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Chitosan-based lifefoam improves survival in lethal noncompressible abdominal bleeding in swine

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ABSTRACT

Background: In military combat settings, noncompressible closed cavity exsanguination is the leading cause of potentially survivable deaths, with no effective treatment available at point of injury. The aim of this study was to assess whether an expanding foam based on hydrophobically modified chitosan (hm-chitosan) may be used as a locally injectable hemostatic agent for the treatment of noncompressible bleeding in a swine model.

Methods: A closed-cavity, grade V hepato-portal injury was created in all animals resulting in massive noncoagulopathic, noncompressible bleeding. Animals received either fluid resuscitation alone (control, $n = 8$) or fluid resuscitation plus intraperitoneal hm-chitosan agent through an umbilical port (experimental, $n = 18$). The experiment was terminated at 180 minutes or death (defined as end-tidal CO_2 <8mmHg or mean arterial pressure [MAP] <15mmHg), whichever came first.

Results: All animals had profound hypotension and experienced a near-arrest from hypovolemic shock (mean MAP = 24 mmHg at 10 minutes). Mean survival time was higher than 150 minutes in the experimental arm versus 27 minutes in the control arm ($P < .001$). Three-hour survival was 72% in the experimental group and 0% in the control group ($P = .002$). Hm-chitosan stabilized rising lactate, preventing acute lethal acidosis. MAP improved drastically after deployment of the hm-chitosan and was preserved at 60 mmHg throughout the 3 hours. Postmortem examination was performed in all animals and the hepatoportal injuries were anatomically similar.

Conclusion: Intraperitoneal administration of hm-chitosan-based foam for massive, noncompressible abdominal bleeding improves survival in a lethal, closed-cavity swine model. Chronic safety and toxicity studies are required.

Introduction

Uncontrolled traumatic hemorrhage is the leading cause of mortality, not only in military combat settings, but also in young adults in civilian settings.[1,2] Reports state that up to 90% of potentially survivable military casualties are attributed to bleeding, whereas up to 50% of combat fatalities in Iraq and Afghanistan occurred before even an evacuation of the wounded soldiers was possible.[2,3] Holcomb et al (2001) published one of the first studies introducing the terminology of potentially survivable and non-survivable injuries in the battlefield after reviewing the autopsy findings of special operations military personnel killed in the wars of Afghanistan and Iraq. The authors showed that potentially survivable truncal injury was the cause of death in 50% of the soldiers, identifying for the first time the importance of noncompressible, uncontrolled truncal bleeding[4]; however, noncompressible bleeding is not a problem exclusively seen in military casualties. Within the United States, mortality rates of patients with noncompressible bleeding who present to level 1 trauma centers are as high as 45%.[5]

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Although noncompressible bleeds are less frequent than their compressible counterparts, they account for the majority of preventable deaths.[5,6] Nearly 80% of the hemorrhage-related deaths are noncompressible, and these very high rates of potentially survivable deaths have not changed over time.[7–9] Thus, research on optimizing hemostasis and developing an effective biomaterial that will prolong survival in noncompressible bleedings has become a priority by the U.S. Army. The suggested biomaterial should be able to effectively control bleeding in injuries that would otherwise lead quickly to death by exsanguination and must prolong survival for several hours to allow time for evacuation and transfer of the injured soldiers to facilities with surgical capability.[10,11]

Chitosan is a biocompatible, biodegradable linear polysaccharide that has the benefit of being easy to produce in abundance and at a low cost.[12–15] Chitosan was first approved for human use in 2002 as a dressing with freeze-dried chitosan indicated for use only in external bleeding.[10] Hydrophobically-modified chitosan (hm-chitosan) is synthesized by the addition of hydrophobic tails to the polysaccharide backbone of chitosan, and it has enhanced hemostatic effects relative to its unmodified counterpart.[16] When an acidic hm-chitosan solution is mixed with an inert biopolymer (gelatin) solution containing concentrated sodium bicarbonate, the resulting material becomes a soft expanding foam that fills cavities rapidly, with enough mechanical integrity to slow severe blood loss from vessels and tissues.[12–14] The theoretical mechanism of action of hm-chitosan is the anchoring of hydrophobic tails into bilayer lipid membranes and consequent formation of a cross-linked network sustained by noncovalent interactions involving polysaccharide polymers and blood cells.[12–14]

