Comparison of 10 hemostatic dressings in a groin puncture model in swine

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Background: The use of mineral (clay) or biologic (chitosan) materials has improved the efficacy of dressings used in the bleeding control of noncompressible areas. A series of novel manufactured products already evaluated in a vascular transection model was further compared in a severe vascular puncture injury model.
Methods: Ten hemostatic dressings were tested in anesthetized Yorkshire swine hemorrhaged for 45 seconds in a femoral arterial puncture model. Application of these dressings was followed by 5 minutes of compression (about 175 mm Hg), and at 15 minutes, 500 mL resuscitation fluid (Hextand) was infused during a 30-minute period. The animals were monitored for a 3-hour experimental observation period. Primary outcomes were incidence of bleeding after dressing application and animal survival.
Results: Blood loss was 18.8% ± 5.2% estimated blood volume (EBV) after 45 seconds of free bleeding. Relative performance of dressings is characterized as groups of dressings that performed similarly. Recurrence of bleeding after application was observed with most dressings and was lower with Woundstat, Celox, X-Sponge, and ACS+ (35% ± 49%) compared with FP-21, Hemcon, Chitoflex, and Bloodstop (79% ± 43%; $P < .01$). Blood loss after treatment was 25.3% ± 18.4% EBV for the top four dressings and 53.0% ± 18.4% EBV for the bottom four ($P < .05$). Survival was higher for top four vs bottom four dressings (78% ± 12% vs 25% ± 0%, respectively; $P < .01$). Overall performance of these dressings according to survival, incidence of bleeding, and post-treatment blood loss, yielded similar ranking as with a previously tested transection injury model.
Conclusions: The findings indicated that the efficacy of Woundstat, Celox, X-Sponge, and ACS+ were similar and superior in improving survival, hemostasis, and maintenance of mean arterial pressure in an actively bleeding wound caused in this severe vascular injury model. (J Vasc Surg 2009;50:632-9.)

Clinical Relevance: Major improvements have been made in the development of novel dressings with hemostatic properties to control heavy bleeding in noncompressible areas. Hemostatic dressings offer promise in the military and civilian surgical environment for hemorrhage control in difficult situations. This animal-based study identified dressings with good absorption and good clotting abilities that ranked superior in terms of control of rebleeding. Also, these dressings might be beneficial in well-attended or remote surgical theaters as well as for first aid bandaging in extreme sport.

Methods to suppress massive external hemorrhage should be provided immediately after injury and should control active bleeding within minutes to prevent death or consequences of hypovolemia and hypoperfusion.[1-3] The amount of blood lost relates to the type of injury and dictates survival. Compression points remain a very effective method to stop bleeding in extremities.[4-6] In most noncompressible cases, standard of care treatment of a slow hemorrhage involves using cellulose-based compressed gauze (eg, Kerlix dressings) as the first action to absorb blood.[7] The use of these simple dressings assumes that hemostasis will eventually be restored naturally by clot formation, vessel constriction, or retraction, but subsequent bleeding (rebleeding) may occur and require additional dressing applications.

As an alternative to bleeding control, tourniquets, used efficiently for extremities, are not suited for noncompressible areas such as the neck, peritoneum, or groin. Promising solutions for controlling rapid bleeding in these areas could come from novel hemostatic dressings with maximum efficacy in a single application.[4,8] By adding an active hemostatic agent to a supportive matrix, the first generation of improved dressings has contributed to better bleeding control in noncompressible areas.[9-16] The selection of dressings for military conflict-related situations, including non-

Table I. Description of hemostatic products tested in wound puncture model by form and type[a]

