Hemostatic Efficacy of Modified Amylopectin Powder in a Lethal Porcine Model of Extremity Arterial Injury

Michael Kilbourne, MD
Kaspar Keledjian, MD
John R. Hess, MD, MPH
Thomas Scalea, MD
Grant V. Bochicchio, MD, MPH

From the Department of Surgery, Division of Clinical and Outcomes Research, R. Adams Cowley Shock Trauma Center, Baltimore, MD (Kilbourne, Keledjian, Scalea, Bochicchio); and the Department of Pathology, University of Maryland School of Medicine, Baltimore, MD (Hess).

Study objective: Rapid hemostasis is crucial in controlling severe extremity hemorrhage. Our objective is to evaluate the hemostatic efficacy of a newly modified amylopectin powder in a model of severe extremity arterial hemorrhage.

Methods: Anesthetized pigs underwent severe, reproducible femoral artery injuries. Animals were randomized (nonblinded) to either modified amylopectin powder (n=10) or standard gauze application (n=6). Each hemostatic agent was applied through a pool of blood with manual compression for 3-minute intervals until hemostasis was achieved. Fluid resuscitation was infused as necessary to reestablish a mean arterial pressure within at least 80% of the preinjury mean arterial pressure if possible. The primary measured outcome was total blood loss. Secondary endpoints were survival, time to hemostasis, resuscitation mean arterial pressure, and resuscitation volume.

Results: Pretreatment blood losses were similar in both groups. Median (absolute average deviation of the median) posttreatment blood loss was significantly less in the modified amylopectin powder group than in the gauze group, 275 (108) mL versus 1,312 (171) mL. Resuscitation mean arterial pressure at 180 minutes after injury was 68% of preinjury mean arterial pressure in the modified amylopectin powder group and undetectable in all control animals. Fluid volume required for resuscitation was 1,962 (258) mL in the modified amylopectin powder group and 2,875 (150) mL in the gauze group. Time to hemostasis was 9.0 (2.1) minutes in the modified amylopectin powder group. Hemostasis was not achieved in any animal in the gauze group. Survival was 100% in the modified amylopectin powder group, whereas no animals survived in the gauze group.

Conclusion: Modified amylopectin powder demonstrates the ability to control major vascular bleeding in a lethal arterial injury model during a 3-hour period. [Ann Emerg Med. 2009;53:804-810.]

Provide feedback on this article at the journal's Web site, www.annemergmed.com.

INTRODUCTION

Background

Uncontrolled bleeding is the second leading cause of mortality in civilian trauma and the leading cause of death in soldiers during wartime.[1-3] Extremity bleeding, compared with intrathoracic or intraabdominal hemorrhage, is more accessible to the first responder in the field. Therefore, extremity hemorrhage has significant potential for early control by the emergency medical technician or the military medic.[4]

Chitosan dressings, which are freeze-dried preparations of deacetylated chitin derivatives, have demonstrated the ability to stop hemorrhage in various scenarios since studies in the early 1990s.[5,6] Thereafter, the chitosan dressing HemCon Bandage (HemCon Medical Technologies, Inc., Portland, OR) was Food

and Drug Administration approved in 2002 and has been used by medical personnel in both Operation Iraqi Freedom and Operation Enduring Freedom. Thus, amylopectin agents are time-tested and effective in bandage form, but other delivery forms remain less studied, especially in potentially salvageable severe extremity injury models.

Importance

Effective hemostasis in the field can improve survival and reduce the complications of early blood loss. The ideal hemostatic agent has several key features besides its inherent hemostatic ability. It should be easy to use and store, require no cumbersome mixing, be lightweight and bioabsorbable, and have no adverse effects.[1] Several other hemorrhage control

Editor's Capsule Summary

What is already known on this topic

Amylopectin-impregnated bandages have been used to control severe extremity bleeding in trauma. Amylopectin powder is lightweight and easily transportable but has not been studied.

