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# Hydrophobically modified chitosan gauze: a novel topical hemostat



Apurva Chaturvedi, BS,<sup>a</sup> Matthew B. Dowling, PhD,<sup>b</sup> John P. Gustin, PhD,<sup>c</sup>  
 Thomas M. Scalea, MD,<sup>b</sup> Srinivasa R. Raghavan, PhD,<sup>b,c</sup>  
 Jason D. Pasley, DO,<sup>a</sup> and Mayur Narayan, MD, MPH, MBA<sup>a,\*</sup>

<sup>a</sup>Program in Trauma, R Adams Cowley Shock Trauma Center, University of Maryland, School of Medicine, Baltimore, Maryland

<sup>b</sup>Fischell Department of Bioengineering, University of Maryland, College Park, Maryland

<sup>c</sup>Department of Chemical & Biomolecular Engineering, University of Maryland, College Park, Maryland

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## ABSTRACT

**Background:** Currently, the standard of care for treating severe hemorrhage in a military setting is Combat Gauze (CG). Previous work has shown that hydrophobically modified chitosan (hm-C) has significant hemostatic capability relative to its native chitosan counterpart. This work aims to evaluate gauze coated in hm-C relative to CG as well as ChitoGauze (ChG) in a lethal *in vivo* hemorrhage model.

**Methods:** Twelve Yorkshire swine were randomized to receive either hm-C gauze ( $n = 4$ ), ChG ( $n = 4$ ), or CG ( $n = 4$ ). A standard hemorrhage model was used in which animals underwent a splenectomy before a 6-mm punch arterial puncture of the femoral artery. Thirty seconds of free bleeding was allowed before dressings were applied and compressed for 3 min. Baseline mean arterial pressure was preserved via fluid resuscitation. Experiments were conducted for 3 h after which any surviving animal was euthanized.

**Results:** hm-C gauze was found to be at least equivalent to both CG and ChG in terms of overall survival (100% versus 75%), number of dressing used (6 versus 7), and duration of hemostasis (3 h versus 2.25 h). Total post-treatment blood loss was lower in the hm-C gauze treatment group (4.7 mL/kg) when compared to CG (13.4 mL/kg) and ChG (12.1 mL/kg) groups.

**Conclusions:** hm-C gauze outperformed both CG and ChG in a lethal hemorrhage model but without statistical significance for key endpoints. Future comparison of hm-C gauze to CG and ChG will be performed on a hypothermic, coagulopathic model that should allow for outcome significance to be differentiated under small treatment groups.

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## Introduction

Trauma deaths are spread indiscriminately across racial and economic backgrounds.<sup>1</sup> In the United States, trauma injuries are responsible for more years of life loss than heart

disease and cancer combined.<sup>2</sup> Hemorrhage has consistently been a major cause of mortality from traumatic injuries in both the civilian and military setting.<sup>3-7</sup> Despite these significant mortality rates from hemorrhage, little technological advancement in hemorrhage-controlling

\* Corresponding author. Program in Trauma, R Adams Cowley Shock Trauma Center, University of Maryland, School of Medicine, Baltimore, MD 21201. Tel.: +1-214-648-6586; fax: +1-214-648-5477.

E-mail address: [mayur.narayan@utsouthwestern.edu](mailto:mayur.narayan@utsouthwestern.edu) (M. Narayan).  
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materials had been made over standard cellulosic gauze or tourniquets until the beginning of the 21st century.<sup>8</sup> However, much effort has been placed into the development of advanced hemostats over the past 15 years. First-generation advanced hemostats, Quickclot Powder and Hemcon Bandage, had technical issues with safety<sup>9,10</sup> and efficacy respectively.<sup>11,12</sup> These gave way to a second generation of advanced hemostats, which included Woundstat, the FAST dressing, and Quickclot Combat Gauze (CG). Of this second generation, CG has proven quite effective in treating severe hemorrhage and has become standard of care for use in the US military.<sup>13</sup>

