Comparison of Hemostatic Efficacy of ChitoGauze and Combat Gauze in a Lethal Femoral Arterial Injury in Swine Model

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ABSTRACT

Uncontrolled hemorrhage is the leading cause of death of soldiers in wartime. Quickly accessing and stabilizing the wound with effective hemostatic techniques is the key to saving lives on the battlefield. There exists a need for a hemostat that is efficacious in achieving hemostasis in severe traumatic combat wounds and easy to apply.

The ChitoGauze dressing is composed of polyester/rayon blend non-woven medical gauze that is coated with chitosan. The four inch by four yard (4" x 4 yds) dressing is z-folded and packaged in a peelable foil pouch and is terminally sterilized. The hemostatic properties of chitosan enhance the ability of the medical gauze to control bleeding. ChitoGauze also offers antibacterial properties against a wide range of gram positive and gram negative organisms, including methicillin resistant Staphylococcus aureus ATCC33591 (MRSA), Enterococcus faecalis ATCC51299 (VRE) and Acinetobacter baumannii ATCC15308.

In this study, we evaluated the hemostatic efficacy of two advanced hemostatic wound dressings: ChitoGauze™ (HemCon Medical Technologies Inc., Portland, OR) and QuikClot® Combat Gauze™ (Z-Medica Co., Wallingford, CT), in a swine femoral arterial injury model. Surgical information including body weight, pre-treatment blood loss, vessel size and MAP change were similar between the two treatment groups. Average post treatment blood loss over three hours or survival was less in the ChitoGauze group than the Combat Gauze group (430 mL vs. 1180 mL). In the ChitoGauze group, seven (87.5%) animals achieved hemostasis and survived with minimal blood loss or oozing. Only two (25%) animals achieved immediate hemostasis and five (63%, $p = 0.04$) survived in the three hours observation time in the Combat Gauze group. In the survived animals, five out of seven animals had complete hemostasis in first attempt using the ChitoGauze; two out of five animals achieved hemostasis in first attempt with the Combat Gauze. Average time to achieve complete hemostasis in the survived animals was three minutes with the ChitoGauze and 12 minutes using Combat Gauze.

Both ChitoGauze and Combat Gauze demonstrated hemostatic effectiveness in this lethal extremity hemorrhage model. Both dressings were easy to apply into the femoral wound geometries. While both bandages performed similarly in this small sample, we did note a trend toward more blood loss among the successful Combat Gauze applications as compared to ChitoGauze. ChitoGauze had greater success in achieving immediate hemorrhage control with less blood loss than Combat Gauze in this model.

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14. ABSTRACT
Uncontrolled hemorrhage is the leading cause of death of soldiers in wartime. Quickly accessing and stabilizing the wound with effective hemostatic techniques is the key to saving lives on the battlefield. There exists a need for a hemostat that is efficacious in achieving hemostasis in severe traumatic combat wounds and easy to apply. The ChitoGauze dressing is composed of polyester/rayon blend non-woven medical gauze that is coated with chitosan. The four inch by four yard (4 x 4 yds) dressing is z-folded and packaged in a peelable foil pouch and is terminally sterilized. The hemostatic properties of chitosan enhance the ability of the medical gauze to control bleeding. ChitoGauze also offers antibacterial properties against a wide range of gram positive and gram negative organisms, including methicillin resistant Staphylococcus aureus ATCC33591 (MRSA), Enterococcus faecalis ATCC51299 (VRE) and Acinetobacter baumannii ATCC15308.

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INTRODUCTION

Uncontrolled hemorrhage is the leading cause of fatality of soldiers in time of war [1]. Many wound dressings have been developed for use in emergency traumatic situations and potentially fatal haemorrhages. Two of the original haemostatic dressings were the HemCon 4in x 4in chitosan pad (HemCon Medical Technologies Inc., OR) and the QuikClot Zeolite mineral-based powders (Z-Medica Co. CT) [2].

Several novel topical hemostatic dressings have been developed many ustilising different delivery systems and different haemostatic agents. [3], [4], [5], [6], [7], [8] and [9]. Currently the two primary types being utilised are chitosan or zeolite mineral based. When zeolite comes into contact with blood, it rapidly adsorbs water from the blood and holds the water molecules in the pores by hydrogen bonds [6]. According to Z-Medica Corp. literature, this has the effect of locally concentrating the proteins and cellular elements to further catalyze clot formation. Additionally, the nano-engineered negative charge surface beads provide key surface chemistry, rapidly activating the coagulation process [10] and [11].

The polycationic nature of chitosan is such that the substance possesses natural antimicrobial properties [11], and the use of chitosan acetate also allows for the material having two highly desirable properties in a dressing in hemostasis and antibacterial activity. HemCon produced a freeze dried pad of chitosan acetate that was the hemostatic choice of the US military for a number of years and it demonstrated itself to be a safe and efficacious product [17]. Also importantly it moves away from the freeze dried pad product to fabric gauze that is already familiar to care providers. HemCon has now created and released an improved next generation hemorrhagic bleeding dressing for use as an emergency traumatic situation dressing and for potentially fatal haemorrhage applications called ChitoGauze.