What question this study addressed

Whether the direct application of amylopectin powder to severely injured pig femoral arteries would achieve more rapid hemostasis than standard gauze with manual compression.

What this study adds to our knowledge

The 10 pigs randomized to amylopectin all survived and had faster hemostasis, better blood pressure, and lower fluid resuscitation requirements than the 6 pigs randomized to standard treatment, none of whom achieved hemostasis or survived.

How this might change clinical practice

This study suggests that amylopectin powder may be a useful treatment for severe extremity bleeding, especially under battlefield conditions.

Research we'd like to see

The study repeated with less aggressive fluid resuscitation.

Figure 1. Femoral artery injury after application of modified amylopectin powder and 6 minutes of gauze compression. There is complete hemostasis at this point. The powder nearly fills the entire wound and easily conforms to all contours of the injury site. *MA,* Modified amylopectin.

agents, included dry fibrin sealant dressings, pure amylopectin patches, and zeolite mineral-based powders (QuikClot, Z-Medica Corporation, Wallingford, CT) have been developed. The products available are applicable to the battlefield, as well as civilian trauma. In animal models, these hemorrhage control agents have been tested in severe extremity arterial injuries, with mixed results.[7-9]

Hemorrhage control agents continue to evolve. Hemostatic powders confer several important advantages to dressings and bandages. Powders are lightweight and easy to transport. The powder granules can conform to irregular wound surfaces, easily achieving complete contact with origin of bleeding. In contrast to patches or dressings, powders can be reapplied several times to the same area without concern for disrupting previous hemostatic areas, which makes it relatively easy to precisely apply to small residual areas of continued bleeding or oozing after first application to the larger wound surface.

However, the hemostatic efficacy of previously tested products, especially powders, is only marginal in large animal models of severe extremity arterial injuries.[1] In addition, the adverse effects appear to be significant, specifically the exothermic reaction and resultant high wound temperatures involved in zeolite granular powders, as previously reported by
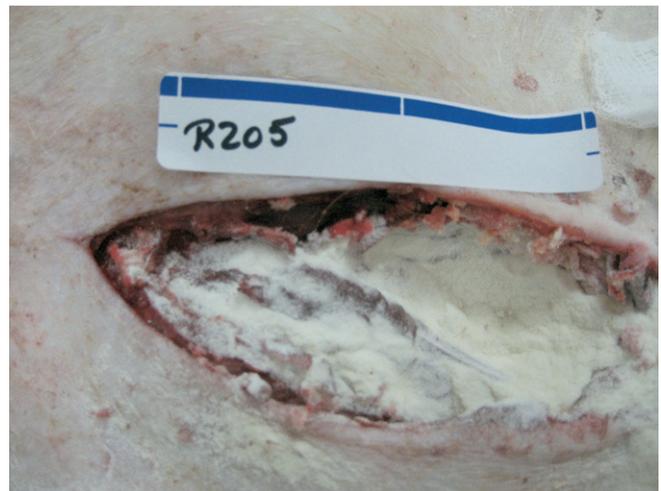
Alam et al[9] in 2004. As such, new powder technology that is potentially both effective and lacking in adverse effects was evaluated in this study.

Goals of This Investigation

Our objective in this study was to evaluate the hemostatic efficacy of a newly modified amylopectin powder (Hemostasis, LLC, St. Paul, MN) in a model of severe extremity arterial hemorrhage that could not be stopped with standard gauze treatment.

MATERIALS AND METHODS
Study Design

The design implemented was a nonblinded randomized animal study that compared 2 treatments for surgically created, reproducible, lethal extremity arterial bleeding. The treatment arm consisted of a modified amylopectin powder, whereas the control arm was standard gauze compression to the injuries.

Setting

Female Yorkshire crossbred swine, aged 2.5 months, were screened by a veterinarian to ensure that they were in good health. Animals were allowed free access to water and to commercial laboratory swine food. Food was withheld the night before the study. All animals were maintained in an Association for Assessment and Accreditation of Laboratory Animal Care International–accredited facility, and all experimental manipulations 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 Institutional Animal Care and Use Committee.

