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COMPARISON OF NOVEL HEMOSTATIC GAUZES TO QUIKCLOT COMBAT GAUZE IN A STANDARDIZED SWINE MODEL OF UNCONTROLLED HEMORRHAGE

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14. ABSTRACT: Uncontrolled hemorrhage is one of the leading causes of death in the battlefield. This study aims to determine which of the most current hemostatic gauzes is the most efficacious in controlling hemorrhage. Pigs were sedated and anesthetized before isolation and puncture of the femoral artery. Free bleeding was allowed to proceed for 45 seconds before packing of Quikclot Combat Gauze (QCG), Quikclot Combat Gauze XL(QCX), Celox Trauma Gauze(CTG), Celox Gauze(CEL), or Hemcon ChitoGauze(HCG), into the wound. After manual compression, the animals were resuscitated to keep MAP above 60 mmHg. Animals were observed for 150 minutes or until death. Animal survival, hemostasis, and blood loss were used as primary endpoints. 60% of the QCG-treated animals survived through the entire 150-minute observation period. QCX, CEL, and HCG all had higher rates of survival (70%, 90%, and 70% respectively), while CTG had a 50% survival rate. Immediate hemostasis ranged from 30% with QCG to 80% with QCX. Post-treatment blood loss varied from an average of 64 ml/kg with CTG to 29 ml/kg with CEL, but no significant difference amongst groups was observed.

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The experiments reported herein were conducted in compliance with the Animal Welfare Act and in accordance with the principles set forth in the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animals Resources, National Research Council, National Academy Press, 1996.

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EXECUTIVE SUMMARY

Problem: Uncontrolled hemorrhage is one of the leading causes of death in the battlefield. The development, testing, and application of novel hemostatic dressings may lead to a reduction of pre-hospital mortality through enhanced point of injury hemostatic control.

Objective: This study aimed to determine the efficacy of currently available hemostatic dressings as compared to the current Committee for Tactical Combat Casualty Care Guidelines standard of treatment for hemorrhage control (QuikClot Combat Gauze-QCG).

Approach: This study utilized the Department of Defense consensus swine model for uncontrolled hemorrhage. Briefly, Yorkshire swine were anesthetized and instrumented for telemetry. Following a femoral cut-down, a 6 mm punch injury was created in the femoral artery and free bleeding was allowed to occur for 45 seconds. For each swine, one of five hemostatic gauzes (QCG, QuikClot Combat Gauze XL-QCX, Celox Trauma Gauze-CTG, Celox Gauze-CEL, or ChitoGauze-HCG) was packed into the wound site. Direct pressure (3 min) was then applied, and the animals were rapidly resuscitated to achieve and maintain a MAP $\geq$ 60 mmHg for 150 minutes or until death. Animal survival, hemostasis, and blood loss were assessed as primary endpoints.

Findings: Animals had an average weight of 36.6 ± 2.2 kg, a mean arterial pressure of 67.5 ± 8.2, and pretreatment blood loss of 15.4 ± 3.1 ml/kg. 60% of QCG-treated animals (controls) survived through the entire 150-minute observation period. QCX, CEL, and HCG demonstrated higher rates of survival when compared to QCG (70%, 90%, and 70% respectively). Immediate hemostasis was achieved in 30% of QCG applications, 80% of QCX, 70% of CEL, 60% of HCG, and 30% of CTG-treated animals. Post-treatment blood loss varied from an average of 64 ml/kg with CTG to 29 ml/kg with CEL, but no significant difference amongst groups was observed.

Conclusions: Novel FDA-approved hemostatic dressings exist that perform equally to the current standard of care based on hemostasis, survival, and blood loss measured in the DoD concensus model of swine femoral uncontrolled hemorrhage. One product, QCX was identified as outperforming the current standard in achieving immediate hemostasis, while two products, QCX and CEL were identified as outperforming the current standard in achieving 10-minute hemostasis. These results suggest that the current standard for point-of-injury hemorrhage control (QCG) may need to be re-evaluated or alternatively the standard of care expanded to include QCX, CEL, CTG and HCG.

INTRODUCTION

Problem

Uncontrolled hemorrhage remains the most common cause of death on battlefield (1-3). The majority of uncontrolled hemorrhage deaths are a result of injuries that are either non-compressible (torso) or are not amenable to tourniquet (neck, groin; (1)). Many of these deaths were due to the increased incidence of injuries sustained from the detonation of improvised explosive devices, deployed against coalition forces in Operation Iraqi Freedom and Operation Enduring Freedom (1-3). In order to reduce mortality from injuries resulting in uncontrolled hemorrhage, more effective means to achieve early hemostasis must be developed and implemented. One such means, capable of mitigating hemorrhage at point-of-injury care is hemostatic gauze.

Objective

The aim of this study was to determine the efficacy of novel hemostatic gauze products as compared to the current Committee on Tactical Combat Casualty Care (CoTCCC) standard, QuikClot Combat Gauze (QCG; Z-Medica, Wallingford, CT) in a groin puncture model of hemorrhage in swine (4). The model implemented in this study is an application of the United States Department of Defense (DoD) standardized model for uncontrolled hemorrhage, described in Kheirabadi et al. 2011, based on the recommendations of a panel of DoD medical experts who convened on June 30, 2009 (5).

Background

In recent years, many new externally applied hemostatic agents have been developed that show promise in reducing hemorrhage. These agents vary in form from gauzes and sponges to powders and granules formulated from materials including aluminum silicates, chitosans, starches, smectite, and proprietary formulations (6, 7). However, gauze has several aspects that make it a superior agent for treatment of uncontrolled external hemorrhage on the battlefield. It is familiar and easily applied to self or other casualties. Gauze is also less affected by elements such as wind or rain and is easily applied in low-visibility conditions. Finally, gauze conforms to an injury site unlike sponges or wafers.

