

Evaluation of NuStat[®], a Novel Nonimpregnated Hemostatic Dressing, Compared With Combat Gauze[™] in Severe Traumatic Porcine Hemorrhage Model

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ABSTRACT

Background: Uncontrolled hemorrhage remains one of the most challenging problems facing emergency medical professionals and a leading cause of traumatic death in both battlefield and civilian environments. Survival is determined by the ability to rapidly control hemorrhage. Several commercially available topical adjunct agents have been shown to be effective in controlling hemorrhage, and one, Combat Gauze[™] (CG), is used regularly on the battlefield and for civilian applications. However, recent literature reviews have concluded that no ideal topical agent exists for all injuries and scenarios. The authors compared a novel nonimpregnated dressing composed of cellulose and silica, NuStat[®] (NS), to CG in a lethal hemorrhagic groin injury. These dressings were selected for their commercial availability and design intended for control of massive hemorrhage. **Methods:** A complex penetrating femoral artery groin injury was made using a 5.5mm vascular punch followed by 45 seconds of uncontrolled hemorrhage in 15 swine. The hemostatic dressings were randomized using a random sequence generator and then assigned to the animals. Three minutes of manual pressure was applied with each agent after the free bleed. Hextend[™] bolus (500mL) was subsequently rapidly infused using a standard pressure bag along with the addition of maintenance fluids to maintain blood pressure. Hemodynamic parameters were recorded every 10 minutes and additionally at critical time points defined in the protocol. Primary end points included immediate hemostasis upon release of manual pressure (T0), hemostasis at 60 minutes, and rebleeding during the 60-minute observation period. **Results:** NS was statistically superior to CG in a 5.5mm traumatic hemorrhage model at T0 for immediate hemostasis ($p = .0475$), duration of application time ($p = .0093$), use of resuscitative fluids ($p = .0042$) and additional blood loss after application ($p = .0385$). NS and CG were statistically equivalent for hemostasis at 60 minutes, rebleeding during the study, and the additional secondary metrics, although the trend indicated that in a larger sample size, NS could prove statistical superiority in selected categories. **Conclusions:**

In this porcine model of uncontrolled hemorrhage, NS improved immediate hemorrhage control, stability, and use of fluid in a 60-minute severe porcine hemorrhage model. In this study, NS demonstrated equivalence to CG at achieving long-term hemostasis and the prevention of rebleed after application. NS was shown to be an efficacious choice for hemorrhage control in combat and civilian emergency medical service environments.

KEYWORDS: EMS, hemostatic dressing, uncontrolled hemorrhage, severe hemorrhage, traumatic injuries, NuStat, NS, CG, silica, bamboo, cellulose, Combat Gauze, kaolin

Introduction

Despite advances in combat casualty care and civilian emergency medical service (EMS) response and training, almost 50% of current combat fatalities in Iraq and Afghanistan before evacuation and up to 80% of civilian trauma fatalities within the United States are attributed to uncontrolled hemorrhage.¹⁻⁴ Efficacious and rapid hemorrhage control is crucial for decreased morbidity and mortality. 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 researched and adopted many newly developed agents, including redesigned tourniquets, hemostatic agents, and wound dressings to address these needs.⁵⁻⁷ Given the intended use of the dressings in trauma and battlefield applications, these dressings and agents are also applicable for civilian use and application.

QuikClot[®] (ZMedica Corp.; www.z-medica.com/healthcare), HemCon Bandage[®] (HemCon, Inc.; <http://www.hemcon.com/>), ChitoFlex[®] (HemCon, Inc.; <http://www.hemcon.com/>), and Combat Gauze[™] (Z-Medica Corp.; www.z-medica.com/healthcare) have been used by the military for battlefield trauma applications. These hemostatic agents were effective in external hemorrhage control, but varying limitations were found with human

use. These limitations include difficulty placing them at the exact site of a hemorrhage and reactions that can potentially cause tissue damage. 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 (CG).⁸⁻¹⁰