Table. Baseline data for animals treated with modified amylopectin powder (n=10) versus standard gauze packing (n=6).

Baseline Data	MA Powder Group	Gauze-Packing Group
Animal weight, kg	44 (6)	42 (3)
Preinjury MAP, mm Hg	104 (10)	106 (12)
Free-bleeding MAP, mm Hg*	57 (11)	60 (8)
Pretreatment blood loss, mL	410 (77)	429 (60)

MAP, Mean arterial pressure.
All values reported as mean (SD).
*MAP after 1 minute of free bleeding before hemorrhage control treatment begun.

Figure 2. Parallel univariate plots of posttreatment blood loss for animals treated with modified amylopectin powder (n=10) and standard gauze packing (n=6). Modified amylopectin powder application to femoral artery injuries reduces posttreatment blood loss (median values [average absolute deviation of median] 275 [108] versus standard gauze packing 1,312 [171]). The horizontal lines represent median values for each group.

Sixteen swine were anesthetized with 1.5 mL buprenorphine and 0.3 mL glycopyrrolate intramuscularly. They were then intubated and received mechanical ventilation at a tidal volume of 12 mL/kg, a rate of 10 breaths/min, and 100% oxygen. Anesthesia was maintained with isoflurane.[10]

The animals underwent midline laparotomy, and a unilateral groin incision was made over the femoral canal. Exposure and isolation of at least 5 cm of the femoral artery was performed. Control of the femoral artery was achieved atraumatically in both the proximal and distal locations. The artery was then transected, with the femoral vein and nerve being spared.

Free bleeding was allowed for 45 seconds. Blood loss was collected by suction for this period and was designated pretreatment total blood loss. Animals were randomized to receive either modified amylopectin powder treatment (n=10) or standard gauze packing (n=6).

In the modified amylopectin powder group, 100 g of powder was applied to the arterial bleeding, followed by manual compression with 4×4 gauze pads for 3 minutes at a pressure sufficient to occlude distal flow, as measured by arterial Doppler. Figure 1 demonstrates the modified amylopectin powder applied to a severed femoral artery. After 3 minutes, the gauze was carefully removed and hemostasis was evaluated. Hemostasis was judged to be complete if there was no oozing of any kind after 20 seconds of visual observation by 2 separate surgeons. If hemostasis was not completely achieved, 40 to 50 g of additional powder was applied and compression was reinstituted for another 3-minute interval. In the gauze packing group, 5 large gauze pads were placed on the injury site. Manual compression intervals were initiated in similar fashion to that previously described. If hemostasis was achieved at the end of any 3-minute interval, compression was stopped. This series of compressions was permitted for a maximum of 12 minutes.

The 3-minute intervals were chosen for 2 reasons. First, previous studies investigating the efficacy of hemostatic agents in injury models have used this interval. As such, we chose the same for the sake of consistency with regard to accepted models. In broader terms, the interval of compression is somewhat arbitrary. However, a first responder has many tasks to complete when 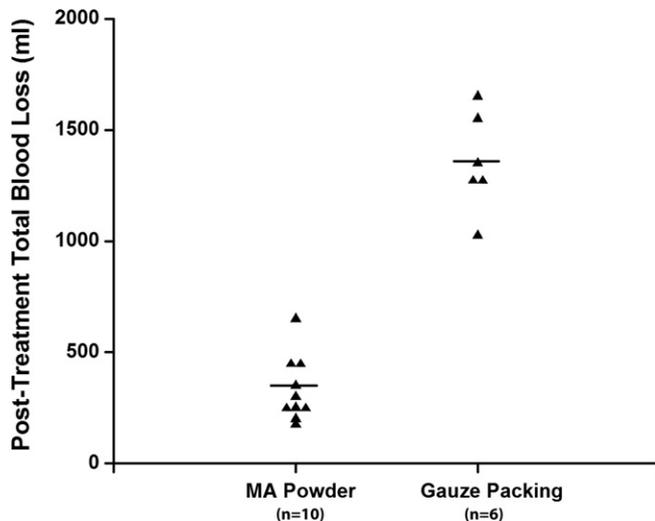evaluating and treating a bleeding patient, including basic airway management, intravenous line starts, and transportation. Thus, having a long interval of manual compression is not realistic, given the various responsibilities involved in initial trauma care.