Ideally, an advanced hemostat for topical use in treating severe hemorrhage would be both inexpensive and effective in treating the highest mortality patients who suffer from coagulopathy and hypothermia. A novel fibrin-based hemostat, the FAST dressing, was demonstrated to be quite effective in treating coagulopathic and hypothermic swine in a lethal hemorrhage model.<sup>14</sup> However, as is the case with all other fibrinogen-based hemostats, the FAST dressing will likely be significantly more expensive and less durable than competing nonfibrinogen-based topical hemostats. In the same study that illustrated the FAST dressing's effectiveness in a coagulopathic and hypothermic swine model, CG was shown to have limited effectiveness under these conditions.<sup>14</sup> Considering these historical data, there still remains an unmet need for the development of a further generation of advanced hemostats which would be both effective in treating the highest mortality hemorrhages and also cost competitive with other topical hemostats.<sup>15</sup>

In past work, hydrophobically modified chitosan (hm-C) and alginate (hm-A) have been shown to promote hemostasis, decrease blood loss, and increase survival in lethal animal models.<sup>16-18</sup> Both hm-C and hm-A are hypothesized to form a nonbiological clot resulting from hydrophobes on the biopolymer backbone interacting with the cell membrane via self-assembly, thereby using blood cells as crosslinks in the formation of a polymer matrix. In addition, hm-C has been shown to increase tissue adhesion, which likely further enhances the modified biopolymer's ability to act as a hemostatic dressing relative to the native biopolymer.<sup>16</sup> It should be noted that although past use of hm-A helps provide historical context to the use of hydrophobically modified polymers for hemostasis, neither hm-A nor any alginate or alginate derivatives were used in the present study.

Because hm-C has previously been effectively used in the form of both a pad and foam for treating lethal hemorrhages in animal models,<sup>16,18</sup> we hypothesize that hm-C would be efficacious in other form factors, such as gauze. This work was undertaken to evaluate hm-C processed into a flexible, packable gauze, which is the most common form of hemostatic dressing encountered by a first responder. Here, we compare hm-C gauze with commercially available CG and ChitoGauze (ChG) with respect to *in vitro* clotting capability, *in vitro* tissue adhesion, and *in vivo* effectiveness in a swine hemorrhage model. Overall, this study illustrates the hm-C Gauze to be at least as effective as CG or ChG in a lethal hemorrhage model.

## Materials and methods

### Materials

Chitosan (Molecular Weight 190-310K) and *n*-decyl aldehyde were obtained from Primex (Iceland) and Sigma Aldrich (St. Louis, Missouri), respectively. Band-Aid First Aid Covers Kling Rolled Gauze, ChitoGauze, Combat Gauze, and Woundstat were purchased from Johnson & Johnson (New Brunswick, New Jersey), HemCon Medical Technologies (Portland, Oregon), Z-Medica (Wallingford, Connecticut), and Trauma-Cure (Bethesda, Maryland) respectively. L-929 Mouse fibroblast cells were purchased from ATCC (Manassas, Virginia). Adult Bovine whole blood with sodium citrate was purchased from Lampire Biological Laboratories (Pipersville, Pennsylvania). Lactated Ringer's Injection USP was obtained from Baxter (Deerfield, Illinois). Hextend Solution was purchased from BioTime, Inc. Eagle's minimal essential medium (EMEM), fetal bovine serum (FBS), penicillin, and streptomycin were obtained from ThermoScientific (Waltham, Massachusetts). Live/Dead assay kit for mammalian cells was purchased from Invitrogen (Grand Island, New York).

### hm-C synthesis and preparation of hm-C gauze

Hm-C was synthesized as previously described.<sup>17</sup> Briefly, six percent of the available amines along the chitosan backbone were modified with *n*-decyl aldehydes to create Schiff bases. The unstable imine groups were reduced to stable secondary amines by sodium cyanoborohydride. Product was precipitated out of solution via dropwise addition of 0.1-M NaOH, and the precipitate was rinsed 10× with ethanol. For gauze preparation, 2 wt% percent solutions of hm-C in 0.1-M lactic acid were prepared. Band-Aid First Aid Covers Kling Rolled Gauze was then soaked in this hm-C solution for 2 h. Subsequently, excess hm-C solution was removed, and hm-C gauze was allowed to air dry overnight. After air drying, hm-C gauze was z-folded and vacuum sealed in airtight aluminum packaging. hm-C gauzes were then sterilized via gamma irradiation at a dose range of 25-40 kGy at Steris Corporation. Figure 1 below shows a picture of the hm-C gauze.