Test products	Manufacturer	Description
Powder/granular agents		
ACS+ (ACS+)	Z-Medica Corp Wallingford, Conn	Mineral Al/Si Zeolite with attenuated exothermic reaction
Celox (CEL)	Sam Medical Products Newport, Ore	Chitosan based granules
Instaclot (IC)	Emergency Medical Devices Loxahatchee, Fla	Proprietary composition powder
WoundStat (WS)	TraumaCure, Inc. Bethesda, Md	Polymer/mineral Al/Si granules
Solid (flexible) agents		
Alpha Bandage (AB)	H & H Bena, Va	4" × 4" interwoven fabric, bamboo and fiberglass
BloodStop (BLS)	LifeScience, Santa Clara, Calif	Coated deoxidized cellulose based gauze
X-Sponge (XS)	Z-Medica Corp Wallingford, Conn	4" × 4" gauze coated with proprietary hemostatic formulation
Solid (rigid) agents		
Chitoflex (CHI)	HemCon Medical Technologies, Inc.	3" × 28" roll made of chitosan
HemCon (HC)	Portland, Ore	4" × 4" pad made of chitosan
Polymem FP-21 (FP-21)	Ferris Mfg. Corp. Burr Ridge, Ill	3-layer Dextran/chitosan based 4" × 4" wafer

[a]The abbreviations used in this article for each product are indicated in parenthesis.

compressible wounds, requires identification of specific products that exhibit superior performance in these challenging injuries.

Recently, major developments have been made in the composition, texture, and active constituent materials of hemostatic dressings.[11,17-20] The relative efficacy of these dressings may be influenced by their form, nature, and application, especially in trauma patients with bleeding wounds. These improved hemostatic dressings were evaluated in an animal vascular transection injury model and showed better performance than standard compressed gauze, but the difference between dressings was minimal.[19]

Owing to different modes of action and presentation of these diverse products, it is reasonable to assume that effectiveness will vary with the severity of injury. For example, some hemostatic dressings may perform better in high-pressure bleeding injuries, whereas others may be more effective in slower bleeding injuries. Therefore, efficacy of these dressings needs to be confirmed for bleeding control and long-term secure placement on the wound (when the casualty is moved through difficult terrains and situations or experiences a long delay for evacuation) in other types of severe injury models.

Even though in the transection model the rupture of vessels and muscles is designed to represent multiple injuries associated with combat injury,[21] application of bandages may not be sufficiently challenged by active bleeding in this model. In a continuous effort to find the best performing dressings, we evaluated products in a severe vascular puncture injury model with a high initial bleeding rate and low survival.[20,22]

The objective of this study is to compare the efficacy of hemostatic dressings of different nature (eg, granular, fabric-based) with standard gauze. The specific hypotheses of this project are that the new products are more efficacious than standard gauze in (1) arresting bleeding, (2) preventing additional bleeding over time, and (3) improving survival. Further, they should have comparable or better efficacy

than Zeolite and ACS+ hemostatic dressings currently approved for use in United States (U.S.) military.

MATERIALS AND METHODS

Hemostatic dressings. The products used for this study and their abbreviations referred to throughout are listed in Table I. The hemostatic products and the standard compressed gauze bandage (SD; H&H compressed gauze, H&H Associates, Bena, Va) were categorized according to nature, form, and composition. Hemostatic dressings were made of a wide range of material from mineral-based products made of zeolite, smectite, and kaolin to biologically based products made of chitin. We tested 10 different hemostatic agents and one control SD (11 groups in total). A more detailed description of each of the products was reported by Arnaud et al in 2009.[19]

Animal model. The study adhered to the principles in the *Guide for the Care and Use of Laboratory Animals,* (National Research Council, 1996 Edition). The study was approved by the Naval Medical Research Center/Walter Reed Army Institute of Research Institutional Animal Care and Use Committee (IACUC). All procedures were performed in accordance to the Animal Welfare Act and in an animal facility approved by the Association for Assessment and Accreditation for Laboratory Animal Care International (AAALAC).