HemCon ChitoGauze stops hemorrhagic bleeding by controlling the rate of blood flow through the dressing and allowing for significant erythrocyte and platelet interaction with the uniformly chitosan coated surface. ChitoGauze is optimized to maximize hemostatic performance. The robust uniformly applied chitosan coating on the gauze, allows for significant chitosan blood interaction in conjunction with optimized fluid handling performance. The chitosan coated surface of ChitoGauze helps to retard blood flow through the dressing thereby diminishing rapid bleeding. The chitosan coating on the gauze further reduces blood loss by helping to adhere the dressing to the wound site providing a physical barrier to prevent bleeding. Significant aggregation of erythrocytes and activation of platelets promotes localized clotting within and on the gauze to stop bleeding.

ChitoGauze provides effective hemostasis outside of the body's normal clotting cascade and has natural antibacterial properties. Unlike the previous HemCon freeze dried pad, ChitoGauze is highly flexible and suitable for easy application to superficial as well as deep and narrow wounds. It readily conforms to wound surfaces with complex geometries to allow efficient staunching of all bleeding. The ChitoGauze dressing is also designed to aid with rapid deployment to the wound by a z-folded configuration that speeds application time when hemostasis is critical.

The purpose of this study was to compare ChitoGauze and Combat Gauze (Figure 1), with a lethal femoral arterial injury in a swine model.

Figure 1: ChitoGauze™ (HemCon Medical Technologies Inc., Portland, OR) and QuikClot Combat Gauze™ (Z-Medica Co., Wallingford, CT).

METHODS

In vivo animal study:

Animal Preparation

All testing was carried out on healthy castrated Yorkshire crossbred male swine with an average weight of 37±3 kg according to previous description.4? The experiments were performed in accordance with the 1996 Nation Research Council, "Guide for the Care and Use of Laboratory Animals" and applicable Federal regulations. The animal protocol was approved by the Institutional Animal Care and Use Committee at the Legacy Clinical Research and Technology Center (LCRTC) of Legacy Health System.

Sixteen swine were cycled in this study. Animals were fasted starting the evening prior to the surgical procedure with water allowed *ad libitum*. The animals were premedicated at approximately 30 minutes prior to anesthesia induction with Glycopyrrolyte (0.01mg/kg) through intramuscular injection for blocking vagal stimulation and were then transported to the prep room and injected with Telazol at 4-6 mg/kg. Isoflurane was given up to 5% in 100% oxygen via face mask. The animal was intubated, and an ear catheter and a jugular line were placed for resuscitation. The animal then was connected to the respirator machine with 1-2% Isoflurane in 100% oxygen. Buprenorphine at a dose of 0.025 mg/kg was injected intramuscularly. The ventilation setting was adjusted in maintaining the end tidal PCO2 between 38-42 mmHg. Anesthesia was maintained with 1% to 2% isoflurane added to oxygen by the ventilator. Lactated Ringer's (LR) maintenance fluid was administered at 5ml/kg/hr through a venous line placed in an ear vein. The temperature of the swine was maintained at 37 ºC - 39 ºC (98.6 ºF – 102.2ºF).

After induction of general anesthesia, the swine was placed in the dorsal recumbent position. A splenectomy was performed via midline laparotomy to minimize any hematological changes that may occur from autotransfusion by contractile spleen. The removed spleen was weighed and warm LR solution

(37 ºC) was given three times the splenic weight to replace the approximate volume of blood contained in the spleen. A cystostomy was performed for the drainage of urine. The abdomen incisions were then closed with conventional suturing and stapling.

Surgical Procedure

The swine was secured to allow a flat exposure of the injured leg. An approximate 10-15 cm skin incision is made over the groin area and overlying adductor longus muscle is excised to exposure the femoral canal. Then, 5-cm of femoral artery is dissected free from surrounding tissues. The vessel is bathed in a 2% lidocaine solution for vessel dilation. After replacement fluid is administered for splenectomy, the animal is then preconditioned at mean arterial pressure (MAP) above 65 mmHg, PCO2 between 38-42 mmHg, body temperature at 37 ºC - 39 ºC, and femoral artery diameter larger than 6-mm for a 10-minute stabilization prior to create the femoral injury. If initial MAP was less than 65 mmHg, Hextend was administered intravenously to elevate the pressure. To create the injury, the proximal and distal ends of the femoral artery were clamped and an arteriotomy made on the anterior wall of the femoral artery using a 6.0 mm IBC vascular punch by a second surgeon.