### Diluted blood gelation

A 50/50 solution of bovine heparinized blood and Lactated Ringer's Injection USP was made. This 50/50 solution was then mixed with a one weight percent solution of hm-C or chitosan at a ratio of 1 to 2. After vortexing the mixtures for 30 seconds, the vials were inverted to test for gelation. Similar procedures were followed in which Lactated Ringer's Injection USP was replaced with either normal saline or Hextend.

### Thromboelastography (TEG)

TEG was performed with Woundstat, CG, ChG, and hm-C gauze with bovine blood in a similar manner as previously described.<sup>19</sup> These studies were undertaken to see if any of the gauzes activated the clotting cascade.



**Fig. 1** – Photograph of hm-C gauze. Gauze was aired dried after coating with hm-C solution and then Z-folded for better ease of packing into the wound. (Color version of figure is available online.)

### Biocompatibility studies

L-929 Mouse fibroblast cells were seeded into a 24-well plate at 70,000 cells per well and allowed to grow for 48 h in incomplete EMEM (incomplete EMEM + 5% FBS with 100 I.U./mL penicillin and 100  $\mu$ g/mL streptomycin). Extracts of hm-C gauze were prepared by first testing the absorption of the gauzes. This was achieved by contacting samples with 50 mL incomplete EMEM per gram of sample for 24 h at 37°C and 60 rpm in a glass vial. After 24 h, the volume of the remaining fluid was measured and used to determine the amount of fluid absorbed per gram of sample (mL/g). After sample absorption is determined, 1 g of sample was contacted with complete EMEM in a glass vial at a ratio 5 mL/g greater than the calculated absorption. Extracts were then incubated at 37°C and 60 rpm for 24 h. Extracts were then tested on previously seeded L-929 Mouse fibroblast cells. Each well on the plate was aspirated and replaced with 1.0 mL of extract. The cells were then incubated with the extracts for 72 h at 37°C in humid air with 5% CO<sub>2</sub>. Cells were then imaged observed under 100 $\times$  optical magnification on a confocal microscope (Leica SP5 X). Cells were then washed once with 1.0-mL PBS buffer per well and contacted with 1.0-mL PBS containing 4- $\mu$ M Calcein AM (live stain) and 4- $\mu$ M Ethidium Homodimer (dead stain). Each well was then imaged at 100 $\times$  using a GFP fluorescence filter (ex, 473; em, 520) to observe the live stain and a Texas Red fluorescence filter (ex, 562; em, 624) to observe the dead stain. Fluorescent images were then overlaid to create a composite live-dead image.

### Tissue adhesion studies

Tissue adhesion studies were conducted on CG, ChG, and hm-C gauze in the same manner as described by Wu *et al.*<sup>20</sup> These studies were undertaken to see if any of the gauzes were more adhesive than the others.

### Surgical preparation, instrumentation, procedures

Twelve female Yorkshire pigs, weighing  $37.2 \pm 2.2$  kg, were obtained from the Thomas D. Morris Institute of Surgical



**Fig. 2** – Gelation of blood on the bench. hm-C at 0.6 wt% (left) holds its weight upon vial inversion when mixed with a 50/50 (v/v) blend of blood and Hextend Solution. In contrast, native chitosan at 0.6% (right) remains as a freely flowing viscous liquid when mixed with the same blend. (Color version of figure is available online.)

Research (Reisterstown, MD). All animals were maintained in a facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, and all experiments were performed in accordance with the National Research Council's Guide for the Care and Use of Laboratory Animals. The protocol was approved by the IACUC at the University of Maryland, School of Medicine. The swine were prepared, anesthetized, incubated, placed on mechanical ventilation, and maintained as described in DeCastro *et al.*<sup>16</sup>

Surgical procedures were performed as previously described.<sup>16</sup> All the surgical procedures were performed using standard aseptic methods. The ear vein was cannulated with a Teflon catheter (21-gauge), and lactated Ringer's solution was administered (5 mL/kg/h) throughout the operation to compensate for fluid evaporation. The left femoral artery and vein were cannulated for arterial blood sample collection and intravenous drug and fluid infusion. The arterial line was also connected to a telemetry device that permitted monitoring and recording of blood pressure, heart rate, electrocardiogram, and body temperature of the animals without restricting movement. The animals were randomized to chitosan, hm-chitosan, or gauze dressings. The surgeons were blinded to the type of dressing being used for each animal.