Yorkshire swine, weighing 25 to 35 kg (Animal Biotech Industry; Danboro, Pa), were refrained from food the night before the experiment but had free access to water. Surgical techniques and animal monitoring are described in a previous study by Arnaud et al.[19] Briefly, anesthesia was induced with intramuscular injection of ketamine HCl (30 mg/kg; Henry Schein, Inc, Melville, NY) and inhalation of isoflurane (3% to 4%). The animals were allowed to breathe spontaneously or through mechanical ventilation when necessary (Narkomed M ventilator, North American Dräger Telford, PA). An 18- to 20-gauge angiocatheter (Schein Care Corp, Irvine, Calif) was placed in the right

carotid artery and a 7F or 9F Introflex introducer (Edwards Life Sciences, Irvine, Calif) in the right external jugular vein, and vital signs were continuously monitored and recorded. Pulse oximetry (Datex-Ohmeda; SurgiVet, Waukesaha, NJ) was obtained from the tongue, tail, or ear.

Puncture model. The skin of the inguinal area of the right thigh was incised longitudinal to the groin approximately 12 cm to expose the femoral vasculature. The femoral artery was invasively isolated for approximately 4 cm on the distal side of the groin, free of fascia tissue. Lidocaine (Henry Schein, Inc) (1% to 2%) was spread on the artery to maximize dilation of the vessel. Any proximal side branches from the artery were ligated to avoid redirection of blood to the main circulation. After a brief period of stabilization and recording of baseline data, bulldog clamps were placed on the artery and a 4-mm hole was perforated by an aortic vascular punch (APU440, Medtronic, Minneapolis, Minn).

After this injury, the clamps were removed to produce uncontrolled hemorrhage (time 0 [T0]) resulting in immediate release of high-pressure arterial blood. Shed blood was removed by suction directed towards the blood accumulating in the groin cavity and not directly to the injured vessel area. Blood was aspirated into a vacuum bowl that was continuously weighed. The collected blood was recorded as pretreatment blood loss. Because of the high shear stress bleeding and shed blood accumulation, blood was still present in the wound cavity at the time of the dressing application (T1).

Treatment and monitoring. The animals were randomly assigned to one of the 11 bandage regimens (n = 8 per group). Rectal temperature was monitored and maintained between 37°C and 39°C using a Bair Hugger device (Model 505, Bair Hugger, Minn). After uncontrolled bleeding for 45 seconds (T1), the treatment consisted of placement of the test dressing on the injured site with a SD placed over it. The test dressings were applied according to the manufacturer's directions for use.

A constant pressure of 175 ± 28 mm Hg (measured by HM 28 pressure monitor, Dwyer, Calif) was manually applied over the wound with the dressings for 5 minutes. The pressure was then released and the skin apposed over the dressing, which resulted in a residual pressure of 30 ± 5 mm Hg. At 15 minutes (T15), resuscitation was initiated by intravenous administration of 500 mL of isotonic colloid (Hextend, Biotime, Abbott, Abbott Park, Ill) through the external jugular vein over 30 minutes until T45 using a pump set (Masterflex, Cole Parmer, Vernon Hills, Ill). No additional fluid was administered regardless of the mean arterial pressure (MAP) or the blood loss.

The dressings remained in place on the wound, untouched for the remainder of the experiment. All animals were monitored until the end of the third hour (T180), at which time the dressings were removed, and the injured site was inspected for presence of clots. Animals that survived until T180 were euthanized with Euthasol solution (0.3 mL/kg; Virbac AH, Inc, Fort Worth, Tex).

Recording and data acquisition. Shed blood volume was collected in a sealed container (2 L MediVac;

CardinalHealth, Dublin, Ohio) and continuously weighed on a top-loading scale (PS 5100, Mettler-Toledo Inc, Columbus, Ohio). Rectal and wound temperature at the interface of wound blood and dressing were continuously recorded using temperature probes (BAT 12, Physitemp, Clifton, NJ). Blood pressure (MAP) and heart rate were continuously measured on a blood pressure analyzer (BPA; Micro-Med Inc, Louisville, Ky). Data were organized in an Excel spreadsheet (Microsoft Corp, Redmond, Wash) for further analysis. Arterial and mixed venous samples were taken at T0, T5, T15, T45, T60, T120, and T180. Blood samples were analyzed for complete blood count (Pentra 60C+, HoribaABX, Irvine, Calif), thromboelastography (TEG; TEG5000, Haemoscope, Niles, Ill), coagulation parameters (Sta Compact, Diagnostica Stago, Parsippany, NJ), and blood gas using the ABL 750 analyzer (Radiometer, Copenhagen, Denmark).

