± 6.0 seconds; P < .01) and CG (mean, 43.5 ± 5.6 seconds; P = .01).

CONCLUSIONS: XG demonstrated shorter application time and decreased secondary blood loss in comparison with both SG and CG. These differences may be of potential benefit in a care-under-fire scenario.

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Despite improvements in prehospital care, the creation of organized trauma systems, and new techniques in resuscitation, hemorrhage continues to be the leading cause of preventable death in trauma patients.1-5 Although truncal hemorrhage is the leading cause of hemorrhagic death, compressible extremity bleeding continues to contribute significantly to trauma mortality.1,5 Until recently, manual compression with or without gauze was the primary method of hemorrhage control in the prehospital setting. In recent
years, advances in biotechnology have allowed the development of advanced hemostatic dressings. Numerous studies have attempted to compare the effectiveness of these dressings, with varying results.\(^1,3,6,7\)

Prior granular hemostatic agents had serious complications, including local tissue destruction and distant embolization.\(^1,4\) Gauze-based dressings avoid these problems, but manufacturers recommend applying manual compression of the dressing on the wound for 2 to 5 minutes.\(^1\) This can be problematic in certain scenarios.

Military combat is one of the most common settings in which these dressings are used. Providing aid in a combat setting poses unique challenges. While caring for the injured, medics are instructed to return fire if able and to extricate the wounded to an area of cover. Current tactical combat casualty care guidelines recommended only tour-ningue placement for initial treatment of extremity hemorrhage in the care-under-fire setting.\(^1\)

Prior research in our lab has compared available advanced hemostatic dressings.\(^1,7,8\) Most recently, we examined hemostatic dressings used by both US and UK military personnel and compared them with standard gauze (SG). In that study, we demonstrated that neither of the advanced dressings tested were superior to SG in relation to survival, dressing failure, or postapplication blood loss in the groin arterial hemorrhage no-hold model.\(^7\)

Since the completion of that study, new generations of these dressings have been developed. Combat Gauze (CG; Z-Medica, Wallingford, CT), a kaolin-impregnated gauze dressing used by US forces, is now available in a Z-pack formation, which is intended to improve ease of application and reduce application time. Celox Rapid gauze (XG; Medtrade, Crewe, UK), used by UK forces, is a chitosan granule–impregnated z-fold gauze strip, which is also intended to shorten application time compared with prior products.

Again, the aim of the present study was to compare these products with SG in a compressible arterial hemorrhage model invoking a care-under-fire scenario by applying no additional manual compression after dressing application.

**Methods**

This protocol was approved by the Institutional Animal Care and Use Committee at Oregon Health & Science University. This facility follows the guidelines set forth in the National Institutes of Health Guide for the Care and Use of Laboratory Animals (1996).

**Swine hemorrhage model**

This was a randomized controlled study using 36 Yorkshire-crossbred female swine randomized to 1 of 3 groups: SG, CG, or XG. Swine were anesthetized, intubated, and mechanically ventilated. Left cervical cut-down was performed. Left carotid artery catheterization for continuous blood pressure monitoring and external jugular vein cannulation for fluid resuscitation were carried out. Right femoral artery exposure through a standardized 8-cm incision was performed. A small celiotomy was made for the purpose of placing a suprapubic catheter for urine output monitoring.

After suprapubic catheterization, baseline laboratory assessments were performed. These included spun hematocrit (i-STAT System; Abbott Point of Care, Princeton, NJ) for point-of-care electrolyte and acid-base laboratory measurements and thromboelastography (TEG5000 Hemostasis Analyzer System; Hemoscope, Niles, IL) for in vivo coagulation status evaluation.

The right femoral artery was then bathed in 1 mL of 2% lidocaine solution for 60 seconds. Baseline heart rate and mean arterial pressure were recorded. Using a 6-mm punch biopsy instrument, a sidewall femoral arterial injury was created. Free hemorrhage was allowed for 60 seconds, with suctioning of the wound base into a canister on a continuous weighing scale to calculate initial blood loss.