CG is a thin, flexible, nonwoven rayon–polyester blended (50% rayon and 50% polyester) gauze impregnated with mineral kaolin powder (Figure 1). Kaolin is a naturally occurring, inorganic mineral that does not contain any botanicals, biologic material, or shellfish products, and has not been shown to produce an exothermic reaction or cause vascular complications.^{8,9,11}



Figure 1 *Combat Gauze is an impregnated rayon–polyester blend dressing currently used by civilian emergency medical service and battlefield medical personnel.*

NuStat® (NS) (Beeken Biomedical; <http://www.beekenbiomedical.com/nustat.html>) is a novel hemostatic dressing with a unique combination of two fibers: silica-based fibers and regenerated cellulose (Figure 2). NS contains no pharmaceutical or biologic agents. The mechanisms underlying NS's hemostatic properties encompass both cellular and molecular interactions, including platelet activation and amplification of the intrinsic coagulation cascade through activation of factor XII.¹² The silica- and cellulose-based fibers enhance platelet recruitment to the wound site via surface-charge-mediated interactions and amplify the activation and aggregation of platelets. The dressing also triggers the coagulation cascade by amplification of protein–enzyme reactions in the blood and accelerates the formation of platelet–fibrin networks.^{13,14}

Figure 2 *NuStat is a knit dressing of regenerated cellulose and silica currently used for emergency medical service, burn, and trauma applications by medical, law enforcement, and civilian personnel.*



Our study compared the efficacy of CG with NS in controlling arterial hemorrhage in a well-published and validated porcine traumatic femoral wound model.

Methods

Study Design

This study was a randomized, controlled, unblinded preclinical trial using a swine model of acute femoral hemorrhage. Two intervention groups of eight animals and seven animals were examined using two different hemostatic agents, NS and CG, respectively. Standard dosages and techniques, as recommended by the manufacturer, were used. Prior to the study, the individual applying the dressing (applicator) had previously done so with each dressing more than 50 times. NS was provided directly from the manufacturer and CG was purchased from Boundtree Medical (www.boundtree.com).

The protocol was approved by the Institutional Animal Care and Use Committee. All research was conducted in compliance with the Animal Welfare Act. The animals received humane care in accordance with the “Guide for the Care and Use of Laboratory Animals.”¹⁵

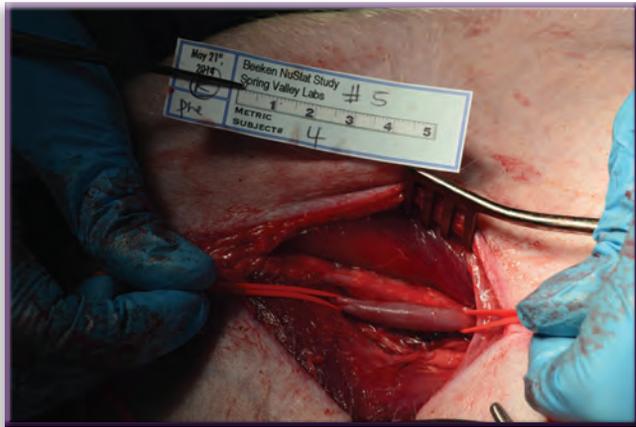
Animal Subjects

The study was conducted in the controlled environment of a veterinary surgical suite designed to accommodate up to two subjects at any one time. Farm-raised, Yorkshire swine (*Sus scrofa*, Blackwater Farms, Franklin, VA, USA) were chosen as study subjects. This choice was made due to the reliability of swine as a cardiovascular model and the ease of accessibility. Subjects were randomized and required to be within age and weight ranges per the protocol.