Previous studies performed on rat models showed that hm-chitosan, when used as a sprayable foam directly on injured liver surfaces, significantly decreased blood loss and prolonged survival.[15,16] Moreover, hm-chitosan bandages have been shown to be effective in treating high-pulsatile, compressible bleeds from femoral artery injuries in swine.[16] Studies that would assess the efficacy of hm-chitosan in noncompressible, closed-abdomen bleeding on larger animals are needed. We hypothesized that administration of an injectable, expanding hm-chitosan-based foam to control or reduce the bleeding without the requirement of external compression, would improve survival in a highly lethal swine model. The aims of our study were (1) to test different formulations of the foam and identify the one that combines the best mechanical integrity properties with prolonged survival, and (2) to test the efficacy of the foam in prolonging survival when used as a locally injectable hemostatic agent in a lethal, closed-abdomen, noncompressible grade V hepato-portal injury in a swine model.

Methods

Animals utilized

Female Yorkshire swine (Sus scrofa domestica) 3 to 4 months old, 40 to 50 kg were purchased from Tufts University Cummings School of Veterinary Medicine, North Grafton, MA. Animals of the same sex were used to ensure consistency across experiments. Animals were housed in a facility accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care International. All work presented herein was conducted with approval from the Massachusetts General Hospital Institutional Animal Care and Use Committee. All animals were cared for following the Guide for the Care and Use of Laboratory Animals. All animals received no per oral (P.O.) feeding the night before surgery, but access to water was not restricted.

Liver injury protocol

A previously validated lethal, noncoagulopathic, noncompressible, closed-cavity hepatoportal injury model was used to evaluate the efficacy of the hm-chitosan–based foam.[17] Briefly, all animals underwent instrumentation for hemodynamic monitoring. A midline neck incision was performed at the beginning of the procedure to access the internal carotid artery for placement of an arterial line, and the internal jugular vein for placement of a central line used for fluid resuscitation.[17] Subsequently, a lateral neck incision to expose the external jugular vein was performed and a Swan-Ganz catheter was placed. Hemodynamic monitoring and recording was performed throughout the experiment.

A Foley catheter was placed in the urinary bladder for intra-abdominal pressure monitoring. A grade V, closed-cavity, hepatoportal injury was created by percutaneous wire distraction leading to a >10-mm tear in the bifurcation of the portal vein.[17] Resuscitation with Lactated Ringer's solution (LRS) through a peristaltic pump, at a rate of 165 mL/minute, was initiated when mean arterial pressure (MAP) <60 mmHg and turned off when MAP >65 mmHg. Maximum fluid resuscitation was set at 10 L LRS. High-volume resuscitation was used in this animal model to prevent formation of blood clots at the sites of vessel injury and maintain bleeding. Time of injury (time of percutaneous wire distraction) was considered to be timepoint 0. At 10 minutes after injury ($t = 10$), animals were included in our model if MAP had dropped <30 mmHg (if preinjury MAP = 60–65 mmHg) or if there was a drop in the MAP of at least 30 mmHg (if preinjury MAP > 65 mmHg). Animals who did not meet these inclusion criteria were excluded from our study. At 10 minutes after injury, control animals continued to receive fluid resuscitation alone, whereas experimental animals also received the hm-chitosan–based foam (delivered from a large double-barrel syringe system: 500 mL of aqueous, acidic hm-chitosan solution in Barrel A and 500 mL of gelatin solution containing concentrated sodium bicarbonate in Barrel B) injected intraperitoneally through a mixing nozzle-trocar placed at the umbilicus incorporated in the midline laparotomy incision created for liver instrumentation. Upon mixing, the acid-base reaction releases CO_2 gas, resulting in foaming of the biopolymer mixture; the foam structure is stabilized by the hydrophobic groups along the backbone of the hm-chitosan.