Currently, QuikClot Combat Gauze is the CoTCCC recommended standard hemostatic agent in the U.S. military. QCG is a non-woven, kaolin-coated surgical gauze that has shown equal or higher efficacy for hemorrhage control in laboratory tests when compared to other hemostatic agents including TraumaStat (Ore-Medix, Salem, OR), Celox-D (SAM Medical, Portland, OR), and Hemcon RTS bandage (HemCon, Portland, OR) (8-12). Kaolin is an aluminosilicate clay that activates the intrinsic coagulation

pathway (6, 7). QCG was observed to not produce any short term vascular damage compared to standard gauze in an animal model (13). Finally, no adverse reactions were found during its use on the battlefield during the Israeli Operation Cast Lead in the Gaza Strip (14).

We chose to compare four of the most promising hemostatic gauzes to QCG in a swine uncontrolled arterial hemorrhage model. These gauzes include QuikClot Combat Gauze XL (QCX, Z-Medica, Wallingford, CT), Celox Gauze (CEL, MedTrade Products, Crewe UK), Celox Trauma Gauze (CTG, MedTrade Products, Crewe UK), and ChitoGauze (HCG, Hemcon, Portland, OR). QCX is similar to QCG and only differs in that XL is 2-ply created by folding a larger piece of gauze in half during packaging. CEL and HCG are chitosan-coated gauze dressings, while CTG is made entirely of chitosan made flexible through its manufacturing process. As opposed to dressings that utilize kaolin, chitosan dressings do not directly activate or stimulate the coagulation pathway, but rather promote the cross-linking of red blood cells to form a physical barrier (6, 7). Information regarding each of the hemostatic gauzes is summarized in Table 1.

Table 1. Characteristics of tested hemostatic gauzes

Product	Package	Abbrev	Form	Size	Weight	Chemistry	Mechanism
QuikClot Combat Gauze		QCG	Z-folded gauze	3 in X 12 ft	21.4 g	Non-woven kaolin (Al-silicate)	Activates intrinsic coagulation
QuikClot Combat Gauze XL		QCX	Z-folded 2-ply gauze	4 in X 12 ft	49.5 g	Non-woven kaolin (Al-silicate)	Activates intrinsic coagulation
Celox Trauma Gauze		CTG	Rolled gauze	3 in X 6 ft	19.5 g	Non-woven chitosan fibers	Cross-links RBCs to form clot
Celox Gauze		CEL	Rolled gauze	3 in X 10 ft	53.1 g	Chitosan-coated gauze	Cross-links RBCs to form clot
Hemcon ChitoGauze		HCG	Z-folded gauze	3 in X 12 ft	20.1 g	Chitosan-coated gauze	Cross-links RBCs to form clot

METHODS

All procedures involving animals were approved by Tri-Service Research Laboratory's Institutional Animal Care and Use Committee, Fort Sam Houston, TX. Animals were utilized in accordance with the *Guide for the Care and Use of Laboratory Animals* (15).

Animals

Healthy, male, Yorkshire cross-bred pigs, weighing 34-45 kg, purchased from Oak Hill Genetics (Ewing, IL) were used in all procedures. Animals were housed on-site with enrichment and quarantined for at least four days for acclimation prior to experimentation.

Surgical Procedures

Animals were fasted for 12 hours prior to surgery, but allowed access to water *ad libitum*. The animals were then sedated with 8 mg/kg Telazol (Tiletamine and Zolazepam). Buprenorphine (0.01 mg/kg IM) was administered for alleviation of pain and glycopyrrolate (0.004 mg/kg IM) to reduce mucous secretion. Anesthesia was induced with 2-4% isoflourane in pure oxygen initially and then decreased to 1-2% once a stable plane of anesthesia was reached. The ventilator was adjusted to maintain an end tidal CO_2 partial pressure between 38 and 42 mmHg.

The right carotid artery was cannulated via cutdown for blood sampling and invasive blood pressure measurements. Blood pressure was continuously monitored using a Cardiocap (GE Healthcare, Waukesha, WI). The right internal jugular was vein was cannulated for administration of resuscitation fluids. A midline laparotomy was then performed to simulate soft tissue injury and to allow bladder catheterization. Maintenance fluid in the form of lactated Ringer's solution (LRS) was administered at a rate of 5-10 ml/kg/min for a total of 10 ml/kg during surgical procedures.

Injury and Hemorrhage

Two research surgeons performed all study injuries and were blinded to the identity of the randomly chosen test gauze until just before application. Each test gauze group consisted of ten randomized animals. Randomization was accomplished by picking a sealed envelope that contained the name of the test gauze by a staff member not involved in the creation of the injury or application of the gauze. The gauze was then given to the investigator during the free bleeding portion of the experiment immediately before application.

The injury procedures used in this study were developed by Kheirabadi *et al*. as a standardized model for hemostatic gauze efficacy testing and has been described in detail elsewhere (5). Briefly, to expose the femoral artery, a 10-cm incision was made in the groin above the artery. The thin overlying adductor muscle was excised followed by careful dissection and removal of the adventitia surrounding the artery. Finally, all small branches stemming from the artery were cauterized or suture ligated. Once all surgical manipulations were completed and maintenance fluids were infused (lactated ringers, 10 mL/kg/hr, total 10 mL/kg). The artery was covered with a small piece of gauze and bathed in 10 ml of 2% lidocaine solution for 10 minutes to promote arterial dilation (Figure 1). The incision site was covered with saline soaked gauze to prevent drying. Following this 10-minute stabilization period, the artery was clamped both proximally and distally using atraumatic bulldog clamps. A 6.0-mm aortic punch (International Biophysics Corp., Austin, TX) was then used to create an arteriotomy in the femoral artery. The clamps were then removed, and hemorrhage was allowed to proceed unobstructed for 45 seconds, while blood was collected by suction and weighed in real time. Next, the test article was packed quickly into the wound site along with enough cut and pre-folded Kerlix backing to fill the cavity as determined by the applying investigator. The time taken to pack the injury site was measured and recorded. Manual pressure was then applied for 3 minutes, followed by gentle release. Post-injury blood was collected by vacuum suction and by pre-weighed absorbent pads for calculation of total blood loss throughout the experiment. Hemostasis was defined as a lack of visible blood pooling outside injury site. Immediate hemostasis was defined as hemostasis occurring within 3 minutes after compression.