Study Protocol

The 15 swine were fed a standard diet and observed for a minimum of 5 days. Animals were fasted the night prior to the procedure and water was provided ad libitum. Anesthesia was induced with an intramuscular injection of ketamine (20mg/kg) and 5% inhaled isoflurane via face mask for 3 minutes. Maintenance anesthesia was set at 2% isoflurane after endotracheal intubation, and the animal remained breathing spontaneously on 21% oxygen and air administered from an MDS Matrx VMC small-animal anesthesia machine (Matrx Medical; www.henryschein.com/us-en/Matrx/default.aspx) for the duration of the procedure. The animal was placed supine on the operating table with the front legs secured to allow adequate access to the neck. The right carotid artery and external jugular vein were then exposed via a cutdown technique. A 22-gauge catheter was used to cannulate the carotid artery for continuous arterial blood pressure monitoring. The external jugular vein was cannulated

Figure 3 Dilated right femoral artery prior to injury.



with a 20-gauge catheter for infusion of resuscitative fluid. Continuous temperature monitoring was achieved via placement of an indwelling rectal probe, and an electric table warmer and blankets maintained a core body temperature of 36°C to 38°C.

After preparation of the animal, baseline vital signs were recorded every 10 minutes before proceeding with the creation of the groin injury. During this period, anesthesia was titrated to adequate surgical pain threshold, as determined by a motor response to hoof and jaw tone stimulation.

Subjects were placed in dorsal recumbency. A complex injury to the right femoral artery was then created to produce uncontrolled hemorrhage, as previously described by Keirabati et al. (Figure 3).¹⁶ A No. 10 blade scalpel was used to create an oblique superficial skin incision perpendicular to the right inguinal crease, 10cm long, at a inferiolateral angle from the inferiormost right nipple. Subsequently, gentle, blunt dissection exposed the adductor fascial layers. This plane was then followed cranially toward the inguinal canal, until faint direct visualization of the femoral vascular bundle was achieved. On visualization, the belly of the adductor muscle was removed and a minimum of 3cm of femoral artery was exposed. A fasciotomy was performed on the right femoral artery prior to a 2% lidocaine bath for a minimum of 2 minutes to achieve a diameter of 5mm or greater. Atraumatic vessel loops were applied proximal and distal to the exposed section of femoral artery to ensure vascular control during creation of injury. Skin incision length, vessel exposure length, vessel predilation and postdilation diameter, mean arterial pressure (MAP), temperature, heart rate (HR), and oxygen saturation levels were recorded prior to injury to ensure all metrics were within study parameters. Cavity volume was measured using displacement weight of saline (1mL equals 1g of saline). When subjects met the appropriate criteria, a hemorrhagic injury was created in the following manner: Using a No. 11 blade, a direct 3mm to 4mm incision

of the femoral artery was made followed by insertion of a 5.5mm vascular punch into the incision to create an arteriotomy wound in the vessel. Once the wound was inflicted, the subject was allowed to bleed, unimpeded, for 45 seconds before treatment was applied. All blood was collected for the first 30 seconds of free bleeding and recorded to calculate a blood loss per minute rate to ensure no statistical difference between injuries and groups. The remaining 15 seconds of free bleeding was used to fill the wound cavity. Any excess blood not remaining in the wound cavity was collected and used to calculate additional blood loss after the 30-second bleed.

After creation of the injury and 45 seconds of hemorrhage, the randomized predetermined dressing was handed to and applied by the applicator (Figures 4 and 5). Application time and blood loss during application was recorded. After application, direct pressure was applied for 3 minutes. On release of compression, a 500mL bolus of Hextend® (Hospira Inc.; <http://www.hospira.com/>) was infused via rapid-infuser pressure bag and maintenance fluids were given at a rate of 2mL/kg/hr. Vital signs (MAP, HR, temperature, and oxygen saturation level) were recorded prior to injury, at time of dressing application, after the 3-minute compressive

Figure 4 Combat Gauze application.



Figure 5 NuStat application.



hold (T0), at 5 minutes, 10 minutes, and every 10 minutes thereafter, until study termination at 60 minutes (T60). A minimum MAP during hemorrhage period was also recorded to ensure no statistical differences between groups. The dressing was visually evaluated for immediate hemostasis, defined as no visible blood leaving the wound cavity, as well as for continued hemostasis for the duration of the test period.