The experiment was terminated either at 3 hours or at death (defined as end-tidal carbon dioxide [ETCO2] <8 mmHg or MAP < 15 mmHg), whichever occurred first. At the end of the experiment, the laparotomy was reopened, the foam was manually removed, and macroscopic examination of the abdomen was performed by a veterinary surgeon. Postmortem explantation of the liver was performed in all animals as a quality control measure for the injury in the portal vein. Animals meeting death criteria before 10 minutes, and animals that did not have a portal vein laceration on postmortem explanation of the liver were excluded from our analysis.

Hm-chitosan–based foam and different formulations

Hm-chitosan is an amphiphilic biopolymer, which is composed of a hydrophilic polysaccharide backbone and hydrophobic grafts attached to that backbone. This structure is known as a "comb-graft" structure, as the hydrophobic tails along the backbone are akin to a hair comb. In this case hm-chitosan has palmitoyl (C-16, ie,

16 carbons in length) tails attached to the backbone at grafting density of 0.8% by mol of available amines along the chitosan backbone. The raw chitosan used in this case is medium molecular weight chitosan ~250 kDa in size. The in vivo blood clotting time for hm-chitosan is much faster than chitosan. This was first shown in a rat femoral vein injury model by Dowling et al[16] (2011). In that evaluation, it was shown that hm-chitosan solution clotted blood in 4.5 seconds, whereas chitosan solution at an equal concentration took 47 seconds to form a clot. Another study completed on freeze-dried versions of both hm-chitosan and chitosan, showed that hm-chitosan dressings achieved 100% survival over an acute observation period of 3 hours in swine after femoral arterial injury.[13] In contrast, none of the chitosan dressings resulted in 3-hour survival, even though most initial applications resulted in temporary hemostasis before the dressing failed due to lack of tissue adhesion.[13] Further details on the preparation and properties of the hm-chitosan foam is provided in the Supplemental Digital Content.

In the first phase of our study, using the same lethal liver injury model, different hm-chitosan foam formulations were tested to identify the formulation that combined optimal mechanical integrity with a survival benefit. Formulations were down-selected based on prolongation of survival and MAP recovery after injury, without necessitating continuous fluid resuscitation (fluid independent). Any formulation that led to 3-hour survival was re-examined in vitro and re-tested in vivo. Critical properties that varied among the formulations were: (1) hydrophobicity of the hm-chitosan molecule; (2) concentration of the hm-chitosan in the solution; (3) concentration of the inert biopolymer in the solution; and (4) type of inert biopolymer.

Statistical analysis

Univariate analyses were performed between the control and experimental populations. Categorical variables were compared using the Fisher exact test, and the results are presented as absolute numbers with their respective percentages. Continuous variables were compared using the two-sample *t* test and results are presented as means with the respective standard deviations (SD). Kaplan-Meier curves were used to graph survival over time and comparison between the 2 groups was performed using the log-rank test. Statistical analyses were performed using Stata v15.1 (StataCorp 2017. Stata Statistical Software: Release 15. College Station, TX: StataCorp LLC).

This injectable foam is intended for use in the temporary control of bleeding from noncompressible abdominal injuries that are not amenable to traditional bleeding control interventions in trauma and battlefield conditions. The product is not used commercially and is still under investigation before Food and Drug Administration (FDA) approval.

Author contribution

L.N., A.D.G., H.N.A.M., B.B.K., J.B., J.B.P., K.B., G.C.V., and D.R.K. were responsible for examining the animals the day before surgery, animal anesthesia, and for carrying out the surgical procedure. M.D. and B.W. were responsible for the alterations in the formulation and after identification of the optimal formulation for verifying consistent chemical and mechanical properties during the case-control study.

Results

Sixty-two animals were used to answer the 2 aims of our study. Of those, 9 animals were excluded for injury model failure. In addition to that, 2 experimental animals were excluded because of anatomical variations. The first animal had an anatomical variation of the liver with a truncated middle lobe and dense adhesions around the hepatoduodenal ligament. Injury created in this animal was more severe than per protocol and MAP <20 mmHg at 10 minutes right before deployment, nearly meeting exclusion death criteria. However, after deployment recovery of MAP was observed with a 38-minute survival. The second animal had very low oxygen saturations (<30%) before injury along with very high pulmonary artery pressures (54 mmHg/30 mmHg) indicating the presence of a pulmonary circulation variation and inability to sustain our injury model (40-minute survival).