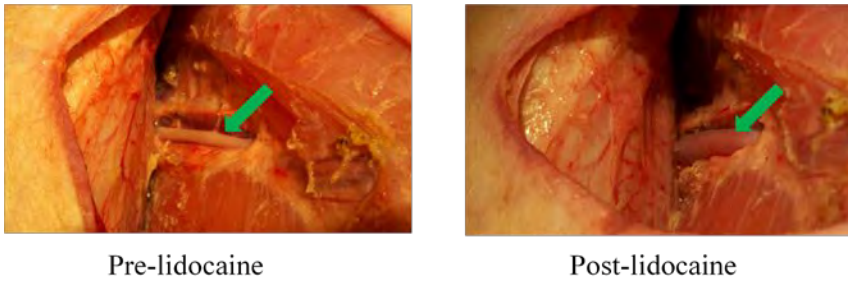

Figure 1. Lidocaine application. Lidocaine application results in maximal dilation of target artery following surgical manipulation. Notice the increase in diameter of the post-lidocaine artery.

Resuscitation

Immediately following the 3-minute compression period, 500 ml of warmed Hextend (6% Hetastarch, Lactated Ringer's, 5% dextrose) was administered using a pressurized infuser bag (Ethox, Buffalo, NY) via jugular vein catheter. Upon completion of Hextend infusion, LRS was also administered using a pressurized infuser bag through the jugular vein catheter for resuscitation as needed throughout the entire procedure to maintain a MAP between 60 and 65 mmHg. A maximum of 10 L of LRS was given following the injury. Death was defined when MAP and ET CO_2 fell below 20 and 15

mmHg respectively and were maintained for two minutes. Animals were euthanized using Beuthasol (Sodium Pentobarbital) after 2.5 hours or when death due to exsanguination occurred.

Real-time Blood Collection

Suctioned blood was weighed with a Mettler Toledo MS6002S precision balance (Mettler Toledo, Columbus, OH), modified with a bracket to hold a 1L suction collection bucket. Changes in weights on the balance were recorded to file every second during the procedure and graphed for simple visualization with custom software (BalanceChart, v1.1).

Biochemical Analysis

For each animal, blood samples were was taken prior to surgical manipulation, immediately prior to initiation of injury, then 10, 30, 60, 90, 120, and 150 minutes subsequent to the injury. Analysis included functional coagulation (ROTEM, TEM Systems Inc, Durham, NC), CBCs using AcT Diff 2 (Beckman Coulter, Inc., Brea, CA), standard clinical coagulation panels including PT, PTT, INR, Fibrinogen, and D-dimer using BCS XP (Siemens, Deerfield, IL), and blood gas analysis using ABL 837 Flex (Radiometer America, Westlake, OH).

Postmortem Analysis

At the end of each experiment, the injured leg was moved three times in each axis to simulate walking while looking for signs of hemorrhage. The Kerlix backing, test gauze and any pads that captured blood were weighed to be included in blood loss calculations. Small sections (0.5 to 1.5 cm) of the femoral artery, femoral vein, femoral nerve, and the adjacent muscle proximal to the injury site were isolated and immediately transferred to 10% neutral buffered formalin for at least 48 hours. Tissues were then processed into paraffin using a standard automated tissue dehydration processor, 5-7um sections were placed on glass slides and stained with hematoxylin and eosin on a standard automated stainer. All sections were evaluated by a board certified veterinary pathologist with experience in hemostatic bandage research. Tissues were evaluated for injury and inflammation using a semi-quantitative scale of 0=normal, 1=minimal, 2=mild,3=moderate, 4=severe. Necropsy was performed on all animals that did not survive the entire 150-minute observation period to determine cause of death, if present, outside of observed exsanguination.

Statistics

Differences amongst groups was considered significant when $p < 0.05$. Data is presented as mean ± standard deviation. Animals were excluded if their baseline MAP was < 60 mmHg or pre-treatment blood loss < 10 mL/kg. The number of animals required in each group was determined by the likelihood to attain hemostasis by T 10 of fluid resuscitation. Power analysis was at $\alpha = 0.05$ and power of 80%. Chi-square tests were used to determine significance amongst groups in tests with binary outcomes.

Analysis of variance (ANOVA) with Dunnett's multiple comparison tests were used to compare means amongst groups. Log-rank test was used to determine significance in survival analysis. When appropriate, data was analyzed prior to the first animal death to avoid data censoring. Data analysis was done using Microsoft Excel 2007 (Microsoft, Redmond, WA) and SigmaPlot 12 (Systat Software, San Jose, CA).

RESULTS

Pre-treatment Levels

There were no statistically significant differences amongst groups with respect to pre-injury vitals (weight, blood pressure, etc.) or hemodynamic properties (Table 2, ROTEM, blood gases, etc.). Animals had an average weight of 36.6 ± 2.2 kg and a mean arterial pressure (MAP) of 67.5 ± 5.7. There were also no significant differences amongst groups based on pretreatment blood loss with an average loss of 15.4 ± 3.1 ml/kg and an average rate of 11.6 ± 2.3 ml/kg/min. Hematocrit was significantly different amongst groups by ANOVA, but Dunnett's post-hoc analysis did not indicate any significant differences when compared to control (QCG).