The swine were equally divided into two groups to receiving either ChitoGauze or Combat Gauze for hemostasis. The primary surgeon (applicator) was blinded to the wound site and hemostatic agents. After vessel clamps are released, free bleeding is allowed for 45 seconds. Then the primary surgeon directly applies the dressing into the wound through a pool of blood and holds compression for 2 minutes. The blood loss during the 45-seconds bleeding and excess blood during the application were collected with suction. Vital signs including MAP and pCO2 are monitored at 15 minutes interval. The study allowed one time re-application. If the first application was failure (immediate bleeding) within three minutes, the second surgeon would removed the first dressing and clean out remaining clot and the primary surgeon applies a second dressing into the wound according to the first application (into a pool of blood). If there was no immediate bleeding in first application or after second application, the animal was followed-up for 3-hours observation. Resuscitation begins 30 seconds after dressing application with 500mL of Hextend fluid at 100 mL/min. Following the infusion of Hextend, fluid resuscitation is continued if necessary with pre-warmed LR infused at 100mL/min, to raise the MAP to 65 mmHg. When 65 mmHg is reached, discontinue fluids until pressure drops below 60 mmHg. A maximum of 12L of LR infusion was allowed.

The primary measured outcomes were the immediate hemostasis, total blood loss in three hours, survival rate after dressing rescue. Secondary endpoints were average number of applications, time to hemostasis, change of mean arterial pressure, volume of the 45-seconds pre- blood loss, and size of injured femoral artery.

Statistical Analysis

Categoric variables were analyzed with a chi-squared test unless the value in any cell was less than 5 and then a Fisher exact test was used. A Student t test was used to compare the means of the 2 groups. Any data that did not follow a normal distribution were analyzed with a nonparametric analysis (Mann–Whitney U test). Statistical significance was defined as a P value of less than 0.05.

RESULTS

Sixteen animals were divided into two groups receiving either Combat Gauze or ChitoGauze treatment for hemostasis. Surgical information including body weight, pre-treatment blood loss, vessel size and the change of mean arterial pressure (MAP) were recorded and as can be seen in Table 1, both groups had similar baseline characteristics and blood loss after 45 seconds of uncontrolled hemorrhage. These baseline characteristics are presented in graphical format in Figures 2 and 3.

In the ChitoGauze group, seven (88%) animals achieved hemostasis and survived the 3 hr observation period without appreciable post-compression blood loss or oozing (Figure 4) whereas 5 (63%) of the Combat Gauze group survived.

Table 1: Swine pre-treatment characteristics.

	Combat Gauze	ChitoGauze
Weight (kg)	40 ± 3	41 ± 2
Artery Width (mm)	6.19 ± 0.26	6.06 ± 0.18
45-s blood loss (ml)	736 ± 147	676 ± 160
ΔMAP (mmHg)	36 ± 6	33 ± 6

Data are expressed as means ± SD. MAP = mean arterial pressure. Data are expressed as means ± SD.

Table 2: Hemostatic Efficacy Results.

	Combat Gauze	ChitoGauze
n	8	8
3 hr Survival	5/8 (63%)	7/8 (88%)
Immediate Hemostasis *	2/8 (25%)	5/8 (63%)
Time to Hemostasis (min) [§]	38 ± 42	12 ± 29

[§] Data are expressed as means ± SD.
*Defined as hemostasis on first application out of two possible applications

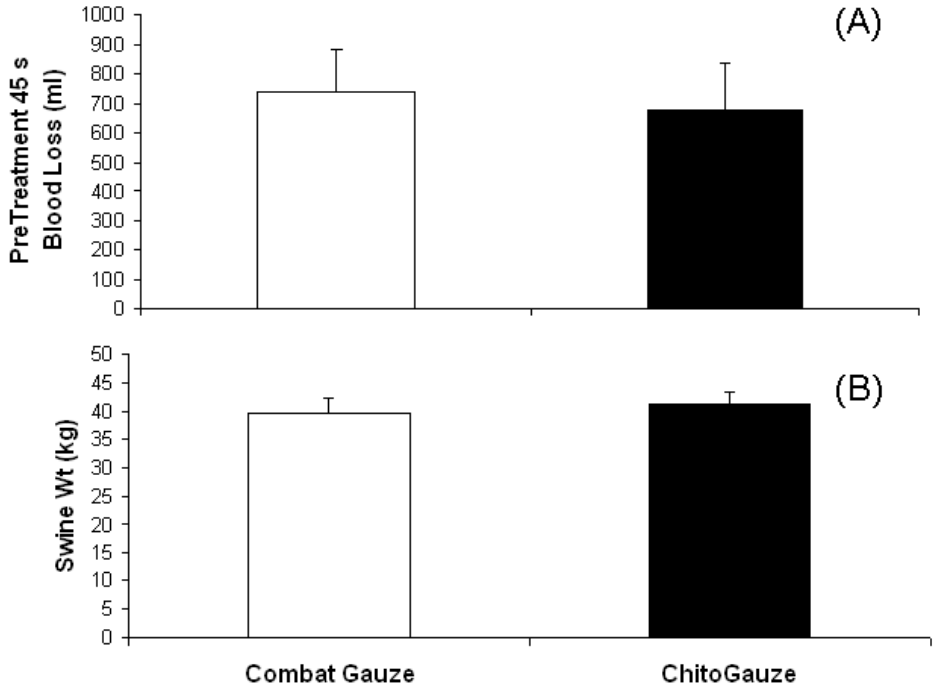

Figure 2: Surgical information including pre-treatment blood loss (A), body weight (B), were similar between the two treatment groups. Combat Gauze ☐ ChitoGauze ■.