Blood loss was divided into pretreatment (initial injury hemorrhage to 45 seconds) and post-treatment (from T1 to end point). Aspirated shed blood was continuously recorded and total post-treatment blood loss was calculated as measured shed blood, blood absorbed in test dressing, and blood absorbed in the SD, each weighed at the end of the experiment.

Recurrence of bleeding (rebleeding) was recorded to assess each product for bleeding control after release of the 5-minute manual compression or when MAP reached 40 mm Hg, or both. Recurrence of bleeding was accounted by incidence of aspiration (ie, no bleeding or bleeding retained in the dressing vs blood oozing from the dressings and able to be aspirated). Given this rebleeding definition, dressing effectiveness was analyzed in terms of percentage of animals that (1) exhibited one occurrence of rebleeding between the application of dressing and end of experiment, (2) exhibited more than one occurrence of rebleeding, and (3) that died before the second rebleeding event.

Statistical analysis. The design of the study was to evaluate each dressing on eight animals, unless there was a survival rate of ≤50% after testing the first four animals in a treatment group. In that case, group size was limited to four animals. This criterion of 50% survival was also applied after testing six animals. Analysis of variance, Mann-Whitney, Kruskal-Wallis, Fisher exact, and χ^2 tests were performed (Statistix, Tallahassee, Fla; SAS Institute Inc, Cary, NC). Data are presented as mean ± standard deviation, and $P <$ .05 was considered significant. The dressings were initially ranked according to survival because survival time of the animals across the 3-hour experiment directly affected quantitative blood loss levels; and secondarily, according to bleeding arrest, which was determined by quantitative assessment of aspirated blood.

RESULTS

The study used 69 of the 88 originally intended animals (n = 8 animals per group). Three pigs died before T10 of excessive initial bleeding and were excluded from analyses. The final analysis included 66 animals. Dressings IC, HC,

Table II. Baseline and treatment variables according to treatment with hemostatic product

	Groups[b]				
Variables[a]	WS	CEL	XS	ACS+	IC
No.	8	8	8	8	4
Pretreatment[c]					
Weight, kg	29.7 ± 2.6	30.7 ± 2.6	30.5 ± 3.5	31.0 ± 2.7	27.5 ± 3.2
MAP, mm Hg	29.9 ± 6.3	29.5 ± 7.6	32.0 ± 8.2	32.8 ± 9.9	35.8 ± 12.1
Blood loss, % EBV	19.0 ± 6.8	19.8 ± 5.1	17.0 ± 5.5	18.0 ± 5.3	18.8 ± 6.9
Rate of loss, mL/min	490 ± 150	437 ± 131	462 ± 106	421 ± 167	445 ± 96
Post-treatment[d]					
Wound temperature	38.5 ± 0.7	39.1 ± 2.2	38.5 ± 0.7	42.0 ± 1.0	38.1 ± 1.0
Lowest MAP, mm Hg	22.5 ± 1.7	20.9 ± 3.9	22.9 ± 3.1	24.4 ± 2.0	20.0 ± 5.7
Blood loss (% EBV)	5.2 ± 1.6	4.3 ± 2.7	6.5 ± 3.4	5.0 ± 2.2	14.2 ± 5.1
Postresuscitation					
Highest MAP, mm Hg	63.8 ± 3.1	60.4 ± 5.8	57.8 ± 6.4	51.6 ± 6.9	62.8 ± 14.3
Blood loss, % EBV	2.8 ± 2.7	2.8 ± 1.9	9.4 ± 3.6	13.3 ± 5.5	4.1 ± 2.0

EBV, Estimated blood volume; *MAP,* mean arterial pressure; *SD,* standard dressing.
[a]Data are presented as mean ± standard deviation.
[b]See Table I for descriptions of products that were tested. Preinjury baseline (time 0) data (not included in the table) were: MAP, 64.0 ± 11.4 mm Hg; wound temperature, 38.2 ± 0.3°C.
[c]Pretreatment, Time 45 seconds except for wound temperature measured at its peak (~T4 min).
[d]Post-treatment ≤Time 15 minutes, time after treatment and before fluid resuscitation, additional post-treatment blood loss after release of compression.