Table 2. Baseline and Pretreatment Values

	QCG	QCX	CTG	CEL	HCG	*p*
Weight (kg)	36.6 ± 1.8	37.6 ± 3.0	37.0 ± 1.9	36.2 ± 2.1	35.9 ± 1.7	0.39
MAP (mmHg)	66.1 ± 7.6	64.8 ± 6.1	66.9 ± 12.2	64.0 ± 8.9	67.3 ± 5.6	0.55
Blood Loss (mL/kg)	16.2 ± 3.5	15.0 ± 3.6	16.3 ± 3.0	15.2 ± 3.0	14.4 ± 2.4	0.62
Rectal Temp (°C)	36.6 ± 1.0	36.7 ± 0.56	36.9 ± 0.83	37.0 ± 0.85	36.9 ± 0.56	0.86
Lowest MAP (mmHg)	33.4 ± 6.3	32.7 ± 6.8	29.5 ± 9.8	33.1 ± 7.0	33.0 ± 7.1	0.76
Hematocrit (%)	29.5 ± 2.1	27.2 ± 2.4	27.8 ± 2.1	30.1 ± 2.8	28.5 ± 2.8	0.04
Platelets (x10^3/µL)	349 ± 57	383 ± 63	311 ± 70	359 ± 48	375 ± 59	0.08
Fibrinogen (mg/dL)	208 ± 31	209 ± 22	211 ± 19	214 ± 12	216 ± 25	0.94
WBC (x10^3/µL)	20.0 ± 3.7	18.5 ± 4.4	18.7 ± 4.1	19.1 ± 5.9	17.7 ± 4.3	0.85
PT (sec)	11.6 ± 0.5	11.4 ± 0.5	11.3 ± 0.6	11.3 ± 0.4	11.4 ± 0.6	0.64
PTT (sec)	17.4 ± 1.0	17.5 ± 0.6	17.1 ± 1.2	17.3 ± 1.4	17.2 ± 0.8	0.94

MAP, mean arterial pressure; temp, temperature; WBC, white blood cells; PT, prothrombin time; PTT, partial thromboplastin time; QCG, QuikClot Combat Gauze; QCX, QuikClot Combat Gauze XL; CTG, Celox Trauma Gauze; CEL, Celox Gauze; HCG, HemCon ChitoGauze

Wound Pack Time

Each test gauze was packed into the injury site as rapidly as possible while still maintaining pressure and contact with the injury site (Figure 2). The overall average time to pack was 38.8 ± 11.0 seconds with times ranging from 32.0 ± 9.2 seconds for CTG to 47.7 ± 11.3 seconds for QCX. QCG was the second fastest packed gauze followed by HCG, CEL, and finally QCX. An ANOVA performed on pack time revealed significant differences amongst groups (p = 0.02), but no differences compared to QCG using Dunnet's multiple comparison test.

Hemostasis

Immediate hemostasis (no visible bleeding from the wound during the first three minutes after compression) ranged from 30% (3/10) of QCG- and CTG-treated animals to 80% (8/10) of QCX (Figure 3A). Chi-squared analysis reveals that these differences were significant (p = 0.02). QCG also had an additional three animals that eventually achieved hemostasis after the immediate hemostasis period ended, with one taking 84 minutes to achieve hemostasis. The other gauzes had either one or two animals reach hemostasis during the observation period except QCX. QCX, CEL, and HCG also had incidences of re-bleeding in a wound that had previously reached hemostasis (Figure 3B), but in only one pig in the QCX and one in the HCG group did this re-bleeding lead to the death of the animal. Total hemostasis time was also measured (Figure 3C). This time, where there was no visible bleeding from the wound, ranged from just over an hour for CTG (64.8 ± 72.1 minutes) to two hours for CEL (120.5 ± 51 minutes). While a strong trend was observed in total time of hemostasis amongst some groups, this parameter was not found to be statistically significant (p= 0.27).

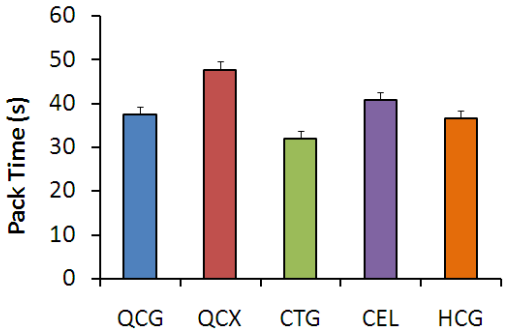

Figure 2. Pack Time. The average time taken to pack each group. Time includes both test gauze and backing. QCG, QuikClot Combat Gauze; QCX, QuikClot Combat Gauze XL; CTG, Celox Trauma Gauze; CEL, Celox Gauze; HCG, HemCon ChitoGauze