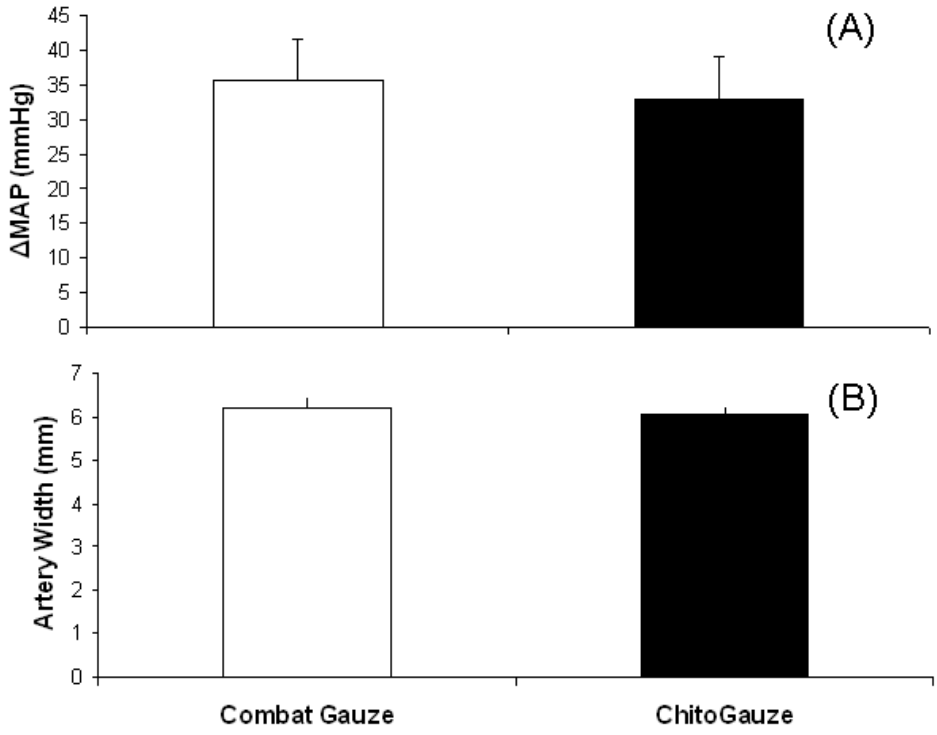

Figure 3: Surgical information including vessel size (A), the change of mean arterial pressure (MAP) (B), were similar between the two treatment groups. Combat Gauze ☐ ChitoGauze ■.

In the ChitoGauze group, 5 (63%) achieved immediate hemostasis while only two (25%, p = 0.04) animals achieved immediate hemostasis in the Combat Gauze group (Figure 5). The mean time to hemostasis for the Combat Gauze group was 38 minutes while the mean time shown by the ChitoGauze group was 12 minutes. The average post treatment blood loss over three hours, presented in Figure 7 was less in the ChitoGauze group than the Combat Gauze group (434 ml vs. 1176 ml).

