Comparison of Two Packable Hemostatic Gauze Dressings in a Porcine Hemorrhage Model

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Abstract

Background. Uncontrolled hemorrhage remains the primary cause of preventable battlefield mortality and a significant cause of domestic civilian mortality. Rapid hemorrhage control is crucial for survival. ChitoGauze and Combat Gauze are commercially available products marketed for rapid hemorrhage control. These products were selected because they are packable gauze that work via differing mechanisms of action (tissue adhesion versus procoagulant). **Objective.** To compare the effectiveness of ChitoGauze and Combat Gauze in controlling arterial hemorrhage in a swine model. **Methods.** Fourteen swine were studied. Following inguinal dissection and after achieving minimum hemodynamic parameters (mean arterial pressure [MAP] ≥70 mmHg), a femoral arterial injury was created using a 6-mm vascular punch. Free bleeding was allowed for 45 seconds, and then the wound was packed alternatively with ChitoGauze or Combat Gauze. Direct pressure was applied to the wound for 2 minutes, followed by a three-hour monitoring period. Resuscitation fluids were administered to maintain an MAP of ≥65 mmHg. Time to hemostasis, hemodynamic parameters, total blood loss, and amount of resuscitation fluid were recorded every 15 minutes. Data were analyzed using the Wilcoxon rank sum test. Histologic sections of the vessels were examined using regular and polarized light. **Results.** No statistically significant differences were found between the groups regarding any measured end point. Data trends, however, favor ChitoGauze over Combat Gauze for time to hemostasis, fluid requirements, and blood loss. There was no evidence of retained foreign material on histologic analysis. **Conclusion.** ChitoGauze and Combat Gauze appear to be equally efficacious in their hemostatic properties, as demonstrated in a porcine hemorrhage model. **Key words:** hemostatic agent; hemorrhage; gunshot wound; ChitoGauze; Combat Gauze; combat medicine

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Introduction

Despite numerous advances in combat casualty care, uncontrolled hemorrhage remains the number one cause of preventable battlefield mortality and a significant cause of domestic civilian mortality.[1–6] Rapid hemorrhage control is crucial for survival. Over the past decade, significant research in both civilian and military sectors has focused on the development of novel hemorrhage control agents and approaches to hemorrhage care. The military has adopted newly developed agents, including redesigned tourniquets, hemostatic agents, and wound dressings, with unprecedented speed. The military and other trauma care experts have even begun to question the "dogma" of applying the "ABC" principles to the management of patients with severe external hemorrhage. During a tactical situation or one in which a patient has massive external hemorrhage, some military educators are now advocating the performance of C (control bleeding) prior to A (airway) or B (breathing). Obviously, these new agents and principles for hemorrhage control are also applicable to the civilian setting.[7–9]

Several different hemostatic agents have been studied and are commercially available. Some are not Food and Drug Administration (FDA)-cleared, appear to be ineffective in severe hemorrhage, or have not been thoroughly studied in human trials. Initially, the U.S. military used three hemostatic agents: QuikClot (Z-Medica Corp., Wallingford, CT), HemCon Bandage (HemCon, Inc., Tigard, OR), and ChitoFlex (HemCon, Inc.). These hemostatic agents appeared effective in external hemorrhage control, but certain limitations were found with human use.[10–13] These limitations include difficulty placing them at the exact site of a hemorrhage and reactions that can potentially cause tissue damage.

QuikClot is an FDA-cleared hemostatic agent consisting of a granular zeolite powder with 1% residual moisture. When placed on a bleeding wound, it adsorbs water in an exothermic reaction, thereby concentrating platelets, erythrocytes, and clotting factors

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at the site of application. Because of its granular nature, it can potentially cover a large surface area. The granules can be difficult to place at the exact site of hemorrhage, however, and can be "blown out" under high-pressure arterial bleeding.[10,11,14–16] Another concern has been the exothermic reaction and its potential to cause tissue damage. According to some case reports, the majority of the tissue damage was superficial burns and did not result in permanent damage.[11,12] Additionally, the heat generated by the exothermic reaction causes mild to severe pain after application. These findings led to quality control (QC) modifications designed to decrease the exothermic reaction and repackaging to prevent the granules from sticking to wounds.