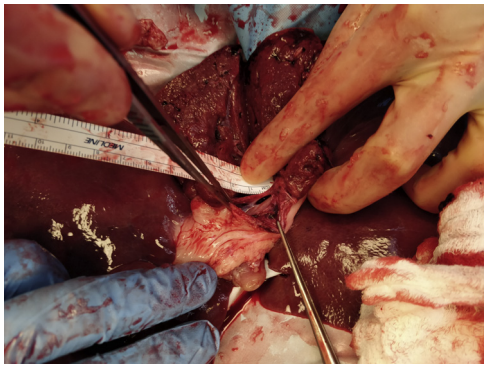

Figure 1. Postmortem quality control examination of the grade V liver injury created in our protocol. This image shows the injury to the portal vein.

We evaluated 15 different hm-chitosan–based foam formulations to identify the one that had optimal mechanical integrity and led consistently to significantly prolonged survival. The leading foam was optimized primarily by alterations of the concentration of hm-chitosan in the solution; this concentration proved critical for the success of the formulation. Higher hm-chitosan concentrations in the solution resulted in reduced expansion of the foam due to high viscosity; too low hm-chitosan concentration resulted in lack of critical mechanical integrity of the foam, resulting in continued bleeding at the injury site. Second, the selection of gelatin as the inert biopolymer was critical for consistent delivery of the formulation via the mixing tip in the abdomen, as well as the ultimate mechanical integrity of the resultant foam. Morphologically, the final foam was white, opaque, and amorphous in nature (ie, takes the shape of its container). While it is a soft material, the foam can hold its shape after being dispensed from its container.

The hepatoportal injury resulted in all animals in a significant vascular injury of the portal vein (Figure 1). As shown in Table I, animals in the 2 arms of the study were comparable regarding their preoperative weight and height. All animals, both in the control and experimental arms, had severe hypotension from hypovolemic shock at 10 minutes after injury, experiencing a near cardiac arrest (mean MAP 23 vs 24 for control and experimental animals, respectively) (Table I). Animals that received the hm-chitosan–based foam had a mean survival time >150 minutes, significantly prolonged than the survival time in control animals (152 minutes vs 27 minutes, $P < 0001$). Overall, 3-hour survival was 72% in the experimental arm of the study (Table I). On the contrary, the injury was lethal in 100% of the control animals with all animals expiring within the first 40 minutes from the time of injury. Figure 2 shows the Kaplan-Meier survival curves comparing control to experimental animals ($P- < .001$).

Figure 3 depicts the MAP of control and experimental animals over time (t = 0 minutes represents the time of injury). Animals in the control arm had a profound hypotension at t = 10 minutes with continuous drop in the MAP until meeting death criteria. On the

Table I
Comparison of control and experimental animals

Variables	Control ($n = 8$)	Experimental ($n = 18$)	P value
Weight (kg), mean ± SD	47 ± 3	49 ± 3	.26
Height (inch), mean ± SD	43 ± 1	43 ± 1	.89
Survival (min), mean ± SD	27 ± 7	152 ± 49	<.001
1-h survival, no. (%)	0 (0%)	16 (89%)	<.001
2-h survival, no. (%)	0 (0%)	13 (72%)	.002
3-h survival, no. (%)	0 (0%)	13 (72%)	.002
Fluid resuscitation (L), mean ± SD	4.4 ± 1.1	7.3 ± 1.5	<.001
Fluid resuscitation (mL/min) alive, mean ± SD	165 ± 11	60 ± 41	<.001
Mean arterial pressure at 10 min (mm hg), mean ± SD	23 ± 3	24 ± 4	.83
Lactate at death, mean ± SD	15.6 ± 1.9	15.6 ± 2.5	.99

SD, standard deviation.