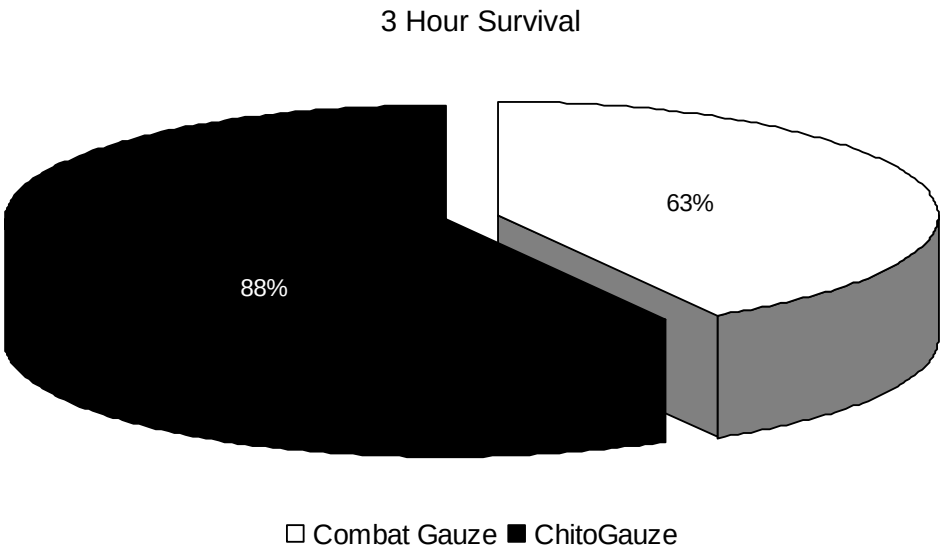

Figure 4: The difference in hemostatic efficacy between the Combat Gauze and Chitogauze as measured by 3 hr survival is shown. Combat Gauze showed a 5/8 (63%) survival rate and ChitoGauze showed a 7/8 (88%) survival rate.

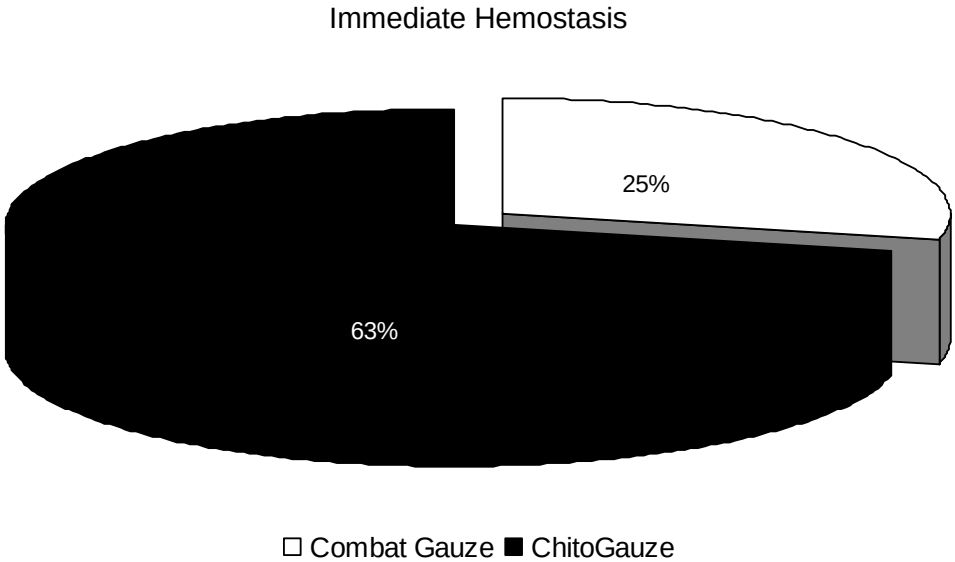

Figure 5: The difference in hemostatic efficacy between the Combat Gauze and Chitogauze as measured by immediate hemostasis is shown. Combat Gauze showed a 2/8 (25%) immediate hemostasis effect and ChitoGauze showed a 5/8 (63%) immediate hemostasis effect.

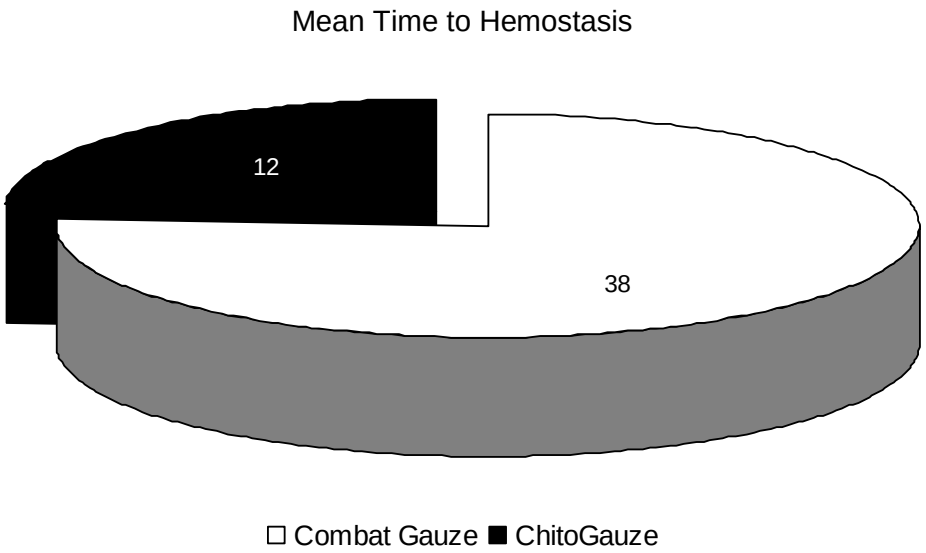

Figure 6: The difference in hemostatic efficacy between the Combat Gauze and Chitogauze as measured by mean time to hemostasis is shown. Combat Gauze showed a mean time to hemostasis of 38 minutes and ChitoGauze showed a mean time to hemostasis of 12 minutes.