The HemCon bandage is a chitosan-based, FDA-approved hemostatic agent utilized in the combat arena and, to a limited degree, in the civilian prehospital environment.[13,17] Chitosan is a biodegradable, nontoxic, deacetylated form of chitin (poly β (1 to 4)-*N*-acetyl D-glucosamine), a naturally occurring substance. The term *chitosan* is generally applied when the extent of deacetylation is greater than 70% and the term *chitin* is used when the extent of deacetylation is insignificant, or less than 20%. In the form of an acid salt, chitosan demonstrates mucoadhesive activity.[18,19] Chitosan has also been shown to promote wound healing and has antimicrobial properties.[20–23] The HemCon bandage has been shown to immobilize wound edges and reduce inflammatory cell infiltrate.[20] The original HemCon wafer had practical limitations largely due to wafer rigidity and the resulting inability of the wafer to fully engage the site of hemorrhage.[13] HemCon was later modified to a more flexible bandage, ChitoFlex, which has been used with some success on the battlefield.[24]

Because of the issues identified with using QuikClot, HemCon Bandage, and ChitoFlex, the current hemostatic agent of choice in combat as recommended by the Defense Health Board Committee on Tactical Combat Casualty Care (CoTCCC) is Combat Gauze (Z-Medica Corp.). Combat Gauze is a 50% rayon and 50% polyester blended gauze impregnated with mineral kaolin powder.[25,26] ChitoGauze (HemCon, Inc.) is another gauze product that was recently cleared by the FDA for external hemorrhage control and is also thought be easier to place and less likely to cause tissue damage. ChitoGauze is a Z-folded chitosan-impregnated gauze. In addition to its hemostatic properties, chitosan also has antibacterial properties that may represent a theoretical advantage over other hemostatic agents.[10] Our study compared the efficacy of Combat Gauze with that of the more recently approved agent, ChitoGauze, in controlling arterial hemorrhage in a porcine model.

METHODS

All research procedures complied with federal laws governing the humane care and treatment of laboratory animals. The Medical College of Georgia's Institutional Animal Care and Use Committee (IACUC) approved the study protocol and all performed procedures. The IACUC uses the Institute of Laboratory Animal Resources National Research Council *Guide for the Care and Use of Laboratory Animals*. As this study has a military relevance, an observer from the Health Affairs Defense Medical Standardization Board (DMSB) was present for one day of the protocol (for seven of the 14 study animals).

Hemostatic agent testing was carried out on healthy female swine (14 total) with a weight range of 35–45 kg. The hemostatic agent being tested was alternated between ChitoGauze (odd-numbered animals) and Combat Gauze (even-numbered animals), with the initial animal randomly assigned to packing with ChitoGauze. A single investigator (RBS) performed wound packing on all animals. Intramuscular injections of glycopyrrolate (0.01 mg/kg) and Telazol (4–6 mg/kg) were given for their anticholinergic and sedation properties. Following the preanesthesia medications, the animals were placed on isoflurane 5% and were intubated. Ventilator settings were adjusted to maintain end-tidal partial pressure of carbon dioxide (pCO_2) between 38 and 42 mmHg, and anesthesia was maintained with 1% to 2% isoflurane added to 100% oxygen throughout the entire observation period. Immediately after intubation, a carotid arterial line was placed for blood pressure monitoring and an internal jugular venous line was placed for fluid resuscitation. Intravenous fluids were initiated using normal saline (NS) at 5 mL/kg/hour.

A 10-cm incision was made in the right groin area overlying the femoral artery. Approximately 5 cm of femoral artery was dissected free from surrounding tissues, and proximal and distal control was obtained using vessel loops. The exposed femoral artery was then completely bathed in 2% lidocaine to mitigate the vasospasm associated with dissection. The femoral artery was considered adequately dilated when it was measured to be 6 mm or more in diameter.

A stable mean arterial pressure (MAP) of 70 mmHg or higher was required prior to initiating the arterial injury procedure. If the MAP was less than 70 mmHg, Hextend was administered until either 500 mL of Hextend had been given or the MAP was at least 70 mmHg. The proximal and distal ends of the femoral artery were controlled using vessel loops and an anterior arteriotomy was performed. A 6-mm vascular punch was then inserted via the arteriotomy and used to create the vascular injury. A second surgeon verified

appropriate injury creation prior to releasing vascular control.