Upon completion of the 1-minute hemorrhage period, dressing application was performed according to prior randomization with preweighed dressing product. The same investigator (N.R.K.) performed every femoral arterial injury and dressing application, to minimize variability. Dressings were removed from their original packaging and placed into an unmarked box so that the investigator was blinded as to which dressing would be applied until the moment of application. Dressings were applied directly to the arteriotomy site, and packing was determined to be completed upon filling of the wound cavity or use of the entire dressing, whichever occurred first. Simultaneously with packing, a 500-mL bolus of Hextend (Hospira, Lake Forest, IL) was initiated and administered over 12 minutes.

Vital signs were recorded continuously and laboratory assessments repeated at 30 and 120 minutes after injury. Dressing failure was defined by hemorrhage spilling beyond the confines of the wound and dressing. After data collection at 120 minutes, dressings were removed from the wound and weighed. The preapplication dressing weight was subtracted from this value to calculate secondary blood loss. Femoral arteries were inspected for evidence of recurrent hemorrhage after dressing removal. Subject animals were then euthanized.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Pretreatment characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variable</td>
<td>XG</td>
</tr>
<tr>
<td>Animal weight (kg)*</td>
<td>31.5 ± 1.7</td>
</tr>
<tr>
<td>Temperature (°C)*</td>
<td>38.3 ± .5</td>
</tr>
<tr>
<td>Preinjury mean arterial pressure (mm Hg)*</td>
<td>78 ± 11</td>
</tr>
<tr>
<td>Baseline hematocrit (%)*</td>
<td>29.5 ± 1.7</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SD. CG = Combat Gauze; SG = standard gauze; XG = Celox Rapid gauze. *Nonsignificant.
Statistical analysis

Chi-square or Fischer’s exact tests were used to analyze categorical variables. Student’s t tests were used to compare the means of continuous variables using a post hoc analysis of variance. Data are presented as mean ± SD. However, nonparametric analysis (Mann-Whitney U test) was performed on data not normally distributed and is presented as median (interquartile range). \( P \) values ≤ .05 determined statistical significance. Analysis was performed using SPSS version 19.0 (SPSS, Inc., Chicago, IL).

Results

Twelve swine were randomized to each study group. Baseline characteristics including weight, temperature, mean arterial pressure, and hematocrit were similar between groups (Table 1). Initial postinjury blood loss was also similar between groups, but posttreatment blood loss was significantly less when using XG compared with either CG or SG (Table 2).

All animals survived to study end. There were 2 dressing failures in both the CG and SG groups, while there were no dressing failures in the XG group, but this did not achieve statistical significance. No difference was seen in the incidence of rebleeding upon dressing removal. Dressing application time was significantly shorter in the XG group compared with both CG and SG (Table 3). Hematologic laboratory measurements at the end of the study were similar between groups (Table 4).

When looking at coagulation status (Table 5), XG had a significantly longer time to initial clot formation (R time) compared with SG at study end. None of the dressings demonstrated significant changes in R time between time points. No difference in the rate of fibrin cross-linking (\( \alpha \) angle) was seen between dressings at any time point. However, significant decreases in \( \alpha \) angle at both 30 and 120 minutes compared with baseline were seen in the XG group. There was no difference in clot strength (maximum amplitude) between groups at any time point. All groups did show significant decreases in maximum amplitude at 30 and 120 minutes compared with baseline. Although both XG and CG had lower percentage clot lysis at 30 minutes compared with SG, only CG achieved statistical significance. Both XG and SG demonstrated significant increase in percentage clot lysis at 30 minutes compared with baseline.

Comments

With the advent of biotechnology came the ability to develop hemostatic agents that either concentrated or directly acted on patients’ red blood cells, platelets, and clotting factors. Initially, many of these agents were developed in granular form. Shortcomings of these formulations included residual granules left within the wound after washout that could act as an inflammatory or infectious nidus, or if placed in a wound with high pressure bleeding, they could be displaced away from the desired site of action.9–11

The natural evolution of advanced hemostatic dressings was clearly to combine the qualities of traditional gauze packing that could be held in place and coat or impregnate the gauze material with the bioactive agents. When used according to manufacturers’ recommendations, numerous studies have demonstrated benefit of these advanced dressings in comparison with SG.1,12 Unfortunately, in certain care-under-fire scenarios, manual compression for 2 to 5 minutes is both unrealistic and would put medics at undue risk of becoming casualties themselves. To be of utility in these combat settings, an advanced hemostatic dressing should be able to demonstrate benefit in comparison with SG without additional manual compression.