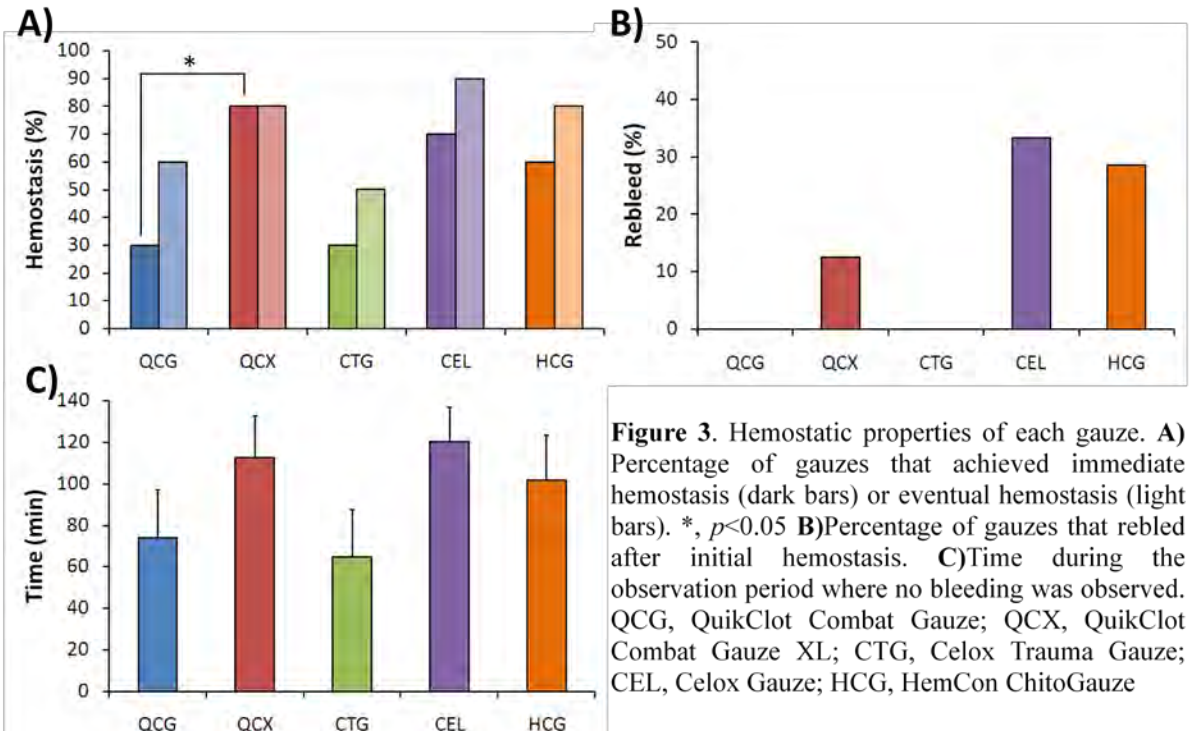

Figure 3. Hemostatic properties of each gauze. **A)** Percentage of gauzes that achieved immediate hemostasis (dark bars) or eventual hemostasis (light bars). *, p<0.05 **B)**Percentage of gauzes that rebled after initial hemostasis. **C)**Time during the observation period where no bleeding was observed. QCG, QuikClot Combat Gauze; QCX, QuikClot Combat Gauze XL; CTG, Celox Trauma Gauze; CEL, Celox Gauze; HCG, HemCon ChitoGauze

Blood Loss

Blood pooling outside the wound was aspirated, collected, and weighed in order to obtain the blood loss volume following the application of the test gauze. Figure 4 graphically displays the differences in blood loss amongst the groups. Figure 4A shows blood loss due to the injury before the gauze packing displayed as mL/kg. However, when blood loss measured at the end of the first ten minutes was analyzed (platinum 10 minutes), differences amongst groups becomes apparent by ANOVA (p= 0.03). Blood loss averaged 5.1 ± 7.8 mL/kg with a range of 1.7 ± 3.8 mL/kg for QCX to 10.8 ± 10.8 mL/kg for QCG. QCG treated-animals shed 6-fold and 4.5-fold more blood than QCX and CEL respectively (p=0.026 vs QCX; p=0.046 vs CEL). At 30 minutes, blood loss averaged 19 ± 27 mL/kg with a QCG losing 3.9- and 2.5-fold more than QCX and CEL respectively, but a one-way ANOVA on this data did not yield significance (Figure 4C). At the end of the experiment (Figure 4D), animals treated with QCG (62 ± 65 mL/kg) or CTG (65 ± 59 mL/kg) lost nearly twice as much blood as QCX (32 ± 52 mL/kg) and CEL (29 ± 64 mL/kg). While again, a strong trend was observed, there was no significant difference between groups at the end of the experiment determined by ANOVA.