At 60 minutes, the dressing was evaluated for hemostasis (Figures 6–8). Range of motion (ROM) testing was conducted on the subjects, involving three each of flexion and extension and three rotational movements. The dressing was again evaluated for continued hemostasis with ROM. Dressing was then removed and ease of removal was evaluated on a 1-to-5 scale, where 1 = very easy to remove and 5 = very difficult to remove. Hemostasis upon removal was evaluated. Study termination vital signs were recorded to include HR, MAP, oxygen saturation level, and temperature. At this time, 20mL of sodium pentobarbital was administered via central line. Animal heart sounds were evaluated.

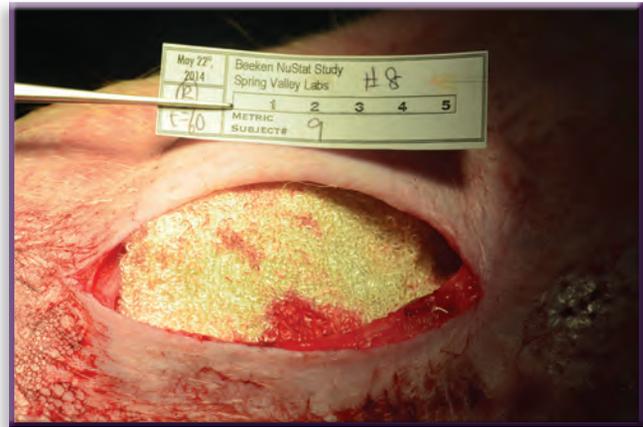
Figure 6 Validation of injury post 60-minute observation period.



Figure 7 Combat Gauze during 60-minute observation window.



Figure 8 NuStat during 60-minute observation window.



End Points

Primary end points for this study included hemorrhage control defined as yes or no at T0 and T60 after release of compression and rebleeding during study. Secondary end points were total blood loss after application of dressing as assessed by the difference between the dry weight and the wet weight of the dressing after 60 minutes; additional blood loss during application; length of application time; blood absorbed by dressing, fluids needed, excluding baseline and Hextend bolus; and hemostasis with ROM.

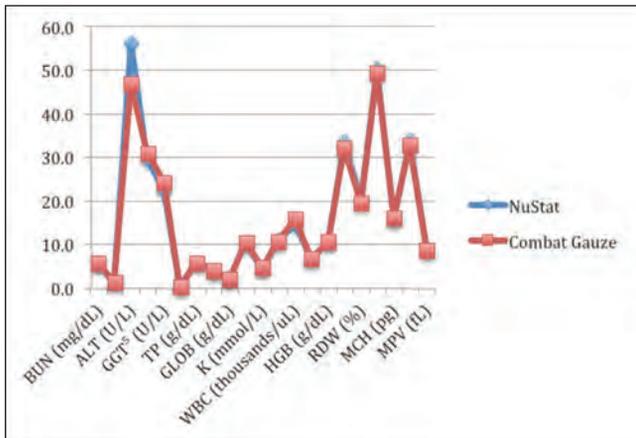
Statistical Analysis

All statistical analyses were performed using Kaleida Graph version 4.5 (Synergy Software; http://www.synergy.com/wordpress_650164087/). Statistical significance was assessed using an α level of .05. Descriptive statistics were determined for each outcome measure within gauze type to examine differences in various outcome measures between NS and CG. Given the small sample size, the Berger binomial two-sided Fisher exact test was used to evaluate the binary data and Wilcoxon paired *t*-test was used to evaluate the continuous data.