In our lab’s prior study comparing the past generations of both CG and XG with SG, no benefit was seen. In fact, the SG dressing application time was significantly shorter than both XG and CG.1 The newest generations that are currently used by US and UK forces have been redesigned as z-fold products instead of gauze rolls. The intent was to improve ease of application. If this could be achieved or additional clinical outcome measures could be significantly improved, these advanced dressings may offer utility in more care-under-fire scenarios.

### Table 2 Blood loss

<table>
<thead>
<tr>
<th>Time point</th>
<th>XG (n = 12)</th>
<th>CG (n = 12)</th>
<th>SG (n = 12)</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>XG vs CG</td>
</tr>
<tr>
<td>Pretreatment</td>
<td>202 (143–289)</td>
<td>166 (134–291)</td>
<td>193 (145–236)</td>
<td>.44</td>
</tr>
<tr>
<td>Posttreatment</td>
<td>13 (9–40)</td>
<td>32 (19–69)</td>
<td>45 (18–85)</td>
<td>.05</td>
</tr>
</tbody>
</table>

Data are expressed as median (interquartile range). CG = Combat Gauze; SG = standard gauze; XG = Celox Rapid Gauze.

### Table 3 Outcomes

<table>
<thead>
<tr>
<th>Outcome</th>
<th>XG (n = 12)</th>
<th>CG (n = 12)</th>
<th>SG (n = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Survival*</td>
<td>12</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Dressing failure*</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Packing time (s)</td>
<td>37.1 ± 6.2</td>
<td>43.5 ± 5.6</td>
<td>45.2 ± 6.0</td>
</tr>
<tr>
<td>Rebleed*</td>
<td>8</td>
<td>9</td>
<td>7</td>
</tr>
</tbody>
</table>

Data are expressed as numbers or as mean ± SD. CG = Combat Gauze; SG = standard gauze; XG = Celox Rapid Gauze.

*Non-significant.

†XG packing time was significantly shorter than CG or SG packing time (\( P \) ≤ .01).
To better test the 3 dressing materials studied as well as create conditions more akin to those in a care-under-fire scenario, the model used in this study varied significantly from the prior model used. While again attempting to challenge the dressings during the postapplication time period, instead of using a continuous infusion of fluid to achieve a specific mean arterial pressure, we used a standard volume fluid bolus. This eliminated the variable volume of fluid administered as seen in the previous study. Current US military policy dictates that fluid boluses should be administered in the field only if there is a change in mental status or loss of radial pulse.13,14 Our model of uncontrolled hemorrhage would likely result in one or both of these findings if encountered in the field. The other significant difference in the present study model was the sole use of study dressings regardless of successful filling of the wound cavity. This eliminated the variable of a laparotomy sponge being used on top of the dressings when they did not fill the wound cavity, as performed in the prior study. We feel that this better tested the current study dressings without the addition of a material with its own absorptive qualities.

As noted in the results, all animals survived to study end, and no difference was seen in the incidence of rebleeding upon removal of the dressings. This may be indicative of dressing equivalence in terms of clinical outcomes, or it may be a reflection of the model’s not being severe enough. Although it did not achieve statistical significance, there were no dressing failures in the XG group, whereas there was a 17% failure rate in both the CG and SG groups. This could be due to a type 2 error with insufficient group size. XG did demonstrate a significantly shorter application time compared with both CG and SG. Not only could this prove clinically useful to injured combatants, with shorter time to hemostatic therapy, it could also help reduce the amount of exposure time for the medics providing care, allowing them to continue engaging enemies as well as tending to other injured soldiers.