Methods of Measurement

Mean arterial pressures were measured by continuous arterial pressure monitoring through a carotid artery catheter, although only specific points were recorded and analyzed. Fluid resuscitation with lactated Ringer's solution was begun immediately after injury through a large-bore catheter in the animal's internal jugular vein. Lactated Ringer's solution was infused as necessary to reestablish a mean arterial pressure within at least 80% of the preinjury mean arterial pressure, if possible. Resuscitation was continued for the entire observation period.

After completion of the 180-minute study period, the groin was examined. Liquid blood was suctioned. Blood clots were removed and weighed. In the gauze-packing group, additional liquid blood loss was calculated by subtracting the wet gauze weight from dry gauze weight. Total blood loss was determined by adding liquid and clotted blood losses. Animal survival was defined as a mean arterial pressure greater than 20 mm Hg at the end of the study period. At 180 minutes, surviving animals were euthanized with 10 mL of Euthasol.

Histologic samples were collected within 2 minutes of injection of Euthasol (Virbac AH, Inc., Fort Worth, TX). Representative sections of the femoral artery and vein were cut at 5 μm and stained with hematoxylin-eosin for microscopic examination.
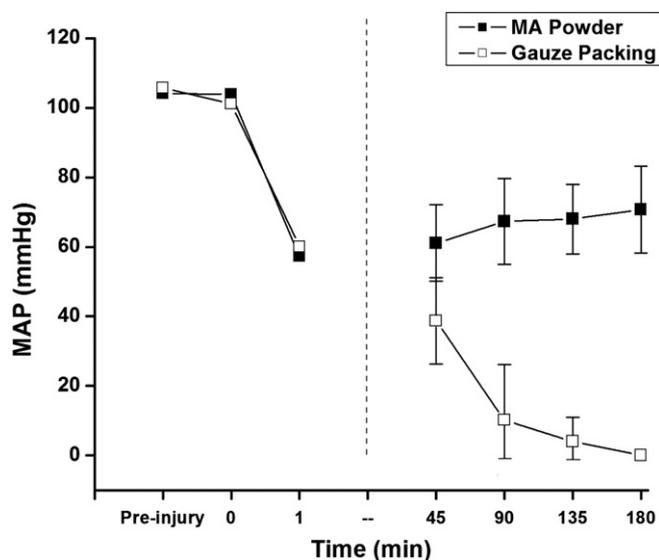
Figure 3. Mean arterial pressures of the modified amylopectin powder group stabilized at about 68% of the preinjury mean arterial pressure after application of the agent and remained stable throughout the 180-minute study period. The gauze group, in contrast, had a steady decline in MAP with no animals having a recordable blood pressure at the end of the study period. Plots show the median MAP values for each time point. Error bars show the average absolute deviation of the median. Lactated Ringer's solution infusion was begun at time 0. No animals in either treatment arm died in the immediate peri-injury period.

Outcome Measures

The primary outcome measured was posttreatment total blood loss. Secondary outcomes included time to hemostasis, survival time, mean arterial pressure, and total resuscitation volume infused. Histologic outcomes included the presence of any femoral vein, artery, or local adjacent tissue inflammation or necrosis.

Primary Data Analysis

A power analysis of pilot data demonstrated that a power of 80% was achieved with a minimum of n=6 animals in each arm. Statistical analysis on baseline data was performed with a Student's *t* test because the distribution of data was normal but the number was relatively small. Data from these sets were expressed as mean (SD). Statistical significance was attained with greater than 95% confidence level ($P<.05$). All posttreatment data were reported as median±average absolute deviation from the median. This statistical method was most appropriate, given the relatively small number of animals used in the study and an assumed non-normal data distribution.