Results

There were no baseline differences between the two groups for vital signs, weight, vessel size, cavity size, or baseline laboratory findings (Figure 9, Table 1). Due to multiple complications and inability to meet baseline criteria, one animal in the CG group was excluded, resulting in seven animals treated with CG and eight with NS. Immediate hemostasis was achieved in four animals in the CG group and eight animals in the NS group, which produced a statistically significant result ($p = .0475$) (Table 2). Additionally, a statistically significant difference was discovered for postapplication blood loss (0 ± 0 vs $1,218.3 \pm 807.7$; $p = .385$), quantity of NS needed to maintain MAP (174 ± 214 vs 842 ± 1291 ; $p = .0042$), and dressing application time (31 ± 7 vs 38 ± 2 ; $p = .0093$) (Table 3). All the animals in both groups survived.

Figure 9 Baseline laboratory measures were taken to ensure no subset of animals had a metabolic or clotting anomaly that might bias the study. No statistical differences between baseline values were found.



Note: ALT, alanine aminotransferase; BUN, blood, urea, nitrogen; GGT, γ -glutamyl transpeptidase; GLOB, globulin; HGB, hemoglobin; K, potassium; MCH, mean corpuscular hemoglobin; MPV, mean platelet volume; TP, total platelets; WBC, white blood cell count.

Discussion

Uncontrolled hemorrhage remains one of the primary causes of morbidity and mortality in civilian and combat trauma. Medical and emergency providers require methods to safely, effectively, and expediently control hemorrhage in the prehospital and battlefield setting. It is well researched and understood that minimizing blood loss improves survival. There are many modalities of hemostatic agent on the market, but the investigators were intrigued by the pliability, size, and packability of NS, and its lack of embedded biologic agent. The investigators sought to perform a comparative study to evaluate equivalence between NS and the currently recommended dressing, CG, for evaluation of a novel adjunct dressing.

Table 2 Primary End Points*

Primary Metrics	NuStat, n = 8 (Y/N)	Combat Gauze, n = 7 (Y/N)	Fisher Exact Test p Value
Immediate hemostasis (T0)	8/0	4/3	.0475**
Hemostasis at T60	8/0	6/1	.4667
Rebled during study	1/7	3/4	.2821

Notes: N, no; T0, time = 0 minutes; T60, time = 60 minutes; Y, yes.
*The primary end points of this study were binary Yes or No criteria at T0, T60, and rebled during the 60-min observation window.
**Statistically significant.

Kheirabadi et al.¹⁶ at the Institute for Surgical Research in San Antonio, Texas, originally developed the 6.0mm femoral punch model. The investigators used a 5.5mm arteriotomy punch, which we do not believe compromised the model, as all injuries across both groups were created equivalently, as demonstrated by comparative baseline metrics. It is important to note that many different groups have tested dressings in a swine femoral punch model. All will attest to the importance of creating an injury in which the porcine tissue will not contribute to the cessation of hemorrhage. For example, it is necessary to make an injury in which the vessel will not retract, vasospasm, or tamponade by the surrounding muscle, or without a sufficient length of vessel exposure. This model addresses those concerns. Although this model is not representative of wounds one may see in the field or in the emergency department, it is reproducible, validated, challenging, and broadly studied.

This model allowed us to successfully demonstrate the differences between commonly used hemostatic dressings and NS. Although this study was intended to show

Table 1 Baseline Metrics*

Baseline Metrics	NuStat (n = 8)			Combat Gauze (n = 7)		
	Mean	SD	Median	Mean	SD	Median
Weight, kg	39.3	2.6	40.7	43.5	1.7	43.5
Temperature, °C	99.4	1.5	99.3	99.2	2.1	99.9
O ₂ sat, %	92.6	5.1	94.0	95.1	1.8	95.5
HR, bpm	82.9	15.7	76.0	78.4	7.7	77.5
MAP, mmHg	84.6	14.2	83.0	88.8	12.5	85.5
Diameter pre-lidocaine, mm	3.7	1.1	3.0	3.8	1.0	3.5
Diameter post-lidocaine, mm	5.7	1.0	5.0	5.4	0.7	5.0
30-sec bleed volume, mL	325.1	188.1	320.4	362.3	155.7	438.6
Cavity volume, mL	43.7	15.4	50.0	40.8	17.4	38.5
Cavity depth, cm	3.3	0.8	3.0	3.0	0.4	2.9

Notes: HR, heart rate; MAP, mean arterial pressure; O₂ sat, oxygen saturation; SD, standard deviation.