CHI, FP-21, and BLS were tested on four animals, and AB was tested on six because these dressings showed repeated failure. Overall, 35 of 66 pigs survived to T180, the experimental end point.

There was no difference between groups for the baseline parameters listed in Table II. These parameters were representative of physiologic and hematologic values for Yorkshire swine in our laboratory. All animals showed comparable hematology and hemostasis baseline measurements: hematocrit, 25.5% ± 1.0%; hemoglobin, 8.6% ± 0.3 g/dL; platelet count, $326 ± 55 × 10^6$/mL; prothrombin time, 14.0 ± 0.3 seconds; fibrinogen, 138 ± 21 mg/dL; reaction time (TEG-R), 593 ± 138 seconds; kinetics (TEG-K), 171 ± 56 seconds; maximum amplitude (TEG-MA), 75.7 ± 2.2 mm; pH, 7.42 ± 0.03; lactate, 0.95 ± 0.11mM; and glucose, 3.4 ± 0.6mM. Hemodynamic parameters after injury but before treatment (T45 seconds) were also comparable between groups (Table II). Owing to blood loss of 18.8% ± 5.2% EBV, MAP was reduced from an average baseline of 64.0 ± 11.4 to 32.3 ± 7.9 mm Hg across groups. The rate of initial rate bleeding (462 ± 128 mL/min) was also comparable in all groups.

Survival. When ranked by the survival rate to the T180 end point, all dressings were superior to SD (Fig 1). We used ACS+ as a benchmark for comparison because it was deployed for combat when these experiments were conducted. Compared with 13% for animals treated with SD, survival rates of animals treated with WS, CEL, XS, and ACS+ were 78% ± 12%, which was significant (Fisher exact test, $P < .001$), and they also significantly outperformed FP-21, HC, CHI, and BLS, which exhibited the lowest survival rates (25% ± 0%, $P < .01$). AB and IC dressings had an intermediate rate of survival (50%) and were not statistically different than the dressings showing the survival rate equivalent to ACS+ or better. There was a gradation in
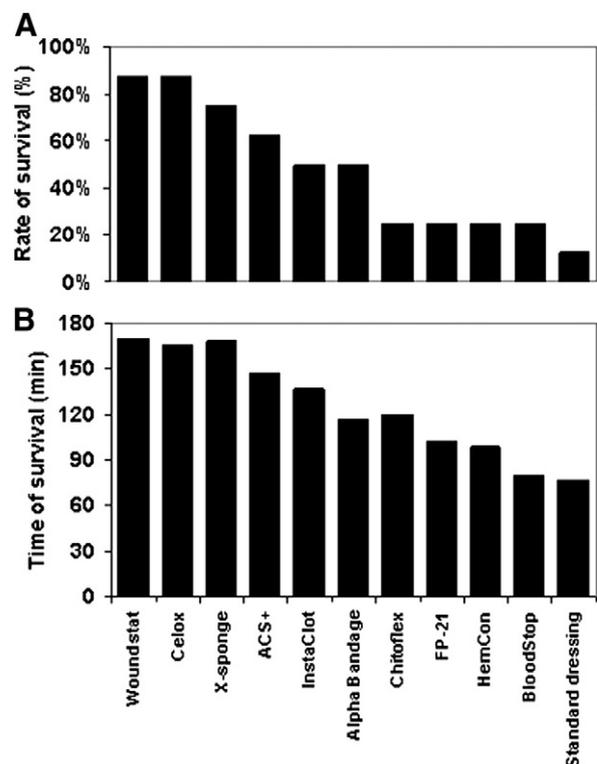
Fig 1. A, Rate of survival (%) across all dressings. **B,** Time of survival (minutes). There were eight animals in each group, except for AB (n = 6) and CHI, FP-21, HC, BLS (n = 4).

survival time that paralleled the survival rates. The time of survival for treatment with the four top dressings averaged 160 ± 13 minutes, whereas the bottom four dressings averaged 114 ± 75 minutes (χ^2 test, $P < .01$).