RESULTS

Pretreatment animal weight, preinjury mean arterial pressure, mean arterial pressure after free bleeding, and pretreatment
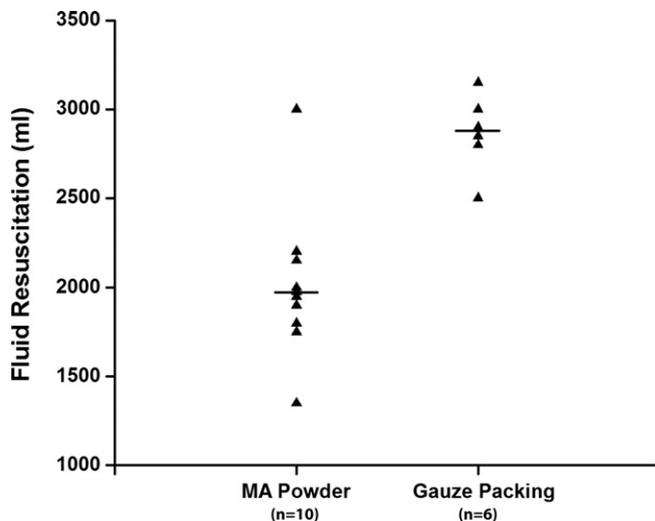

Figure 4. Parallel univariate plots for fluid resuscitation requirements for animals in the modified amylopectin powder group (n=10) demonstrated significantly fewer volume needs, median (average absolute deviation from the median) 1,962 (258), than in the gauze packing group (n=6) 2,875 (175) despite that none of the gauze group survived for the entire length of the study. The horizontal lines represent median values for each group.
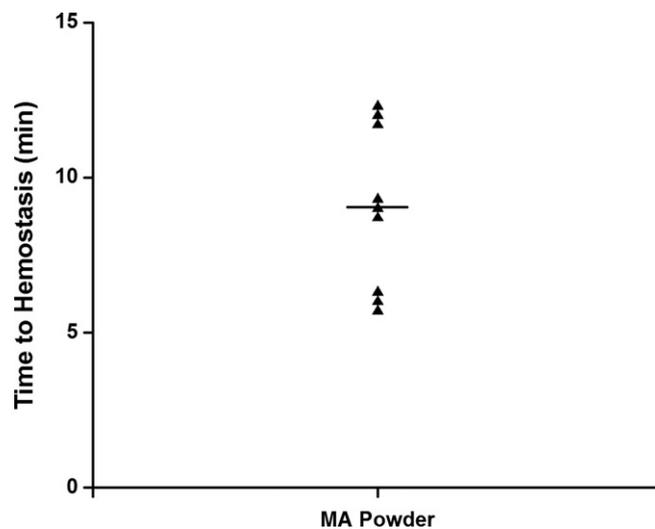

Figure 5. Univariate plot for time to hemostasis for animals in the modified amylopectin powder group (n=10). The horizontal line represents the median value for the group, 9 (2.1) minutes. None of the gauze-packing group achieved hemostasis.

blood loss were similar between the treatment groups. The Table shows these baseline data.

Median (average absolute deviation from the median) posttreatment total blood loss was less in the modified amylopectin powder group, 275 (108) mL versus 1,312 (171) mL in the gauze packing group, as shown in Figure 2.
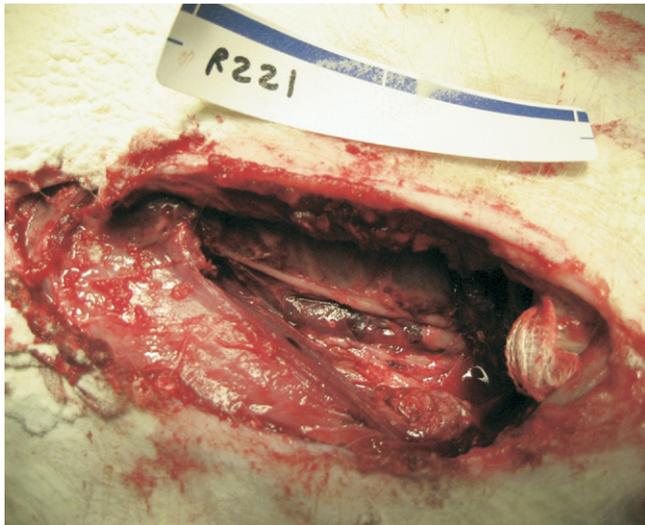
Figure 6. After 180 minutes after application of modified amylopectin powder, the agent was gently irrigated from the injury site. The arteriotomy was clearly visualized here. There was no hemorrhage even after the powder was removed. The vessel ends and surrounding tissues appeared viable, without evidence of necrosis or inflammation.