Another potential clinically important finding was the significantly reduced posttreatment blood loss seen in the XG group in comparison with both CG and SG. In our prior study, although this difference was not statistically significant, the XG group also had less posttreatment blood loss than both CG and SG. Significance may have been

### Table 4 Posttreatment hematologic measurements

<table>
<thead>
<tr>
<th>Variable</th>
<th>XG</th>
<th>CG</th>
<th>SG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematocrit (%)*</td>
<td>22.5 ± 1.7</td>
<td>21.5 ± 3.0</td>
<td>21.2 ± 1.7</td>
</tr>
<tr>
<td>pH*</td>
<td>7.57 ± .03</td>
<td>7.58 ± .03</td>
<td>7.57 ± .04</td>
</tr>
<tr>
<td>Lactate (mmol/L)*</td>
<td>1.32 (1.13–1.38)</td>
<td>1.23 (.99–1.77)</td>
<td>1.32 (1.07–1.92)</td>
</tr>
</tbody>
</table>

Data were collected at 120 minutes and are expressed as mean ± SD or as median (interquartile range).

CG = Combat Gauze; SG = standard gauze; XG = Celox Rapid gauze.

*Nonsignificant.

### Table 5 Thromboelastographic results

<table>
<thead>
<tr>
<th>Variable</th>
<th>XG</th>
<th>CG</th>
<th>SG</th>
</tr>
</thead>
<tbody>
<tr>
<td>R time (min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>11.8 (9.7 to 15.4)</td>
<td>13.8 (11.2 to 16.0)</td>
<td>11.9 (8.9 to 14.0)</td>
</tr>
<tr>
<td>30 min</td>
<td>10.7 (8.3 to 14.5)</td>
<td>14.5 (9.2 to 15.7)</td>
<td>9.1 (8.7 to 11.9)</td>
</tr>
<tr>
<td>120 min</td>
<td>11.5 (10.0 to 18.3)</td>
<td>11.5 (10.0 to 14.6)</td>
<td>9.8 (8.0 to 13.3)</td>
</tr>
<tr>
<td>R angle (°)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>60.1 (45.7 to 65.3)</td>
<td>44.1 (33.7 to 57.9)</td>
<td>58.1 (50.7 to 65.0)</td>
</tr>
<tr>
<td>30 min</td>
<td>36.9 (29.4 to 62.1)*</td>
<td>41.1 (33.1 to 53.8)</td>
<td>57.2 (36.1 to 66.8)</td>
</tr>
<tr>
<td>120 min</td>
<td>38.4 (29.1 to 52.2)*</td>
<td>48.9 (34.9 to 61.4)</td>
<td>46.6 (38.9 to 60.4)</td>
</tr>
<tr>
<td>Maximum amplitude (mm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>78.9 (76.2 to 80.7)</td>
<td>77.5 (74.4 to 80.4)</td>
<td>77.8 (75.1 to 80.4)</td>
</tr>
<tr>
<td>30 min</td>
<td>66.9 (63.1 to 70.0)*</td>
<td>67.1 (62.8 to 70.8)*</td>
<td>68.0 (65.6 to 71.9)*</td>
</tr>
<tr>
<td>120 min</td>
<td>70.3 (67.6 to 72.5)*</td>
<td>68.9 (64.5 to 75.9)*</td>
<td>69.9 (65.9 to 71.6)*</td>
</tr>
<tr>
<td>Percentage clot lysis at 30 min (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>-.1 (-.1 to 1.3)</td>
<td>0 (-.1 to 1.2)</td>
<td>.4 (-.1 to 2.2)</td>
</tr>
<tr>
<td>30 min</td>
<td>.4 (.0 to 2.6)*</td>
<td>.3 (0 to 2.2)</td>
<td>2.4 (1.0 to 3.1)*</td>
</tr>
<tr>
<td>120 min</td>
<td>.6 (.0 to 1.8)</td>
<td>.4 (-.1 to 2.6)</td>
<td>1.3 (-.1 to 2.6)</td>
</tr>
</tbody>
</table>

Data are expressed as median (interquartile range).

CG = Combat Gauze; SG = standard gauze; XG = Celox Rapid gauze.

*Significant change from baseline (P ≤ .05).

†Significant change from 30 minutes (P ≤ .05).
reached in the present study because of the changes made in the study model or the modifications of the dressing by the manufacturer. Regardless, the main purpose of advanced hemostatic dressings is to control or stop ongoing hemorrhage upon application. Decreasing blood loss may have profound effects. It may reduce the need for additional resuscitative fluids, in turn reducing the risk for dilutional coagulopathy as well as worsened hypothermia. It may also minimize the need for blood transfusion and the inherent associated risks of adverse effects. Reducing transfusion conserves blood products for other patients.