Table 3: Blood loss and intravenous fluids.

	Combat Gauze	**ChitoGauze**
Post-injury blood loss (ml)	1176 ± 1374	434 ± 1130
Total-study blood loss (ml)	1913 ± 1433	1110 ± 1029

Data are expressed as means ± SD

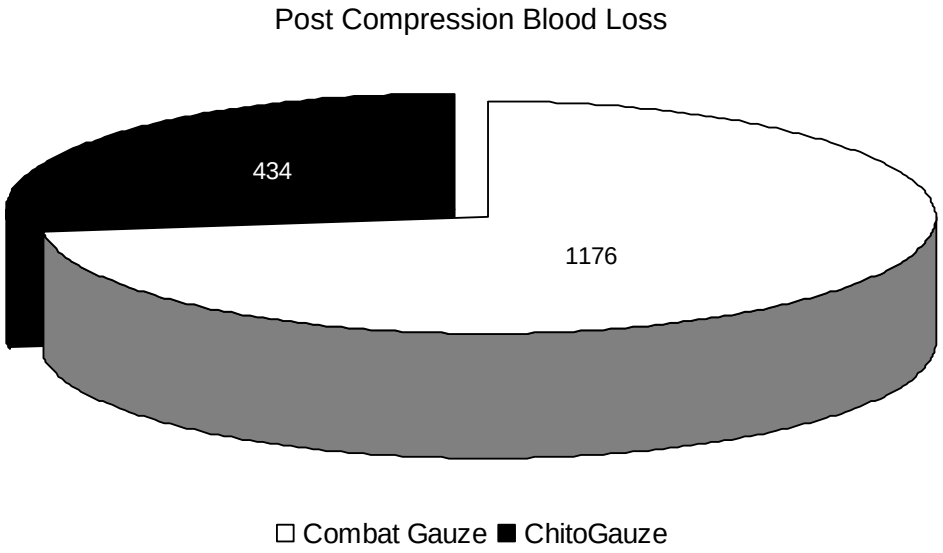

Figure 7: The difference in hemostatic efficacy between the Combat Gauze and Chitogauze as measured by blood lost during treatment is shown. Combat Gauze showed a mean blood loss of 1,176 ml of blood and ChitoGauze showed a mean blood loss of 434 ml of blood.

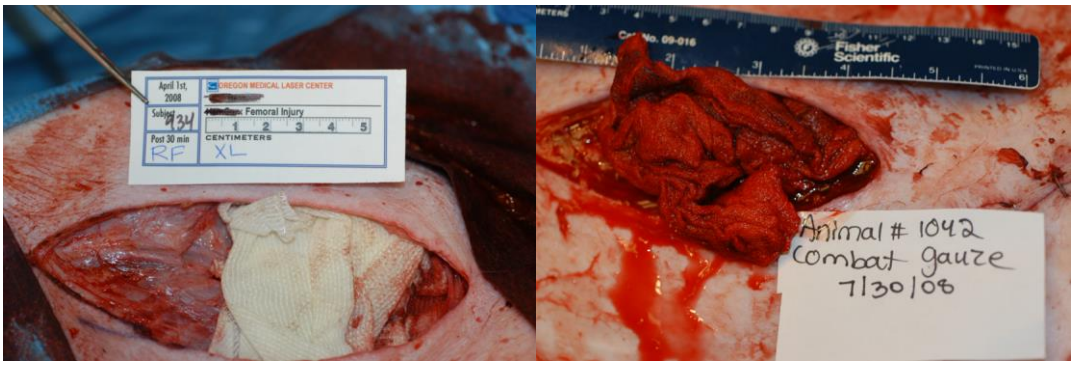

Figure 8: Images show a typical hemostatic effectiveness of ChitoGauze (Left) and Combat Gauze (Right) in the femoral arterial injury model. The ChitoGauze has the capability to achieve immediate hemostasis. The Combat Gauze usually established hemostasis following gradual reduction of haemorrhaging.

Table 4: Hemostatic Results from swine that survived the 3 hours observation period.

	Combat Gauze	ChitoGauze
n	5	7
Post-injury blood loss (ml)	216 ± 368	36 ± 94
Time to Hemostasis (min) [§]	12 ± 18.7	2 ± 4.9

[§] Data are expressed as means ± SD.

The efficacy results from the animals that survived the 3 hours observation period were also examined for insight into the two gauze products differing modes of action. In the survived animals, five out of seven animals had complete hemostasis in the first attempt using the ChitoGauze; two out of five animals achieved hemostasis in the first attempt with the Combat Gauze. The average time to achieve complete hemostasis in the survived animals was two minutes with the ChitoGauze and 12 minutes using Combat Gauze (Figure 9). The average post compression blood loss over three hours for the survived animals was 216 ml for the Combat Gauze group and 36 ml for the ChitoGauze group (Figure 10).