Median resuscitation mean arterial pressure at 180 minutes after injury was 72.5 mm Hg (SD 9.5 mm Hg) (68% of preinjury mean arterial pressure) in the modified amylopectin powder group. No animal in the gauze-treated group had a recordable blood pressure at 180 minutes. Figure 3 demonstrates the trend of mean arterial pressures for each group at specific points. In addition, modified amylopectin powder–treated animals required less fluid resuscitation than did gauze-packing animals. Median fluid volume required for resuscitation was 1,962 (258) mL in the modified amylopectin powder group and 2,875 (150) mL in the gauze group, as shown in Figure 4.

Median time to hemostasis was 9.0 (2.1) minutes in the modified amylopectin powder group (Figure 5), whereas hemostasis was not achieved in any animal in the gauze-packing group. Survival was 100% in the modified amylopectin powder group, whereas no animals survived in the gauze-packing group.

After 180 minutes in the modified amylopectin powder group, the powder was gently irrigated from the arterial injury site (Figure 6). The arterial ends appeared grossly viable, without evidence of necrosis or inflammation. Likewise, histologic evaluation of the femoral vessels in modified amylopectin powder–treated animals revealed no inflammatory or degenerative changes or evidence of vessel wall or smooth muscle necrosis (Figure 7).

LIMITATIONS

The principal technical limitation of this study was the potential arterial spasm or retraction caused by femoral arterial transection. Though we did not specifically observe this phenomenon, an intraluminal hemostatic plug could theoretically form when the artery was severed that would prevent delayed rebleeding (but not initial bleeding). The second model limitation was the focus on arterial injuries only, which does not fully replicate battlefield or complex traumatic soft tissue or venous injuries that can be associated with arterial injuries.

There was the potential for variable compression pressure with application of the hemostatic agents, despite the fact that occlusion was measured by distal Doppler signals. As opposed to using a pressure cuff system or a clamp to compress the agents over the injured site, we chose to use compression by the surgeon's hands. This method was chosen because it replicated a real-life field situation. In addition, a significant element of dexterity was necessary, in our opinion, to manipulate the powder and gauze within a relatively small wound area. Regardless, potential variable compression could have affected end-flow occlusion pressures, and subsequently end-flow hemostasis. Because this model was not blinded, there was a possibility of experimental bias in the compression sequence.

We did not compare modified amylopectin powder to standard amylopectin patches or dressings in this study. Patches have been studied and used effectively in the field to date, so we can make no statement about powder superiority over that of a patch. In fact, we recognize that patches may actually be better for larger surface area battle wounds than powders, but this is unproven.

Specifically, this study was a relative first step to demonstrate that modified amylopectin powder was effective above and beyond that of standard gauze compression. A follow-up study comparing it to patches or dressings would be important. In that follow-up study, the creation of a realistic but reproducible crush/blast injury model that better simulates true traumatic or battlefield injuries, not just arterial bleeding, would be important.