Table II. Continued.

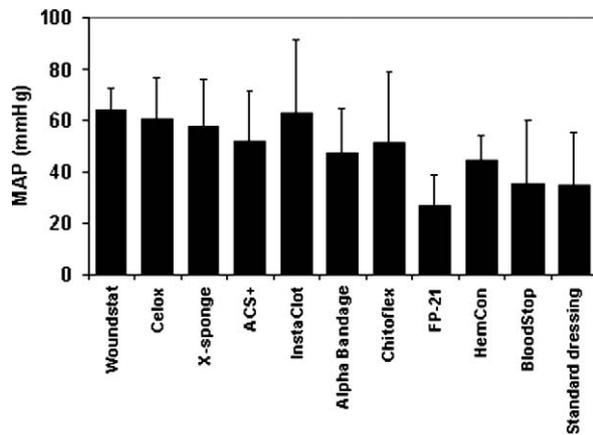|		Groups[b]			
AB	CHI	FP-21	HC	BLS	SD
6	4	4	4	4	8
28.6 ± 4.2	28.8 ± 2.0	29.9 ± 2.9	28.7 ± 0.7	30.0 ± 2.9	29.8 ± 2.6
38.2 ± 9.1	31.8 ± 5.1	27.8 ± 7.5	30.2 ± 4.4	37.2 ± 3.5	35.6 ± 8.6
16.1 ± 3.6	21.6 ± 1.5	20.6 ± 2.1	16.2 ± 2.8	15.7 ± 6.3	21.1 ± 5.7
359 ± 39	562 ± 78	465 ± 117	401 ± 87	473 ± 114	539 ± 151
37.8 ± 0.6	39.0 ± 1.2	38.3 ± 0.4	38.3 ± 0.5	38.2 ± 0.3	38.2 ± 0.7
1.2 ± 2.7	21.5 ± 5.4	15.5 ± 1.6	21.0 ± 3.8	8.7 ± 3.7	20.9 ± 4.9
14.1 ± 1.7	16.1 ± 6.1	22.7 ± 1.5	17.5 ± 8.0	43.3 ± 13.8	12.5 ± 3.8
47.5 ± 7.0	51.3 ± 14.0	27.0 ± 5.8	44.8 ± 4.7	35.8 ± 10.7	34.8 ± 7.2
15.3 ± 5.7	14.1 ± 8.3	26.5 ± 11.5	29.1 ± 4.3	32.1 ± 19.5	16.9 ± 6.8

Fig 2. Restoration of blood pressure: data indicate the highest mean arterial pressure achieved after fluid resuscitation across all groups during the 3-hour experiment time (mean ± standard deviation).

Fig 3. Incidence of rebleeding for each group is shown after release of compression *(black box)* and upon restoration of mean arterial pressure after fluid resuscitation *(white box)*.

Restoration of blood pressure. The highest blood pressure observed within each group reached levels >30 mm Hg after injury and dressing application. MAP >40 mm Hg after application of WS, CEL, XS, ACS+, HC, IC, AB, and CHI; whereas, FP-21, BLS, and SD were unable to restore and maintain blood pressures >40 mm Hg (Fig 2).