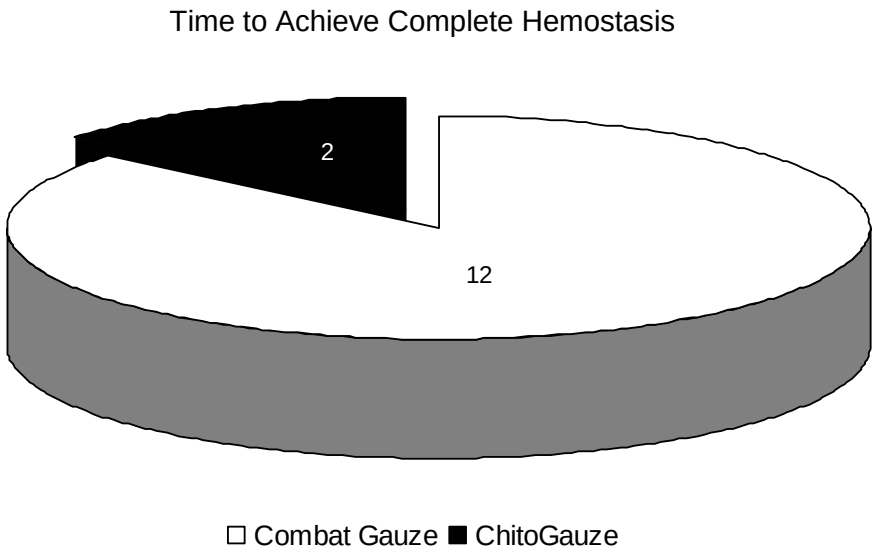

Figure 9: The difference in hemostatic efficacy between the Combat Gauze and Chitogauze as measured by mean time to hemostasis for the swine that survived the 3 hours observation period is shown. Combat Gauze showed a mean time to hemostasis of 12 minutes and ChitoGauze showed a mean time to hemostasis of 2 minutes.

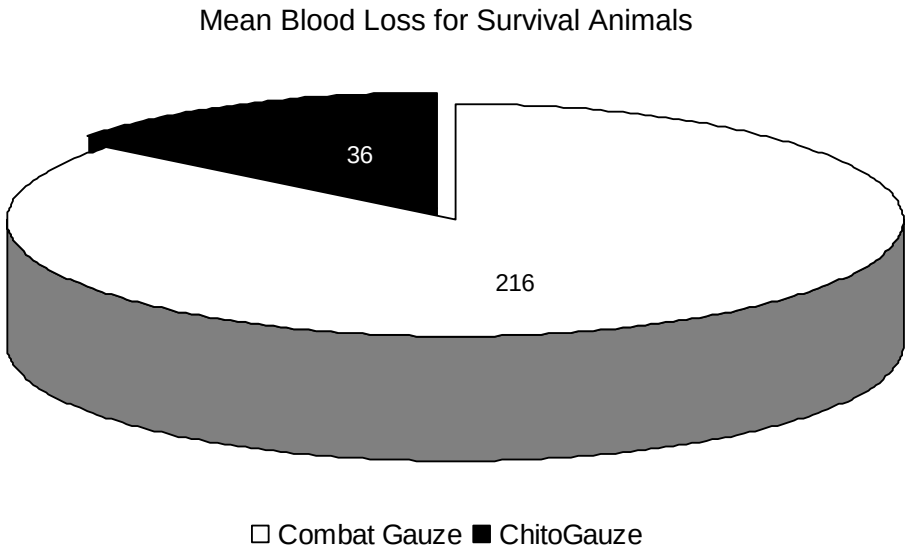

Figure 10: The difference in hemostatic efficacy between the Combat Gauze and Chitogauze as measured by blood lost during treatment is shown for the swine that survived the 3 hours observation period. Combat Gauze showed a mean blood loss of 216 ml of blood and ChitoGauze showed a mean blood loss of 36 ml of blood.

DISCUSSION

Many military groups have sought to reduce the mortality from haemorrhage at various stages of the casualty treatment pathway by introducing many new original treatments. The fundamental hope of these treatments is the commencement of haemodynamic stabilization of the casualty as far forward on the battlefield as possible. Ideally this hemorrhage control commences at the buddy/self-care line of combat use. ChitoGauze and Combat Gauze are two hemostat impregnated fabric-based hemostatic dressings that

clearly show significant potential for battlefield deployment. We have tested them in 6 mm arterial punch to challenge the hemostatic dressing to control bleeding. Overall performance of these dressings according to survival and post-treatment blood loss was measured along with a number of other important efficacy parameters.

Both dressings demonstrated good overall survival with ChitoGauze resulting in an 88% swine survival for the 3 hour observation period. Combat Gauze demonstrated a 63% overall survival time. These results for Combat Gauze are consistent with previously published survival results [13]. The measurement of immediate hemostasis provided an interesting observation in that 63% of the ChitoGauze group stopped bleeding immediately while only 25% of the Combat Gauze stopped bleeding immediately. This distinction is further evidenced by the mean time to hemostasis with the average ChitoGauze time of 12 minutes versus the Combat Gauze value of 38 minutes.