Free bleeding was allowed for 45 seconds following the release of vascular control. Blood loss from the wound site during the 45 seconds was suctioned and measured, with the data being recorded on a standardized data-collection sheet. The lowest MAP was also recorded. Following the 45-second bleed, the dressing was applied to the wound through a pool of blood, covered with a rolled Kerlix bandage, and compressed for 2 minutes using a 75-lb dumbbell weight placed over the wound site. Pressure was then gently released and the animals were observed. The hemostatic agent and Kerlix dressing were left undisturbed for a 180-minute observation period.

The MAP immediately following release of pressure, the time to hemostasis, and any additional blood loss were recorded. Hemostasis was defined as the absence of any residual pooling of blood or seepage of blood around the dressing. Resuscitation began 30 seconds into the product application period with 500 mL of Hextend at 100 mL/min. Following the infusion of Hextend, fluid resuscitation was continued with warmed NS (approximately 38°C) at 100 mL/min and NS infusion was continued until MAP reached 65 mmHg. When an MAP of 65 mmHg was reached, fluids were discontinued and pressure was monitored. If MAP was noted to decrease below 60 mmHg, additional NS was given until an MAP of 65 mmHg was achieved. A maximum of 12 L of NS infusion was permitted per animal.

During the 180-minute observation period time to hemostasis, hemodynamic parameters, total blood loss, and amount of resuscitation fluid were recorded every 15 minutes. Dressing application was considered a success if the swine survived for the 180-minute observation period. Dressing application was considered a failure if the swine died or had a pCO_2 <15 mmHg or an MAP <20 mmHg during the 180-minute observation period. Following euthanasia of the animal, femoral artery samples were obtained in seven animals (three ChitoGauze, four Combat Gauze) and were sent for histological analysis to the Medical College of Georgia's pathology laboratory.

Statistical Analyses

All statistical analyses were performed using SAS version 9.2 (SAS Institute Inc., Cary, NC). Statistical significance was assessed using an alpha level of 0.05. Descriptive statistics were determined for each outcome measure within gauze type. To examine differences in various outcome measures between ChitoGauze and Combat Gauze, Wilcoxon rank sum tests were performed because of the small sample size within each gauze type ($n = 7$) (Table 1).

RESULTS

There were no baseline differences between the two groups (Table 1). One animal in the Combat Gauze group failed to have an initial stable blood pressure of 70 mmHg (68 mmHg). This animal had immediate hemostasis and 35 mL of blood loss following hemostatic dressing placement. These data were included in the analysis, but removing them from the analysis would not have changed our results. Immediate hemostasis was achieved in four animals in the Combat Gauze group and five animals in the ChitoGauze group. The difference was not statistically significant ($p = 0.45$). There were no differences between groups in baseline MAP, volume of prehemorrhage fluids, or the lowest MAP observed following puncture and hemorrhage (Table 1). The mean amounts of blood loss and the mean amounts of NS required for MAP resuscitation were statistically similar in the two groups (Figs. 1–3). One hundred percent of the animals in both groups survived. One of the Combat Gauze–treated animals achieved late hemostasis and remained hypotensive for the entire 180-minute observation period despite maximal fluid resuscitation (12 L NS at 100 mL/min).

The histology samples of the vessels from both groups demonstrated organized clot and scattered

TABLE 1. Hemodynamic Parameters, Blood Loss, and Resuscitation

	ChitoGauze, $n = 7$			Combat Gauze, $n = 7$			
Outcome	Mean	SD	Median	Mean	SD	Median	Wilcoxon Rank Sum p-Value
Baseline MAP, mmHg	74.4	4.7	73	78.3	5.2	79	0.1589
Lowest MAP, mmHg	46.9	6.1	44	47.7	11.6	52	0.5640
MAP after 45-second free bleed, mmHg	56.4	11.6	51	68.3	18.8	76	0.1102
Blood loss during 45-second free bleed, mL	470.3	199.3	504	429.4	121.5	454	0.5653
Blood loss following 45-second free bleed, mL	304.3	675.8	31	796.4	1,223.1	94	0.2774
Blood loss—total, mL	774.6	713.7	642	1,225.4	1,280.0	486	0.6547
Time to hemostasis, min	13.1	28.0	0	32.4	47.2	0	0.4559
Total Hextend, mL	785.7	267.3	1,000	807.1	245.7	1,000	0.8273
Total NS, mL	1,478.6	1,778.8	650	3542.9	4519.3	1,100	0.8480

MAP = mean arterial pressure; NS = normal saline; SD = standard deviation.