Incidence of rebleeding (attainment of hemostasis). After dressing application (post-treatment) and compression release, but before fluid resuscitation, blood loss varied among groups. At least some bleeding was observed after application with each of the dressings. This bleeding was typically observed at compression release and upon MAP increase (Fig 3 and Table III). However, group differences were documented between dressings in the control of bleeding. Bleeding (ie, incidence of aspirated blood) upon
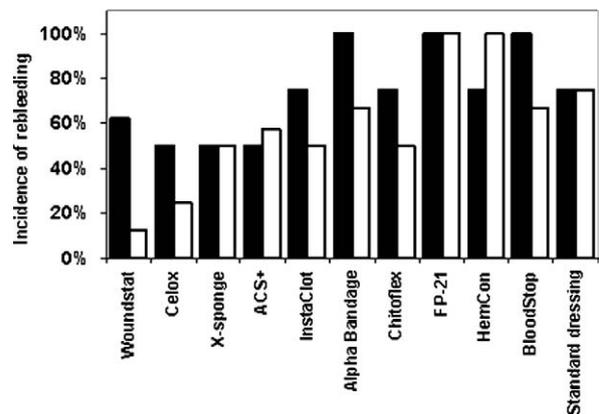
the release of manual compression ranged 50% to 62.5% for XS, WS, CEL, and ACS+, and 75% to 100% for all other dressings. This post-treatment bleeding, with a median 14.1% EBV (range, 5%-43% EBV), decreased MAP in all groups even further than occurred after injury (Table III). Upon compensation, while MAP was restored, the same top four dressings exhibited less incidence of rebleeding. These four dressings, as well as CHI, resulted in no more than a 50% incidence of a second bleeding upon restoration of MAP. The other dressings were less effective, with an incidence of rebleeding upon MAP increase of 66.7% to 100% and the additional blood loss was 3% to 32% EBV. Overall, WS and CEL exhibited the lowest incidence of rebleeding.
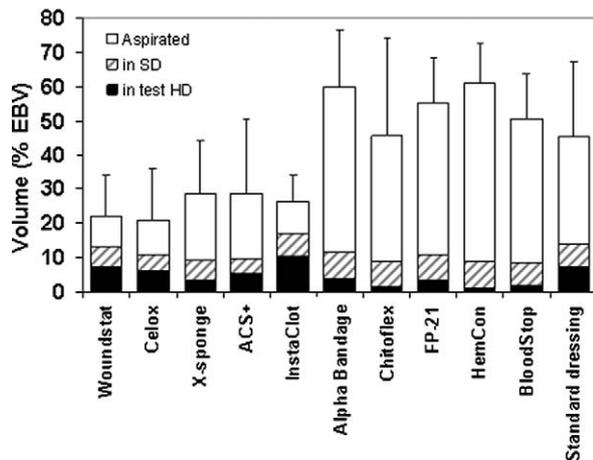
Post-treatment blood loss. Blood loss was divided between blood absorbed in both the test and SDs and the

Table III. Occurrence of rebleeding determined at time of release of compression and upon increase of mean arterial pressure as described in Materials and Methods

Group[a]	Occurrence of rebleeding			Death after first bleed (%)
	None (%)	Once (%)	Twice (%)	
WS	37.5	50	12.5	0
CEL	50	25	25	0
XS	12.5	75	12.5	0
ACS+	25	37.5	37.5	0
IC	25	25	50	25
AB	0	33.3	66.7	0
CHI	25	25	50	0
FP-21	0	25	75	25
HC	0	25	75	0
BLS	0	50	50	25
SD	12.5	37.5	50	12.5

SD, Standard dressing.
[a]See Table I for descriptions of products that were tested.

Fig 4. Post-treatment blood loss. Blood loss was divided in three categories Clear boxes indicate aspirated blood loss, hatched boxes indicate blood in standard dressing and black boxes indicate blood in hemostatic dressings. Error bars indicate standard deviation. *EBV*, Estimated blood volume.

blood aspirated from the wound (Fig 4). Post-treatment blood loss, as quantified by both absorbed and aspirated amounts, is a direct indication of the capacity of the dressing to arrest hemorrhage. Dressings promoting higher survival rates showed better bleeding control with less blood aspirated or absorbed into the dressings. With ranking based solely on aspirated blood, the top dressings were WS, CEL, IC, ACS+, and XS. This order shows consistency with survival ranking, with the exception of IC. In parallel with analysis of the survival rate between the top four dressings and the bottom four dressings, analysis on aspirated blood yielded the same result (Mann-Whitney test, $P < .001$). Blood retained in the hemostatic dressings is illustrated in the black boxes in Fig 4. This suggests that WS, CEL, IC, and SD dressings had the highest absorbing

capacity, whereas CHI, HC, and BLS had the lowest absorbing capacity.