The similar survival results seen, but with disparity in immediate hemostasis time and time to hemostasis, point to different modes of action for ChitoGauze and Combat Gauze. Mechanism of action of these dressings may well be related with their absorption and clotting abilities. Our clinical observations support this hypothesis in that the Combat Gauze generally absorbed blood on contact with the wound thus allowing mixing of the blood with the impregnated hemostatic agent (kaolin). This is consistent with similar observations made by Arnaud et al. [13]. ChitoGauze visibly does not work by such a mechanism. The interaction of the chitosan coated gauze surface with the wound initally seals the wound and immediately helps to retard blood flow through the dressing thereby diminishing rapid bleeding. The chitosan coating on the gauze further reduces blood loss by helping to adhere the dressing to the wound site providing a physical barrier to prevent bleeding. Subsequent to the non-clotting cascade related mode of stopping blood loss, ssignificant aggregation of erythrocytes and activation of platelets promoted by the chitosan can take place helping with the prevention of rebleeding. As noted by Arnaud et al. [13] and others the fabric material from which the gauze is manufactured is an important element of the gauze functionality and the fluid handling properties of the flexible fabric used in ChitoGauze support the sealing of the wound site to diminish blood loss.

The results of this study sustain the different modes of action of the two products discussed. The average post application blood loss for the ChitoGauze group was 434 ml whereas the average Combat Gauze blood loss was 1176 ml. When we further analyzed the animals that survived the 3 hr observation period only it could be seen that the average ChitoGauze blood loss was only 36 ml whereas the average Combat Gauze value was 216 ml. This represents a 6 fold difference in the quantity of blood lost in the survived animals. With hemorrhage induced blood loss being the single major cause of death in potentially salvageable battlefield casualties [14] such a difference in blood loss data between the two tested products is noteworthy.

As mentioned previously, chitosan has antibacterial properties and as such, the original HemCon Bandage had broad spectrum antibacterial action including efficacy against both gram positive and gram negative bacteria. The HemCon ChitoGauze dressing was also produced with such antibacterial action. Although mortality from battlefield wounds has historically declined, war trauma associated infection (WTAI) is still an important issue [15] [16]. One of the key challenges facing military and civilian researchers remains the problem of multidrug-resistant organisms and ChitoGauze brings an effective ability to combat many of these bacteria (Figure 11).

Microorganism	Gram Stain	Log Reduction
Staphylococcus aureus (MRSA) ATCC 33591	+	>4.1
Staphylococcus aureus (MRSA) ATCC BAA-1556	+	>4.2
Staphylococcus epidermidis ATCC 12228	+	>4.2
Pseudomonas aeruginosa ATCC 9027	-	>4.1
Enterococcus faecalis (VRE) ATCC 51299	+	>4.0
Acinetobacter baumannii ATCC 15308	-	>4.4
Citrobacter freundii ATCC 8090	-	>4.3
Enterobacter cloacae ATCC 13047	-	>4.1
Streptococcus mutans ATCC 25175	+	>4.0
Streptococcus pneumoniae ATCC 10015	+	>5.1
Escherichia coli ATCC 8739	-	>4.1
Klebsiella pneumoniae ATCC 4352	-	>4.0
Streptococcus pyogenes ATCC 19615	+	>4.2
Salmonella choleraesius ATCC 10708	-	>4.1
Stenotrophomonas maltophilia ATCC 12714	-	>4.0
Citrobacter koseri ATCC 25408	-	>4.1
Proteus mirabilis ATCC 4630	-	>4.2
Proteus vulgaris ATCC 12454	-	>4.3
Moraxella catarrhalis ATCC 8193	-	>4.1
Clostridium difficile ATCC 9689	+	>4.0
Shigella species ATCC 11126	-	>4.0
Micrococcus luteus ATCC 49732	+	>4.0
Vibrio cholerae ATCC 11558	-	>4.1
Enterobacter aerogenes ATCC 13048	-	4.8
Enterococcus faecalis (VRE) ATCC 700802	+	2.6
Serratia marcescens ATCC 13880	-	5.0

Figure 11: ChitoGauze™ was tested for reduction of microorganisms against the following species. The log reduction data demonstrates the level of antibacterial effectiveness.

CONCLUSION

Both ChitoGauze and Combat Gauze demonstrate hemostatic effectiveness in this lethal extremity hemorrhage model. Both dressings were easy to apply into the femoral wound geometries. While both bandages performed similarly in this small sample, we did note a trend toward more blood loss among the successful Combat Gauze applications as compared to ChitoGauze. ChitoGauze had greater success in achieving immediate hemorrhage control with less blood loss than Combat Gauze in this model.

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