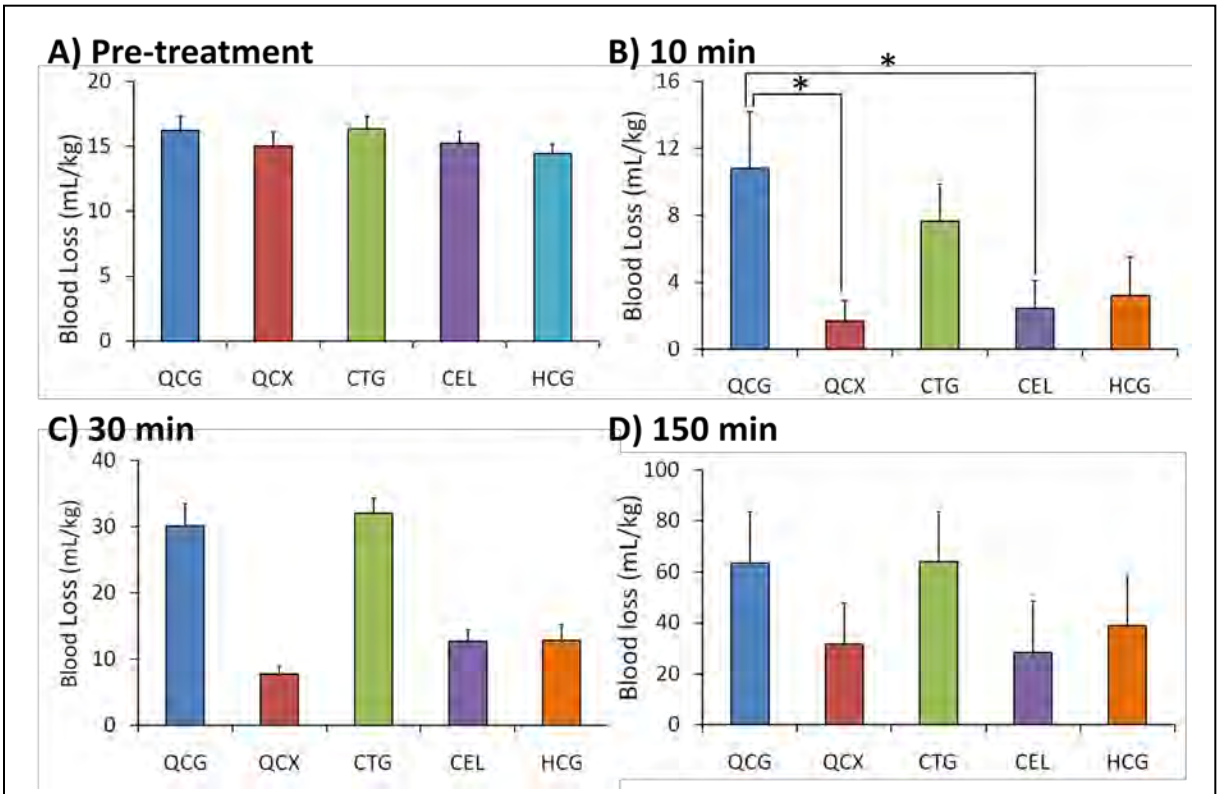

Figure 4. Blood loss **A)** Amount of blood collected prior to treatment given in mL/kg. **B)** Blood loss that occurred during the first ten minutes of treatment. **C)** 30 mins **D)** over the entire experiment *, p<0.05. QCG, QuikClot Combat Gauze; QCX, QuikClot Combat Gauze XL; CTG, Celox Trauma Gauze; CEL, Celox Gauze; HCG, HemCon ChitoGauze

Resuscitation

In these experiments, animals were resucitated with lactated Ringer's solution to maintain a MAP ≥ 60 mmHg. Therefore, the total amount of fluids infused can be analyzed as an indirect measure of the success of the hemostatic agent. Fluids infused averaged 160.2 ± 116.8 mL/kg and ranged from 116 ± 131 for HCG to 207 ± 118 for CTG with no statistically significant differences amongst groups (Figure 5). Interestingly, four animals required the full 10 L of lactated Ringer's, but were hemostatic and survived the entire procedure.

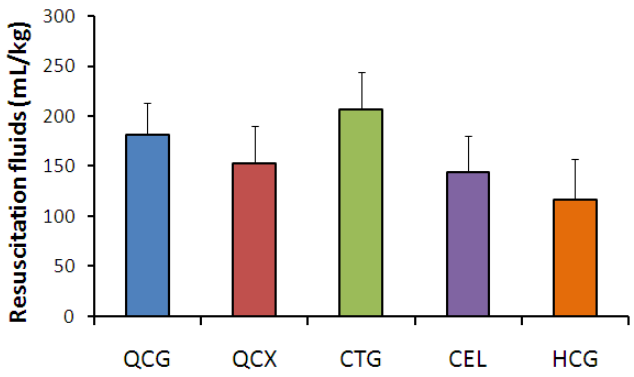

Figure 5. Resuscitation fluids. Values shown include both Hextend and Lactated Ringer's solution and are given as mean ± standard error. QCG, QuikClot Combat Gauze; QCX, QuikClot Combat Gauze XL; CTG, Celox Trauma Gauze; CEL, Celox Gauze; HCG, HemCon ChitoGauze

Coagulation

The extent of coagulopathy is a measurable outcome of the effectiveness of each gauze (as a function of hemostasis and the amount of resuscitation fluid delivered, Figure 6). ROTEM, prothrombin time (PT) and partial thromboplastin time (PTT) were measured in order to determine the coagulation state of each animal. There were no significant differences amongst the groups based on ANOVA before or after the injury in any of the tests for coagulation. Figure 6A and B show the results of ROTEM analysis until 60 minutes after injury. Alpha is a measure of the kinetics of clot formation, while Maximum Clot Firmness (MCF) reflects the strength of the clot. Figure 6C and D show the results of the prothrombin time (PT) and the partial thromboplastin time (PTT). All four tests implicate the trend that QCX-, CEL, and HCG-treated animal's blood performs closer to baseline than the QCG-treated animals. However, CTG-treated animals appear to have worse coagulation parameters than the control.