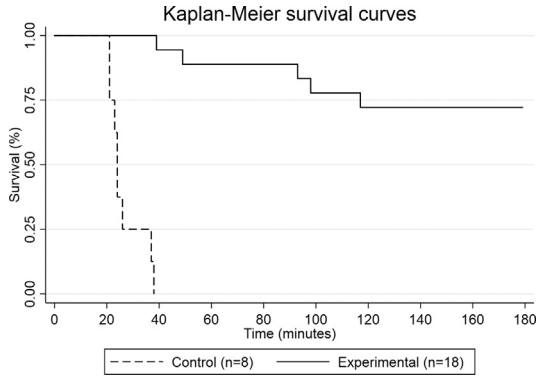

Figure 2. Kaplan-Meier survival curve for the control group (dashed line) and the hm-chitosan–based foam or experimental group (solid line). Intraperitoneal administration of the foam formulation at 10 minutes after injury had a significant survival benefit in a lethal, high-grade, noncompressible liver injury model ($P < .001$).

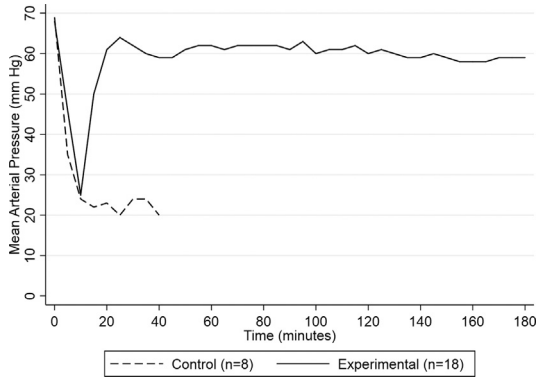

Figure 3. Intraoperative measurement of mean arterial pressure. Injury is created at t = 0 minutes and hm-chitosan–based foam is administered in the experimental arm at t = 10 minutes after injury. All animals had a near cardiac arrest from hypovolemic shock at 10 minutes. Intraperitoneal administration of the foam formulation at 10 minutes led to a recovery of the mean arterial pressure, which was maintained around 60 mmHg for the remainder of the experiment.

contrary, after deployment of the hm-chitosan foam, experimental animals had a return in their MAP, which was maintained around 60 mmHg throughout the experiment. Mean fluid resuscitation with LRS was significantly higher in the experimental arm (7.3L v. 4.4L, $P < .001$) (Table I). However, after adjusting for survival time after the injury, control animals were resuscitated with a mean volume of 165 mL/min alive (per protocol resuscitation; never fluid independent), while experimental animals were resuscitated at a significantly lower volume of 60 mL/min alive (after initial

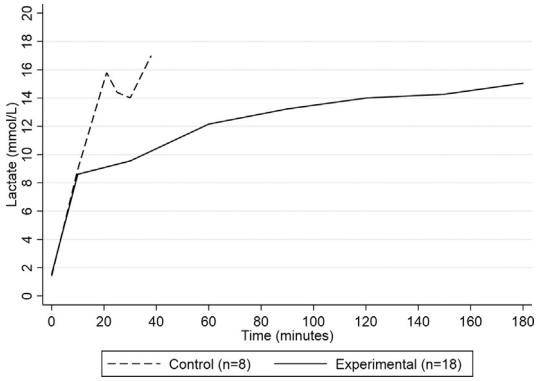

Figure 4. Intraoperative measurement of blood lactate. Injury is created at t = 0 minutes and hm-chitosan–based foam is administered in the experimental arm at t = 10 minutes after injury. Intraperitoneal administration of the foam formulation at 10 minutes led to a stabilization in the rise of lactate preventing acute lethal acidosis in the experimental arm.

resuscitation MAP was restored and animals became fluid-independent for periods of time).

As shown in Table I, no significant difference was documented regarding lactate at the time of death between control and experimental animals (15.6 vs 15.6, $P = .99$). However, as shown in Figure 4, animals in the experimental arm had a stabilization in the build-up of lactate, after deployment at t = 10 minutes, preventing acute lethal lactic acidosis. Moreover, intra-abdominal interaction of hm-chitosan and gelatin leading to the formation of the soft expanding foam was associated with a rapid increase in the intra-abdominal pressure (Figure 5). Immediately after deployment, intra-abdominal pressure increase rapidly and was sustained between 60 to 70 mmHg with a slight downward trend toward the end of the 3-hour experiment.

Removal of the soft foam from the abdomen was performed with a combination of suction and manual removal. No tissue adherence was observed, and at the postmortem examination of the abdomen no lesions were documented in the intra-abdominal organs or diaphragm.