Next, a midline laparotomy was made, followed by a splenectomy to minimize hematologic changes that may occur from autotransfusion by pig's contractile spleen. The blood loss from splenectomy was replaced by infusing LR at three times the weight of the removed organ.

To create a severe hemorrhage in the groin area, approximately 5 cm of the right femoral artery was dissected free from surrounding tissues, and the overlying abductor muscle was removed. Injury to the adjacent femoral vein and nerve was avoided. The vessel was then bathed with a few milliliters of 2% lidocaine to relax vasospasm and dilate the artery to its normal size. A 10-min stabilization period was allowed (no manipulation) and baseline data including mean arterial pressure (MAP) and body temperature were recorded. A stable MAP of 60 mm Hg or higher was required before proceeding with

**Table 1 – Thromboelastogram parameter summary results.**

Dressing type	R (min)	K (min)	Angle deg	LY 30 (%)	A (mm)
Woundstat	4.5 ± 0.3	2.3 ± 0.2	61.3 ± 2.6	0.9 ± 0.2	72.6 ± 1.3
Combat Gauze	4.0 ± 0.2 <sup>*</sup>	2.1 ± 0.2 <sup>*</sup>	65.1 ± 3.3 <sup>*</sup>	1.0 ± 0.2 <sup>*</sup>	71.1 ± 2.0 <sup>*</sup>
ChitoGauze	6.8 ± 0.3 <sup>†</sup>	3.7 ± 0.3 <sup>†</sup>	50.2 ± 2.7 <sup>†</sup>	0.8 ± 0.1 <sup>†</sup>	72.5 ± 1.8 <sup>†</sup>
hm-C gauze	6.5 ± 0.2 <sup>‡</sup>	3.5 ± 0.2 <sup>‡</sup>	55.1 ± 2.3 <sup>‡</sup>	0.9 ± 0.2 <sup>‡</sup>	73.1 ± 1.9 <sup>‡</sup>

Data expressed as mean ± SD.

<sup>\*</sup> versus Woundstat, not significant (NS) (paired t test).

<sup>†</sup> versus Woundstat,  $P < 0.05$ ; versus Combat Gauze,  $P < 0.05$  (paired t test).

<sup>‡</sup> versus Woundstat,  $P < 0.05$ ; versus Combat Gauze,  $P < 0.05$ ; versus ChitoGauze, NS (paired t test).

the rest of the experiment. The artery was clamped proximally and distally and a 6.0-mm diameter arteriotomy was made on the anterior surface of the vessel using a vascular punch. The clamps were then released and free bleeding was allowed for 30 s. The shed blood was collected by suction, weighed and recorded as pretreatment blood loss. Although the femoral artery was bleeding, a dressing was applied to the injury site and manually compressed against the wound with sufficient pressure to occlude arterial blood flow. After a 3-min compression, hemostasis was visually checked; if hemostasis was not achieved or if re-bleeding occurred within the next 10 min, the dressing was removed and replaced with a new dressing of the same type, with an additional 3-min compression. The second dressing was applied under the same conditions as the first one. If hemostasis again was not achieved, a third dressing was applied in the same manner as the second. After failure of a third dressing, hemorrhage was allowed without any additional intervention until the animal exsanguinated. This event was recorded as a failure of the dressing to achieve initial hemostasis; time of death was recorded once MAP and end tidal  $P_{CO_2}$  fell below 20 mm Hg and 15 mm Hg, respectively. The blood and blood clots were collected from the peritoneal cavity and weighed, and the volume of post-treatment blood loss was determined. If hemostasis was achieved and was stable for 10 min, the animal was resuscitated intravenously with warm LR solution (three times the volume of pre-treatment blood loss at 100 mL/min) to its baseline, pre-injury MAP. The abdominal incision was then closed in layers.

Animal survival was defined as the presence of a heart rate at the end of 180 min. Any surviving animals at the end of the study period were euthanized with pentobarbital IV 100–200 mg/kg.