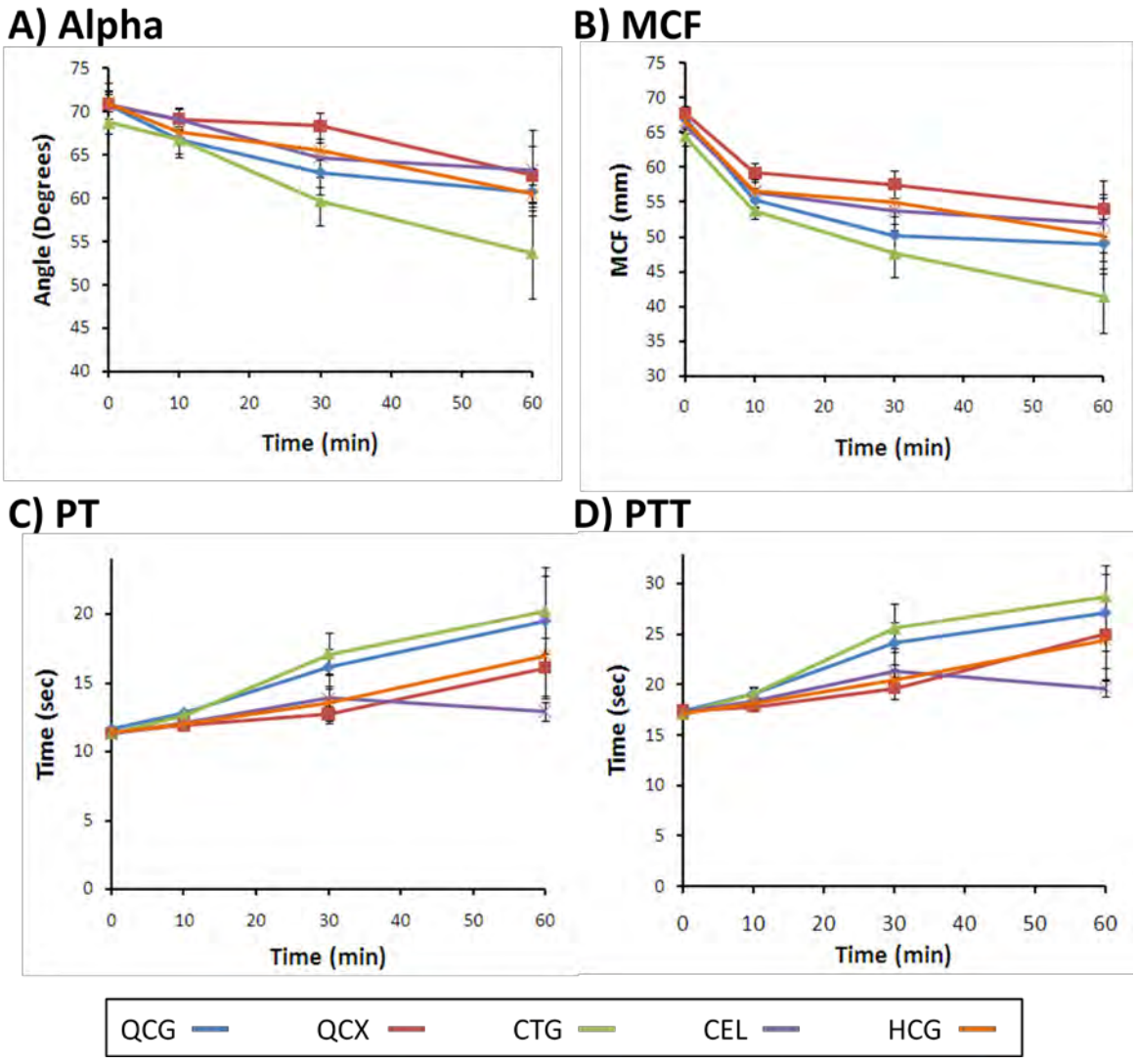

Figure 6. Coagulation parameters. Values are shown only up to 60 minutes due to high level of animal death following 60 minute timepoint **A)** Alpha values obtained from ROTEM analysis indicating kinetics of clot formation. **B)** MCf values indicating the quality of the clot. **C)** Prothrombin time showing the coagulation properties of the extrinsic coagulation pathway. **D)** PTT illustrating the intrinsic pathway of coagulation. QCG, QuikClot Combat Gauze; QCX, QuikClot Combat Gauze XL; CTG, Celox Trauma Gauze; CEL, Celox Gauze; HCG, HemCon ChitoGauze; PT, prothrombin time; PTT, partial thromboplastin time; MCF, Maximum Clotting Firmness

Survival

Survival varied amongst groups with 60% (6/10) of the QCG-treated animals surviving through the entire 150 minutes of the experiment (Figure 7). CEL had the highest observed survival rate with 90% (9/10) of the animals surviving, followed by 70 % (7/10) for both QCX and HCG. CTG ranked lowest with only half (5/10) of the treated animals surviving. However, differences amongst groups were not significant by either log-rank test or by chi-squared analysis.

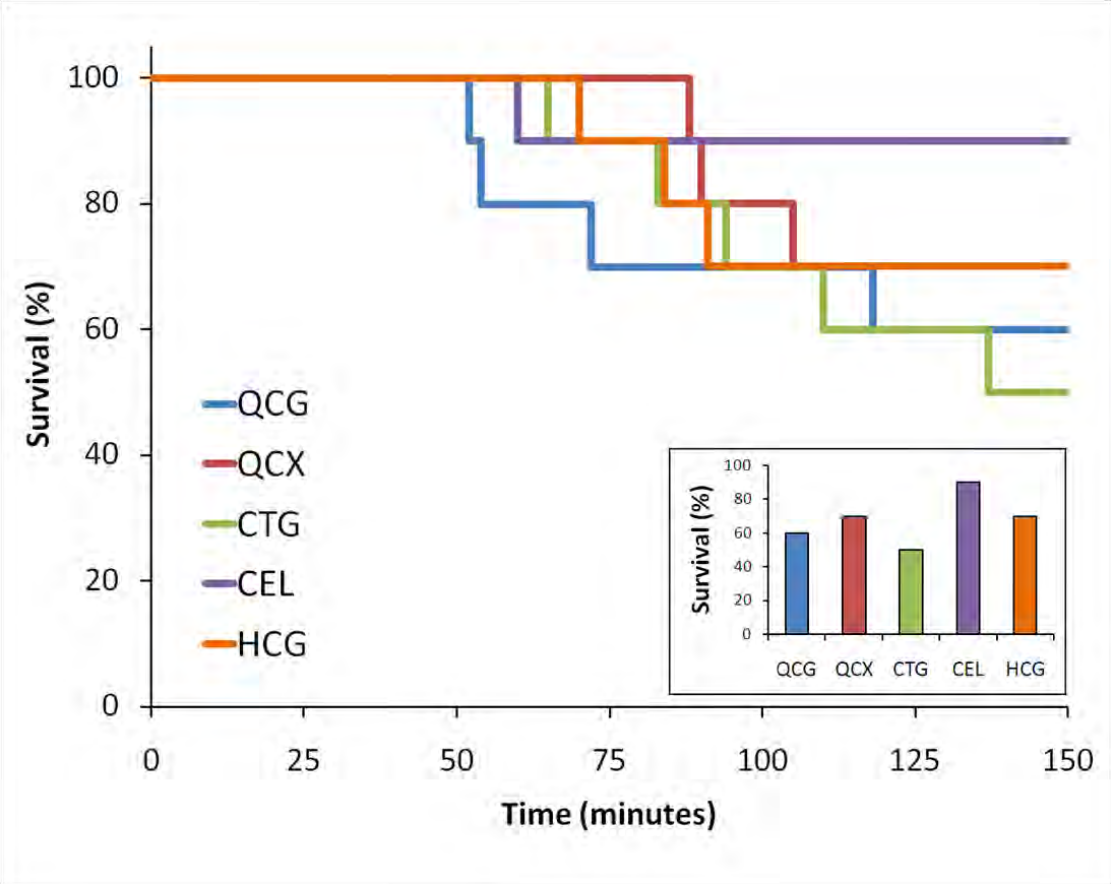

Figure 7. Kaplan-Meier analysis of surviving pigs treated with each test gauze. No significant differences were detected amongst groups (log-rank test). Inset shows survival percentage at end of the experiment.