Discussion

Intraperitoneal administration of an expanding hm-chitosan–based foam in swine with massive noncompressible, closed-cavity abdominal bleeding resulted in improved survival in a lethal hepatoportal injury model. The high lethality of the protocol is highlighted by the short survival period in the control arm of the study (despite high volume resuscitation with LRS) and the

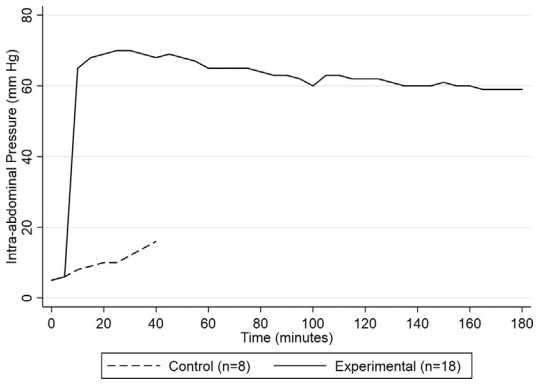

Figure 5. Intraoperative measurement of the intra-abdominal pressure. Injury is created at t = 0 minutes and hm-chitosan–based foam is administered in the experimental arm at t = 10 minutes after injury. Intraperitoneal administration of the soft expanding foam leads to a sudden increase in the intra-abdominal pressure which is maintained around 60 mmHg throughout the experiment.

severely deranged hemodynamics with near cardiac-arrest from hypovolemic shock at the time point of foam deployment. After foam deployment, rapid recovery of MAP was documented, implying improved intravascular volume. Experimental animals that received the hm-chitosan–based foam became fluid independent with in-between periods of time requiring fluid resuscitation, per protocol, suggesting a decrease in the bleeding without complete cessation.

The results of this study signify an important advance in the field of hemostasis research in patients with closed-cavity noncompressible bleeding. Available treatment options for the management of ongoing bleeding in the prehospital setting are limited. While splinting of limbs, pelvic binders, hemostatic suturing, direct external pressure, tourniquets, and hemostatic gauzes are frequently utilized options, the greatest challenge is in the management of noncompressible intracavitary bleeding.[3,15] Survival of these patients depends largely on early intervention with surgical control of the bleeding, which assumes easy access to definitive care facilities.[6] However, extraction of the wounded may be difficult and significantly delayed in more remote rural settings and in the battlefield.[6] This delay places an additional burden on providers at the point of injury increasing the value of developing an intervention that can be applied easily and immediately, by trained, but not necessarily medical, personnel.[4] The 3-hour endpoint used in our study is significantly longer than the contemporary mean time required to transfer a patient to a hospital in civilian settings or evacuate a wounded soldier in the battlefield.[18]

The possible utilization of an intracavitary expanding liquid formulation that would expand in the human body, reaching the bleeding site and controlling blood loss was first introduced by Holcomb et al[19] (2000). Various biomaterials have been assessed so far as potential treatment options for noncompressible bleeding.[15] One of the biggest efforts, led by the United States Army, involved the utilization of fibrin sealant foam in truncal hemorrhage; however, its use has been limited due to its cost, requirements for storing, and necessity for preparation before deployment.[19,20] Moreover, when fibrin sealant foam was used directly on a bleeding rat liver injury a decrease in blood loss was observed but that was not accompanied by a significant survival benefit.[19] In the same direction, Kheirabadi et al conducted 2 experiments using a rabbit liver injury and a swine aortic injury model.[10,20] The authors showed that high-pressure fibrin sealant foam, compared with control animals, significantly reduced blood loss and mortality rates. However, the results were not maintained when the product was tested in a closed-abdomen model.