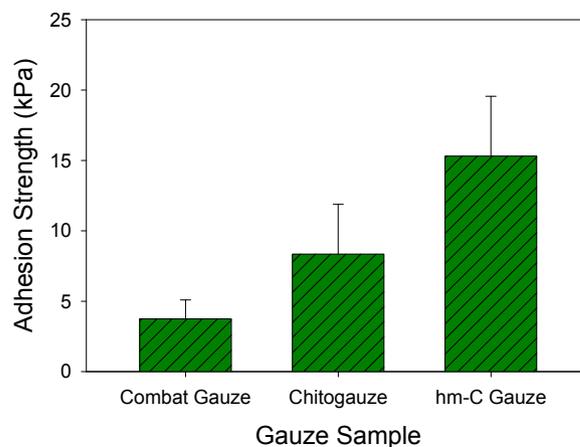
#### Data analysis

Data are expressed as mean ± standard deviation and analyzed by analysis of variance (paired t test), Fisher exact, and log rank for statistical comparisons.  $P$  values were adjusted according to false discovery rate method for bi-group comparison. The data with high variance were log transformed for analysis of variance. Statistical significance was assigned at a >95% confidence level ( $P < 0.05$ ).

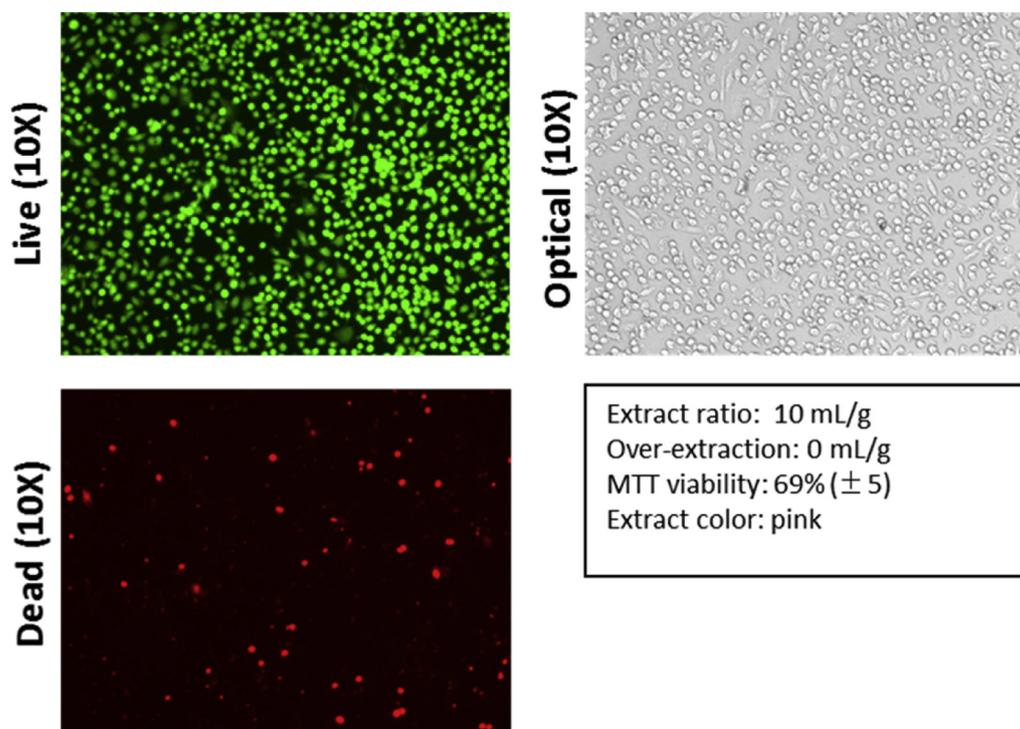
## Results

### In vitro

To demonstrate hm-C's ability to form strong, nonbiological clots even under coagulopathic conditions, hm-C at 0.6 wt% percent was added with 50/50 mixtures of blood with Hextend. The resulting mixing was able to hold its own weight when inverted which suggests the formation of a strong gel (Fig. 2). This result is significant because coagulopathy often occurs during traumatic bleeding; loss of clotting factors in addition to blood dilution with resuscitation fluid creates a very difficult set of circumstances to achieve hemostasis. Agents or materials which can halt blood flow under hemodiluted conditions are of interest because traditional and a number of advanced hemostats have been observed as ineffective under coagulopathic conditions.<sup>14</sup> Furthermore, TEG studies were undertaken to look at the ability of the hm-C gauze to activate the natural clotting cascade. As shown in Table 1, the blood clotting activities of both the hm-C gauze and ChG were decreased when compared to that of Woundstat and CG. This is not all the