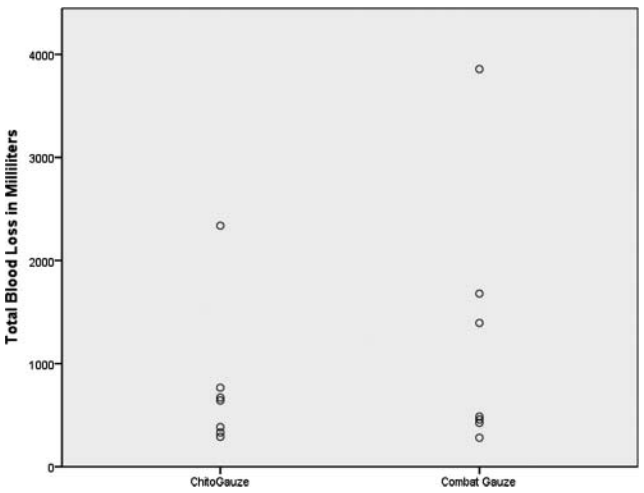

FIGURE 1. Total blood loss, in milliliters.

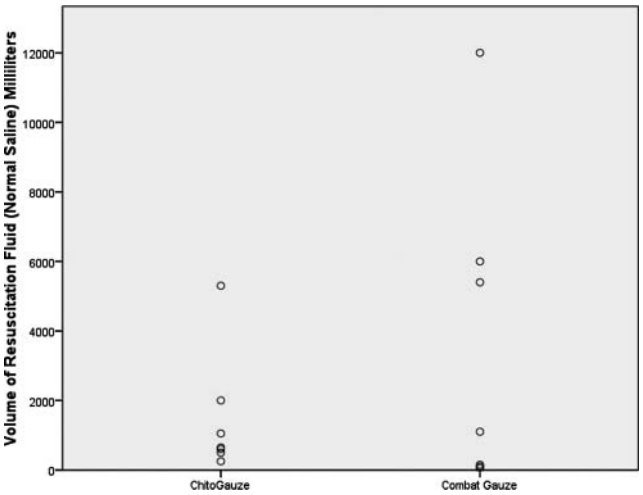

FIGURE 3. Volume of resuscitation fluid (normal saline), in milliliters.

sloughing of the vascular intima. The samples were viewed under normal and polarized light, and there was no evidence of foreign material (kaolin or chitosan) in the clot or blood vessel.

Table 1 provides descriptive statistics and the results of the Wilcoxon rank sum tests. There were no statistically significant differences between gauze types. Box plots (not shown) for each outcome measure show significant overlap in the outcome measures for the two gauze types.

DISCUSSION

One of the primary causes of death in both civilian and combat injuries is uncontrolled bleeding.[24] Thus, emergency care providers need methods to rapidly control hemorrhage in the hospital as well as in the prehospital setting. Effectively controlling blood loss will improve survival and decrease the toll of uncontrolled bleeding. Advances in hemostatic topical agents have increased their use both on the battlefield and in the emergency department. These compact, relatively inexpensive hemostatic agents can be lifesaving, and there is much interest in finding the ideal agent for use in a variety of situations. It is important to note that in all instances during the 2 minutes of compression, there was no apparent ongoing blood loss. This reinforces the importance of direct compression whenever possible.

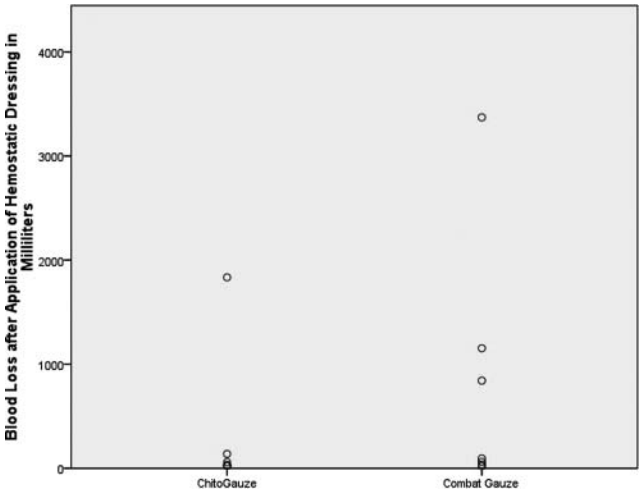

FIGURE 2. Blood loss after application of hemostatic dressing, in milliliters.