Other biomaterials assessed in the literature include lyophilized platelets, fibrinogen-coated albumin microparticles, conjugated red blood cells, modified rapid deployment hemostat bandages, and thrombin based hemostatic agents.[15,21–23] So far, most of the efforts to develop technologies to intervene early on massive intra-abdominal bleeding have either not been successful in prolonging survival, or survival benefit has required an open approach with a laparotomy incision and direct application on the site of injury, reducing feasibility for wider application in remote civilian or military settings.[24]

Similar to our study, Duggan et al (2013) have also evaluated the efficacy of a self-expanding poly(urea)urethane polymer in the same lethal noncompressible swine bleeding model.[24] The 3-hour survival observed in this study is similar to the one observed with the poly(urea)urethane polymer (72% with chitosan-based foam vs 73% poly(urea)urethane polymer foam).[24] However, there are some important differences between the 2 materials. First, chitosan is a readily available natural polysaccharide that is easy to produce in abundance and at a low cost. Regarding the mechanism of action, in addition to expansion and intra-abdominal tamponade, the chitosan-based foam is also hypothesized to reduce bleeding through a biochemical interaction between 3 components: chitosan, gelatin, and blood cells. In addition to that, another important difference is regarding the intra-abdominal pressure. The chitosan-based foam in our experiment reached very high intra-abdominal pressures, as compared with a sudden increase and subsequent quick decrease noted in the poly(urea)urethane foam.[24] Despite the prolonged survival in this study, this feature requires additional research, both in different nonsurvival and in survival animal models, to better determine the acute and long-term sequelae. Lastly, another notable difference is that in the chitosan-based foam no macroscopic bowel lesions that would require repair or excision were noted.[24] This may be the result of various factors, including the lack of a solid component in the chitosan-based foam that may cause pressure-injuries on adjacent bowel loops and the lack of an exothermic reaction, potentially causing small thermal injuries.

Intra-abdominal air insufflation methods have also been studied with the goal of controlling noncompressible intra-abdominal hemorrhage.[25,26] Recently, McCracken et al (2021) assessed the efficacy of an intraperitoneal hemostatic device that consisted of an intra-abdominally inflatable balloon used in noncompressible torso hemorrhage.[27] The device was reported to be successful in stabilizing hemorrhage in 5 swine (vs 3 controls) for a period of 60 minutes (study end-point). Likewise, intra-abdominal application of the XSTAT 30 expanding pellets has been studied in a noncompressible intra-abdominal hemorrhage model.[28] All of these studies rely on the increase of intra-abdominal pressure with direct tamponade of the bleeding source for hemorrhage control, without any known biological hemostatic properties.

The results of this study should be interpreted in view of the following limitations. First, high-volume resuscitation that does not simulate the current standard of care was used to prevent clot formation and increase the severity of the injury. Second, resuscitation was performed only with LRS and no blood products were used, which would have provided a better simulation of current practice. Third, this injury model was not suitable for assessing the long-term effects and toxicity of the hm-chitosan–based foam on tissues and organs and the effects, if any, of the transient intra-abdominal compartment syndrome caused by the high intra-abdominal pressure due to foam expansion. Fourth, in comparison with previous reports in the literature, the more viscous characteristics of our formulation did not allow us to accurately quantify blood loss in our experiments. Fifth, this is the first massive hemorrhage model assessing the efficacy of hm-chitosan–based foams

and more work is required to evaluate its efficacy in additional high-severity intra-abdominal injury models.

In conclusion, we showed that intraperitoneal administration of hm-chitosan for massive, noncompressible abdominal bleeding significantly improves survival and restores MAP in a lethal, closed-cavity swine model. This material has the significant potential to be a treatment option for massive noncompressible intra-abdominal hemorrhage, not only in military settings, but also in any occasion when swift transfer to a facility with surgical capabilities is not possible. Additional efficacy, chronic safety, and toxicity studies in other injury models are required to validate our above results and describe the long-term effects of the interactions of hm-chitosan with organs and tissues.

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Conflict of interest/Disclosure

Dr. Matthew Dowling is a 10% owner and employee of the entity Medcura, Inc., a medical device company which has developed multiple chitosan-based products for bleeding control and wound treatment. Brandon Wallace is an employee of the entity Medcura, Inc., a medical device company which has developed multiple chitosan-based products for bleeding control and wound treatment. Dr. David R. King is on the Clinical Advisory Board of the entity Medcura, Inc., a medical device company which has developed multiple chitosan-based products for bleeding control and wound treatment. For the remaining authors none were declared.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.1016/j.surg.2022.01.016.

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