**Fig. 3 – Tissue adhesion strength of gauze samples. Tissue adhesion strengths of gauze samples were tested via strain-gage instrument. Combat Gauze showed low (< 5 kPa) adhesion strength, whereas ChitoGauze and hm-C gauze displayed appreciable (> 5 kPa) adhesion strengths, with hm-C Gauze at a mean strength of 15.3 kPa. (Color version of figure is available online.)**



**Fig. 4 – Cytotoxicity of hm-C gauze. Left images show live-dead stains of hm-C gauze extracts after 72 h. Right image shows optical transmission of cells after 72 h. Bottom right corner displays the extraction ratio used, as well as MTT viability of cells after 72 h. (Color version of figure is available online.)**

unexpected because chitosan-based products are thought to be effective at stopping hemorrhaging due to chitosan's mucosal adhesive properties not through any inherent hemostatic capability unlike CG and Woundstat, which accelerate the clotting process by concentrating clotting factors.

Additionally, all gauzes examined in this work were subjected to tissue adhesion experiments. Results of these studies are seen in Figure 3. Both chitosan-based gauzes were more adhesive than CG. This is not unexpected due to the different

modes of hemostatic action between CG and chitosan-based products. Furthermore, hm-C gauze was significantly more tissue adherent than ChG ( $15.3 \pm 4.2$  kPa versus  $8.4 \pm 3.5$  kPa).

Initial qualitative biocompatibility studies were undertaken. These experiments took the form of live-dead assays on L-929 Mouse fibroblast cells that had been incubated in extracts of hm-C gauze. These qualitative studies showed no significant cell death from visual inspection as displayed in Figure 4 below.

**Table 2 – Baseline parameters and animal characteristics.**

Variable	Combat Gauze group (mean $\pm$ SD)	ChitoGauze group (mean $\pm$ SD)	hm-C group (mean $\pm$ SD)
Body weight (kg)	40.5 $\pm$ 1.8	40.0 $\pm$ 0.4	41.1 $\pm$ 0.8
Body temp ( $^{\circ}$ C)	37.28 $\pm$ 0.51	37.08 $\pm$ 0.41	37.10 $\pm$ 0.46
Hematocrit (%)	31.6 $\pm$ 1.2	30.8 $\pm$ 1.3	31.0 $\pm$ 1.3
Hemoglobin (g/dL)	10.69 $\pm$ 0.47	11.64 $\pm$ 0.21	10.68 $\pm$ 0.50
Platelets (1000/ $\mu$ L)	304 $\pm$ 44	306 $\pm$ 40	302 $\pm$ 60
PT (s)	10.7 $\pm$ 0.8	10.6 $\pm$ 1.0	10.6 $\pm$ 1.0
aPTT (s)	16.7 $\pm$ 0.93	16.6 $\pm$ 0.97	15.8 $\pm$ 1.22
Fibrinogen (mg/dL)	217 $\pm$ 45	208 $\pm$ 29	219 $\pm$ 24
pH	7.41 $\pm$ 0.04	7.44 $\pm$ 0.06	7.45 $\pm$ 0.04
Preinjury MAP (mm Hg)	66.0 $\pm$ 4.0	68.8 $\pm$ 4.8	65.8 $\pm$ 7.0

PT = prothrombin time; aPTT = activated partial thromboplastin time; MAP = mean arterial pressure.

Data expressed as mean  $\pm$  SD.

Statistical differences between groups was not significant ( $P > 0.05$ ).