*Clinically relevant baseline metrics were recorded and analyzed. There were no statistical differences between groups for any baseline metric.

Table 3 Secondary End Points*

Secondary Metrics	NuStat (n = 8)			Combat Gauze (n = 7)			p Values
	Mean	SD	Median	Mean	SD	Median	
Additional blood loss during application, mL	189.7	107.3	166.3	225.6	171.2	244.3	0.4348**
Additional blood loss during 60-min observation, mL	0	0	0	1218.3	807.7	0	0.0385**†
Application time, sec	31	7	32	38	2	40	0.0093**†
Blood absorbed by dressing, mL	137.6	29.2	137	89.7	31.6	90.2	0.1276**
Resuscitation fluids used to maintain MAP, mL	174	214	100	842	1291	425	0.0042**†
Hemostatic with ROM testing, Y/N	Y=5	N=3	—	Y=1	N=6	—	0.1189§

Notes: MAP, mean arterial pressure; N, no; ROM, range of motion; SD, standard deviation; Y, yes.

*Secondary end points were chosen to reflect stability of the patient as well as resources needed to stabilize the patient for a 60-min observation window.

**Two-tailed paired *t* test.

†Statistically significant (*p* < .05).

§Fisher exact test.

equivalence between NS and CG, statistical superiority of NS at T0 was proven. This study provides data to demonstrate NS's superiority at achieving immediate hemostasis in traumatic hemorrhage. Likely due to initial hemostasis, additional blood loss and fluids needed to maintain MAP during the 60-minute observation were also significantly less. The investigators feel this is also, in part, due to the ability to rapidly apply the NS versus CG. There was a 7-second average differential between the two groups in application time. At the rate these animals were bleeding, that could equate to between 60mL to 100mL of blood. Given what we know about CG's mechanism of action, that relatively small volume of blood is sufficient to begin washing away the impregnated kaolin. It is interesting to note the performance of the two dressings during ROM testing: NS (yes = 5/no = 3) compared with CG (yes = 1/no = 6). Although the *p* value was not significant (*p* = .1189), it is possible that with a larger sample size, NS might show superiority. Given the environments these dressings are applied in and the need to move and transport many of the recipients, this is a significant end point to analyze.

Limitations and Future Research

This study was limited by the small sample size. Using published data of CG and anecdotally obtained data on NS, a power analysis was performed (G-Power; www.gpower.hhu.de/). The power analysis revealed a need for 83 subjects in each group to show superiority for all three primary metrics. This is both logistically and financially unfeasible. Due to these limitations, our study was powered to show equivalence between groups. In the future, it would be beneficial to conduct a study with a larger sample size to achieve significance for all primary outcomes. A second major limitation was blinding

the applicator. Although the surgeon was blinded to the dressing for which they were making the injury, the applicator was not blinded, due to textural and size differences between products. The applicator was not informed which dressing they would apply and had no knowledge of the product until they removed it from the dressing tray after the bleed was complete. However, once the applicator picked up the dressing they were able to discern a difference. A blinded study would be preferable, but there are practical limitations that make this very difficult due to dissimilarity of dressings.

Conclusions

NS is efficacious for immediate control of life-threatening hemorrhage in a well-described and validated, traumatic, 5.5mm punch arteriotomy porcine model. NS required less resuscitation fluid and allowed less blood loss after dressing application over the course of the 60-minute observation window. NS had trends toward superiority over CG in all additional primary and secondary metrics outcomes; however, these trends failed to reach statistical significance. In this study, NS showed superiority or equivalence to CG for all metrics evaluated.

Disclosures

The authors have nothing to disclose.

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