Morphological and Histological Assessment

Following the completion of the experiment, the gauzes were assessed for their ability to maintain hemostasis following a challenge of repeated leg movements by an experimenter. All gauzes tested retained hemostasis during the leg movement challenge. However, all gauzes tested allowed for free bleeding when gently removed from the wound site indicating the requirement for the gauze to remain in place to be continually effective. All animals that died during the experimentation were examined by necropsy to ensure the all deaths were due to exsanguinations and not an underlying physical condition. No comorbidities were found any of the animals examined.

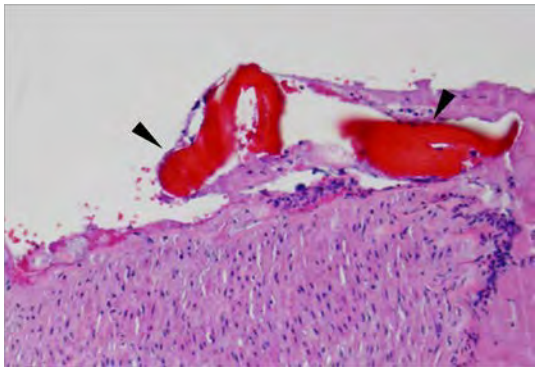

Figure 7. Representative micrograph showing foreign material on the outside of Celox gauze-treated arteries (arrowheads). Hematoxylin and eosin stained (20X).

All animals were subject to histological analysis regardless of outcome. The analysis revealed no significant damage to any of the tissues examined and no differences between groups. All gauzes had some endothelial cell loss near the injury site and minor necrosis of the muscle. There was no apparent lesion in any of the nerve tissue examined. However, linear foreign material was found in all tissues in the CEL group which likely is chitosan, but none was found inside the vessels (Figure 7). The results are summarized in Table 4.

Table 4. Histological Evaluation Summary

	QCG	QCX	CTG	CEL	HCG
Vein	Mild neutrophil transmigration	Mild multifocal neutrophil transmigration	No Significant lesions	Mild neutrophil transmigration	Moderate neutrophil transmigration
Artery	Mild, endothelial loss at injury site	Mild endothelial loss and edema at injury site	Endothelial loss and edema	Moderate endothelial loss and edema	Mild endothelial loss and edema at injury site
Nerve	No Significant lesions	No Significant lesions	No Significant lesions	No Significant lesions	No Significant lesions
Muscle	Mild degeneration and necrosis	Mild degeneration and necrosis	Mild degeneration and necrosis	Mild degeneration and necrosis	Mild degeneration and necrosis

CONCLUSIONS

This study compared the effectiveness of four hemostatic gauzes to the current standard of care, QCG, using a standardized swine model of femoral arterial uncontrolled hemorrhage. Using this model, we compared the efficacy of QCX, CEL, CTG, and HCG to QCG. These test objects reflected the current FDA approved state of the art for hemostatic gauze technology at the onset of this study. All test objects examined in this study performed at least as well as the current standard of care. While some test articles excelled in specific analysis (QCX - significantly better rate of immediate hemostasis and reduced total shed blood, CEL - significantly reduced 10 minute shed blood) no test articles were determined to be deficient as compared to the current standard.

Of particular note, one factor that differentiated QCX and CEL was their mass (nearly twice the mass of other test articles, Table 1). Because QCX exhibited a higher degree of efficacy (immediate hemostasis and 10 minute blood loss) than the traditional and smaller QCG, it may be inferred that the performance differences observed are not necessarily due to the kaolin content of the gauze but rather the total mass of test gauze applied. Unfortunately, this study was not designed to address the question as to whether the differences observed were due to an enhanced tamponade effect produced by increased gauze mass or greater quantities of active ingredients (kaolin, chitosan). Further study may be required to address this question.

Also of note were the measured times for a test article to be fully packed into the injury site. QCG, along with CTG, required the least amount of time while QCX and CEL required the most (Fig 1). These differences in pack time likely result from the larger volumes of gauze present in QCX and CEL (Table 1). Although the pack time amongst gauzes was slight (15 seconds), these differences could prove important during care under fire situations.

The investigators acknowledge that this study has some weaknesses with regard to statistical power and in fact is largely observational. However, upon post-hoc power analysis, we determined that in order to achieve statistical significant amongst groups, at least an additional 15 animals per group would have been required. Therefore it was determined that the n=10 reported here was sufficient to characterize efficacy of the test articles as compared to the current standard. Additionally, it should be noted that the DoD standardized model for

uncontrolled arterial hemorrhage is, to some degree, non-physiologically relevant to clinical/battlefield presentation of hemorrhage. The standardized model incorporates un-realistic resuscitation strategy by design, to align gauze failure with survival outcome, and should be interpreted in the context of testing and evaluation of gauze products in a "worst case scenario". The current study aimed to determine the effectiveness of five hemostatic gauzes and although a practical way to examine these gauzes, the methodology here may not translate directly to use on the battlefield or during emergent care. There are differences between human and swine including blood component ratios and anatomy. Another contrived component of the model is found in the precision of the injury, which is in stark contrast to the battlefield scenario where one would more likely encounter a higher degree of polytrauma and hemorrhage sources not readily amenable to gauze application. Despite these shortcomings, the work here and similar experiments have provided valuable information as to the efficacy of modern hemostatic gauze products.

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