**Table 3 – Outcomes for treatment of a severe arterial hemorrhage with different hemostatic dressings in swine.**

Dressing type	Number of animals	Number of dressings used	Pretreatment blood loss (mL/kg)	% Initial hemostasis achieved <sup>†</sup>	Post-treatment blood loss (mL/kg)	Duration of hemostasis (h)	Survival time (h)
Combat Gauze <sup>*</sup>	4	7	7.9 ± 2.1	75 (3/4)	13.4 ± 15.1	2.25 ± 1.5	2.32 ± 1.37
ChitoGauze	4	7	8.3 ± 2.5	75 (3/4) <sup>‡</sup>	12.1 ± 13.3 <sup>  </sup>	2.25 ± 1.5 <sup>¶</sup>	2.39 ± 1.21 <sup>¶</sup>
Hm-C gauze	4	6	7.7 ± 1.7	100 (4/4) <sup>§</sup>	4.7 ± 3.1 <sup>§</sup>	3 <sup>#</sup>	3 <sup>#</sup>

Data expressed as mean ± SD.

<sup>\*</sup> Sample testing was stopped after 3 unsuccessful experiments.

<sup>†</sup> Initial hemostasis was considered to occur after when bleeding was stopped for at least 3 min after compression.

<sup>‡</sup> versus Combat Gauze, not significant (NS) (fisher exact test).

<sup>§</sup> versus Combat Gauze, NS; versus Chitogauze, NS (fisher exact test).

<sup>||</sup> versus Combat Gauze, NS (paired t test).

<sup>¶</sup> versus Combat Gauze, NS (log-rank test).

<sup>#</sup> versus Combat Gauze, NS; versus Chitogauze, NS (log-rank test).

## In vivo

Table 2 illustrates the baseline parameters and characteristics of the animals used. After these animals' characteristics were measured, the animals were randomly assigned to treatment groups. The outcomes of these *in vivo* experiments are summarized in Table 3. Control groups ( $n = 4$ ) of both CG and ChG were able to achieve initial hemostasis in 75% of animals using a total of seven bandages. Performing slightly better, the hm-C gauze was able to achieve hemostasis in 100% of animals while using only six bandages. In addition, duration of hemostasis was longer for hm-C gauze group (3 h) when compared to CG (2.25 h) and ChG (2.25 h). Although the overall survival, duration of hemostasis, and number of dressings used are not significantly different among treatment groups, the post-treatment blood loss is significantly lower for the

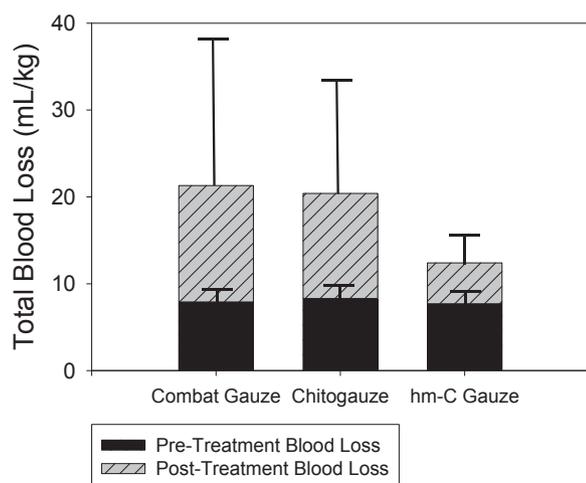
hm-C gauze group when compared to other treatment groups, as displayed in Figure 5.

A Kaplan–Meier curve of survival data is presented in Figure 6. This figure shows that all animals in the hm-G gauze treatment group survived for the entire 3-h duration of the experiments, whereas only three of four survived for the control groups of CG and ChG, where one animal deceased in each group at 16 and 34 min, respectively.