The swine model, in which a deadly femoral artery wound is caused by a 6-mm arterial punch, has been previously described. This model allows for rapid hemorrhage that is not readily arrested by factors such as arterial retraction and spasm.[27–29] Colloid volume expansion immediately after the 45 seconds of free bleeding further tests the efficacy of the hemostatic agent by raising the subject's MAP.[20] Although this model cannot adequately represent all potentially encountered wounds, it is believed that the porcine model is reasonable for evaluating topical agents.

This study failed to demonstrate a statistically significant difference between the ChitoGauze and Combat Gauze groups regarding time to hemostasis, resuscitative fluid requirements, blood loss, and survivability (Table 1). There were, however, favorable trends in all end points in the ChitoGauze group over the Combat Gauze group, including mean time to hemostasis (13 minutes vs. 32 minutes) and mean blood loss following hemostatic agent application (304 mL vs. 796 mL) (Table 1). However, the difference seen between the groups may not have achieved statistical significance because of our sample size. In addition, one of the Combat Gauze–treated animals remained hypotensive for the entire 180-minute observation period, despite resuscitation totaling 12 L of NS. This animal had

an MAP of 25 mmHg 180 minutes after completion of gauze packing.

In an effort to identify the qualities desired in newly developed hemostatic dressings, the U.S. Army Institute of Surgical Research (ISR) held a workshop in June 2009. The identified qualities of the "ideal dressing" include the following: ability to cover large wound areas, ability to stop bleeding from all wound configurations, ability to be applied rapidly, no additional pain on application, no acute or long-term adverse effects, no risk to medics, long shelf life, low cost, and clear superiority to existing fielded hemostatic agents. In our study, both Combat Gauze and ChitoGauze were 100% effective in preventing death in this lethal live animal model and they meet most of the qualities of an "ideal dressing."

Combat Gauze and ChitoGauze have differing mechanisms of action (Combat Gauze acts as a procoagulant and ChitoGauze acts by direct adherence to the tissue and the hemorrhaging vessel). Based on the differing mechanisms of action, there may be a theoretical advantage to the use of a protocol that utilizes both ChitoGauze (tissue adherence) and Combat Gauze (procoagulant). Further work in this area is indicated.

LIMITATIONS AND FUTURE RESEARCH

The primary limitation of the study was the small sample size (14 animals). When we started our study, there were no data available on which to base our sample size estimations, so we planned to use a minimum of 14 swine, but to stop after the first seven had been enrolled to conduct sample size calculations. When data for the first seven swine were analyzed, we found that there was a statistically significant difference between the groups, so we proceeded with the planned sample of 14. However, because of the wide variability within the groups, the result for the larger sample was not statistically significant. Because of the wide variability, our statistical power with a sample size of 14 swine was only 15%. A future analysis should be conducted using a larger sample size that is better powered for detecting no difference between the groups. A sample of 128 swine would be needed to have 80% power to show no difference between the groups given the means and standard deviations that were obtained.

Because of the large amount of variation between the animal subjects in each measured end point, larger numbers would have been required to unmask any statistically significant differences between the ChitoGauze and Combat Gauze groups. Larger studies in the future may be useful in further delineating subtle differences between hemostatic agents.

The research team was not blinded to the agent employed in any given animal. Blinding would be preferred, but there are practical limitations. The studied agents are quite dissimilar in appearance, thus limiting the ability to blind the investigators.

CONCLUSIONS

In this 6-mm arterial punch porcine hemorrhage model, ChitoGauze and Combat Gauze were found to be equally efficacious in controlling life-threatening hemorrhage. ChitoGauze had trends toward superiority over Combat Gauze in all measured outcomes; however, these trends failed to reach statistical significance. It is possible that these trends may become statistically significant with a larger sample size. Additionally, based on histologic analysis, neither product appears to have evidence of embolization of intravascular foreign material. Further research should be conducted to assess the utilization of both hemostatic dressings sequentially and concurrently as different mechanisms of action provide a theoretical advantage over the products used independently.

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