In contrast to our prior study, time to initial clot formation was not significantly shorter in any group compared with baseline, although all groups did trend toward shorter R times at study end. This may be secondary to the differences in study models used, such as longer hemorrhage time or use of Hextend instead of lactated Ringer’s solution. All groups in the present study also developed significant decreases in clot strength compared with baseline. This again is most likely secondary to differences in hemorrhage model and not directly related to the dressing materials. The most notable coagulation difference seen was the significant decrease in rate of fibrin cross-linking compared with baseline in the XG group, which was not seen with CG or SG. Given the known mechanism of action of chitosan, an explanation for this change is not clearly evident. In this study, it did not have a negative impact on the ability of XG to control hemorrhage in this focal injury model. Whether it would have an effect in a polytrauma setting would require further investigation.

Although we feel that our study model provided a well-controlled comparison of the study dressings examined, injuries encountered in a clinical scenario are rarely this focal. As we noted in our past study, it would be extremely difficult to create a standardized complex injury model to replicate the injuries seen in combat or even in the civilian setting to compare advanced hemostatic dressings. Another potential limitation was the unblinded nature of the dressing application, which may have introduced a source of bias into the study. We also alluded to another limitation of our study, the possible underpowering to detect a difference in dressing failure.

**Conclusions**

Although a perfect advanced hemostatic dressing has yet to be developed, this study does indicate that the current chitosan-based dressing in use by UK forces has the potential to significantly reduce blood loss without additional manual compression after application. It can be applied rapidly, and unlike tourniquet application, it does not restrict distal perfusion to the remainder of the extremity. This could also prove invaluable for proximal injuries not amenable to tourniquet application. It could permit quick application in a care-under-fire scenario and help reduce morbidity and mortality of the injured while minimizing exposure time of those caring for the wounded.

Overall, our study findings warrant ongoing consideration of including specific advanced hemostatic dressings in the tactical combat casualty care policy.

**References**


**Discussion**

Paul Lin, M.D. (Spokane, WA): One of the few good things to come out of war is advances in trauma care. We are fortunate in the North Pacific Surgical Association to have members who have experienced first-hand trauma care in the austere setting. I would like to thank Dr Schreiber...
for his service to our country and once again for sharing his research with us at this meeting.

The question that Kunio and colleagues is trying to answer is whether what we pack a traumatic wound with makes any difference in controlling hemorrhage. The answer is – maybe. Their results suggest that chitosan based gauze is better than kaolin based gauze and standard gauze for hemorrhage control if the opportunity to hold pressure to the wound is not there. In previous publications, Dr Schreiber was not able to demonstrate a difference in hemostasis among different types of gauze if pressure was held. Under austere conditions and under enemy fire, however, the opportunity to apply pressure may be limited. I do have some questions regarding the conduct of the experiment.

Since the investigator doing the actual packing of the wound was not blinded to which gauze was being used at the time of packing the wound, which I believe is the crucial moment in the experiment, there is the opportunity for bias. I understand both the chitosan and kaolin gauze were packaged in a Z-pack arrangement while the standard gauze I’m assuming was a roll-type, which would already put it at a disadvantage since you intimate that packaging of gauze does make a difference. Does the packaging itself explain the statistically different time to pack the wound? It seems that 30 to 50 seconds is a long time to pack an 8-cm wound with gauze. Does this time include trying to open the package? Maybe we need better packaging.

Please explain your posttreatment blood loss. I understand the pretreatment blood loss as the 60 seconds of free hemorrhage after the arteriotomy was made. At what point does the posttreatment blood loss start? Does it start at the initiation of packing or at the conclusion of the packing? How do you control for the difference in packing time either way. You will either have more time to hemorrhage or more blood in the gauze at the start of the posttreatment period. Could this explain the difference in the posttreatment blood loss?

Does hemostatic gauze work only if in direct contact with the site of bleeding? Was gauze applied directly to the arteriotomy? In most instances of traumatic wound packing, the major source of bleeding is much deeper than what is accessible to the packing.

And lastly, how does anything impregnated on gauze change the thromboelastogram, for better or for worse? Thank you for the privilege of discussing your work.