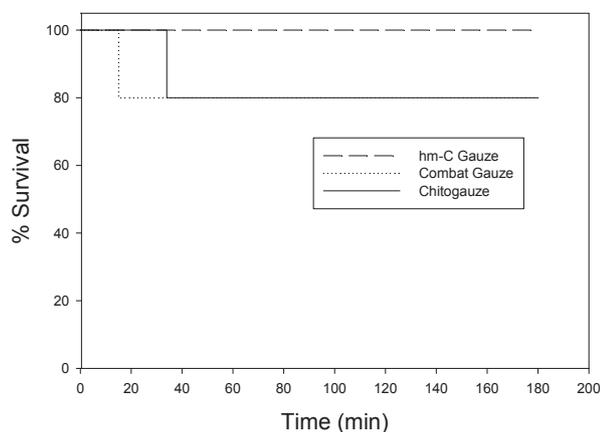
## Discussion

This work utilizes a lethal arterial injury model in swine to evaluate the hemostatic capability of three gauzes: CG, ChG, and hm-C gauze. CG was chosen as a point of comparison because it currently is the current standard of care for hemorrhage control in the military setting.<sup>13</sup> In addition to Combat Gauze, ChG, a gauze coated with native chitosan, was chosen as another hemostatic product to compare to hm-C gauze. Table 3 and Figures 5–6 illustrate the outcomes of these animal model experiments. The hm-C gauze performed at least as well as both CG and ChG in terms of overall survival, numbers of dressings used, and duration of hemostasis. Interestingly, post-treatment blood loss was significantly in the hm-C gauze group when compared the two other gauzes. Post-treatment blood loss has been shown to correlate with survival in other similar studies.<sup>14,16</sup> Given this correlation, one could speculate that if a larger group of animals were studied that hm-C gauze outperformance of CG and ChG would be significant.

In addition to *in vivo* studies, this work undertakes a number of *in vitro* studies which serve to demonstrate both the increased hemostatic capabilities of hm-C gauze and the difference in hemostatic action between chitosan-based and mineral-based hemostatic products. Chitosan-based products work by strongly adhering to surrounding tissue which plugs the wound in a similar fashion to a beaver dam stopping the flow of water in a stream. This mechanism is supported by Figure 3 which shows that both ChG and hm-C gauze have significantly increased tissue adhesion strength when compared to CG. It has been speculated that one of the



**Fig. 5 – Total blood loss in porcine injury models. Average values of pretreatment and post-treatment blood losses of pigs treated with 3 hemostatic gauze dressings were summed to give total blood loss from femoral artery injury. The post-treatment blood loss was less in hm-C Gauze group ( $n = 4$ ) as compared with Combat Gauze ( $n = 4$ ) and ChitoGauze ( $n = 4$ ) controls.**



**Fig. 6 – Kaplan–Meier analysis of survival data. The hm-C gauze (dashed line) maintained hemostasis and supported survival of animals for the full 180 min of observation testing. There was one death in both the Combat Gauze (dotted line) and ChitoGauze (solid) groups at 16 and 34 min, respectively. The remaining animals in Combat Gauze and ChitoGauze groups survived the full 180-min period.**

reasons hm-C itself has consistently outperformed native chitosan in term of hemostatic capability is increased tissue adhesion. Tellingly in this study, the hm-C gauze is significantly more tissue adherent than ChG which suggests superiority in hemostatic potential of the hm-C gauze. Unlike chitosan-based hemostats, mineral-based products work by absorbing blood which concentrates clotting factors and facilitates the activation of the natural clotting cascade. This mechanism is supported by Table 1 which illustrates the TEG results on extracts of hm-C gauze, ChG, CG, and Woundstat. As seen in this table, both CG and Woundstat demonstrate enhancement of the blood's natural clotting cascade when compared to the hm-C gauze and ChG. Overall, both Figure 3 and Table 1 show differences in hemostatic action between chitosan-based and mineral-based hemostatic products.

Early attempts at creating advanced hemostatic products resulted in some products, such as the Quickclot Powder and Woundstat, causing adverse side effects.<sup>9,10,19</sup> For this reason, initial biocompatibility studies were conducted on the hm-C gauze. As seen in Figure 4, no significant cell death was seen when extracts of hm-C gauze were exposed to L-929 Mouse fibroblast cells.

## Conclusions

This work has shown that hm-C gauze was equivalent or better than CG and ChG in performance in treating a lethal hemorrhage model. In this model, hm-C gauze did significantly lower post-treatment blood loss which suggests in a larger study that hm-C gauze may significantly improve overall survival. Initial data demonstrate hm-C can gel hemodiluted blood (Fig. 2), which indicates hm-C gauze may be effective in treating coagulopathic patients. To further demonstrate hm-C gauze's clinical potential, future studies

will be conducted on a hypothermic and coagulopathic hemorrhage model which has shown CG to be ineffective.

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