DISCUSSION

In the current study, we used a model of extremity hemorrhage designed to be 100% lethal in animals treated with standard gauze packing treatment. By applying the modified amylopectin powder and rapidly compressing the wound with regular gauze, hemorrhage could be stopped in all animals even with a pool of blood present. Survival was achieved in all animals treated with modified amylopectin powder. Total blood loss posttreatment was significantly less in modified amylopectin powder–treated animals than in gauze controls. In addition, mean arterial pressures were reestablished above the normal threshold for rebleeding in pigs (65 mm Hg),[11,12] but no modified amylopectin powder–treated animals experienced rebleeding during the 180-minute study period. Finally, the modified amylopectin powder group required statistically less absolute fluid resuscitation than did the gauze group, despite the fact that none of the gauze group survived the duration of the study.

An important feature of modified amylopectin powder is its lack of direct adverse tissue effects. It creates no exothermic reaction and

Figure 7. Histology from femoral vessels. *A*, Femoral vein from injured animal demonstrating normal appearing endothelial and smooth muscle layers. *B*, Femoral artery from MA powder–treated animal without any evidence of necrosis, inflammation, or neutrophil infiltration in the endothelium or myofibers. *C*, Femoral artery with thrombus seen after MA powder treatment.

thus no tissue thermal injury, unlike zeolite-based products. The histologic evaluation of animal femoral vessels in the modified amylopectin powder group confirms the absence of vessel wall necrosis, inflammation, or degeneration.

In conclusion, modified amylopectin powder demonstrates the ability to control major vascular bleeding and promote survival without causing local tissue damage in a porcine model of lethal extremity arterial injuries. This product shows promise as an effective hemostatic product for first responders in the field.

Address for correspondence: Michael Kilbourne, MD, R. Adams Cowley Shock Trauma Center, 22 South Greene St T1R60, Baltimore, MD 21201; 410-328-5377, fax 410-328-7175; E-mail mkilbourne@hotmail.com.

REFERENCES
1. Acheson EM, Kheirabadi BS, Deguzman R, et al. Comparison of hemorrhage control agents applied to lethal extremity arterial hemorrhages in swine. *J Trauma.* 2005;59:865-875.
2. Bellamy RF. The causes of death in conventional land warfare: implications for combat casualty care research. *Mil Med.* 1984; 149:55-62.
3. Hoyt DB, Bulger EM, Knudson MM. Death in the operating room: an analysis of a multi-center experience. *J Trauma.* 1994;37:426-432.
4. Holcomb JB, MacPhee M, Hetz S. Efficacy of a dry fibrin sealant dressing for hemorrhage control after ballistic injury. *Arch Surg.* 1998;133:32-35.

5. Klokkevold PR, Lew DS, Ellis DG. Effect of chitosan on lingual hemostasis in rabbits. *J Oral Maxillofac Surg.* 1991;49:858-863.

6. Fukasawa M, Abe H, Masaoka T. The hemostatic effect of deacetylated chitin membrane on peritoneal injury in rabbit model. *Surg Today.* 1992;22:333-338.

7. Jackson MR, Friedman SA, Carter AJ. Hemostatic efficacy of a fibrin sealant-based topical agent in femoral artery injury model: a randomized, blinded, placebo-controlled study. *J Vasc Surg.* 1997; 26:274-280.

8. Larson MJ, Bowersox JC, Lim RC. Efficacy of a fibrin hemostatic bandage in controlling hemorrhage from experimental arterial injuries. *Arch Surg.* 1995;130:420-422.

9. Alam HB, Chen Z, Jaskille A. Application of a zeolite hemostatic agent achieves 100% survival in a lethal model of complex groin injury in swine. *J Trauma.* 2004;56:974-983.

10. Schreiber MA. The effect of recombinant factor VIIa on noncoagulopathic pigs with grade V liver injuries. *J Am Coll Surg.* 2003;196:691-697.

11. Sondeen JL, Coppes VG, Holcomb JB. Blood pressure at which rebleeding occurs after resuscitation in swine with aortic injury. *J Trauma.* 2003;54:S110–S117.

12. Holcomb JB. Fluid resuscitation in modern combat casualty care: lessons learned from Somalia. *J Trauma.* 2004;57:S46-S51.