Additional characteristics of test hemostatic dressings. Additional observations made during the experiments regarding each dressing are illustrated in Appendix (online only). In addition to survival and hemostasis, the dressings were also evaluated for their ease of application and removal. This was subjectively assessed on a scale: easy, intermediate, or difficult. The difficulty in removing loose granular products was demonstrated by the high number of individual particles left behind, whereas the ease of removing fabric and solid dressings provided a clean wound ready for repair.

DISCUSSION

We tested 10 new hemostatic dressings in a 4-mm femoral arterial puncture swine model. This model was initially developed at the Institute of Surgical Research (ISR), San Antonio, Texas, using a 6-mm punch injury as described by Kheirabadi et al.[11,20,22] These two models, 4- and 6-mm puncture, showed similar levels of severity, causing 100% or 87% death, respectively, in swine treated with standard of care (ie, compressed gauze), with an initial rate of free bleeding blood loss of 462 mL/min in the 4-mm puncture vs 850 mL/min in the 6-mm puncture. With the ISR model, the animal was splenectomized and fluid resuscitation occurred at 30 seconds after the dressing was applied. Additional crystalloid was also provided to re-establish normotension. In our model, restoration of blood pressure is the effect of the dressing and natural physiologic compensation after one 500-mL bolus infusion at 15 minutes without additional fluid.

In our model, MAP decreased immediately after the arterial puncture injury during active bleeding (on average a 19% EBV blood loss caused MAP to drop to 32 mm Hg). MAP decreased further in dressings that did not prevent rebleeding at release of compression and was observed in approximately 50% of the experiments for each of the dressings. This blood loss, in addition to blood lost after resuscitation, brought the total post-treatment blood loss to <30% EBV for WS, CEL, XP, and IC, and to >50% EBV for AB, HC, FP-21, and BLS.

Survival rate was not significantly different among individual treatment groups, but survival rates and times for the top performing dressings as a group (WS, XS, CEL and ACS+) were significantly higher compared with the group of less effective dressings in this model (CHI, FP-21, HC, BLS). This relationship was consistent for post-treatment blood loss and incidence of rebleeding. Most of the dressings exhibited rebleeding during the release of compression or an increase in MAP, or both. Overall, WS, XS, CEL, and ACS+—the best performing dressings in this model as determined by survival—had lower incidences of blood loss at both of these times.

All other dressings, which were better than the SD, were less effective in terms of bleeding control, particularly during the secondary incidence of rebleeding. Most of the SD-treated animals died within about 60 minutes due to

the high incidence of rebleeding and poor hemostasis. This indicates that in high shear-rate bleeding, absorption alone is not sufficient to inhibit rebleeding and promote survival. However, fabric-like dressings that are absorbent and also have additional hemostatic properties (ie, XS) provided better bleeding control by promoting early blood clotting and stopping blood from oozing into the dressings, hence increasing survival.

The incidence of rebleeding after compression is released (first possible incidence) was high in the less effective dressings and was often fatal. It is compelling that the top performing dressings that controlled bleeding in this model corresponded to the same dressings previously observed as superior in the transection model.[19] These dressings, despite being consistently effective in comparison of multiple dressings, did yield more blood loss and less survival in the puncture model than in the transection model. This observation substantiates the severity of the hemorrhagic injury in this model despite there being no soft tissue injury, as is the case in the transection model.

Treatment with IC and AB dressings resulted in intermediate levels of survival (50%). Additional animals were allowed in the AB group because the initial performance was positive (3 of 4 survived). However, massive bleeding occurred with both of these dressings due to insufficient clotting. As blood filled the wound cavity, AB seemed unable to promote rapid clotting. Also, the IC membrane did not seem to allow ready absorption, which resulted in significant blood loss around the dressing edges in failed cases. Of the four other dressings, which yielded 25% survival, CHI, FP-21, and HC exhibited little initial absorption, followed by a high incidence of rebleeding. BLS, owing to the small size of the dressing unit, was unable to contain massive bleeding.

These findings suggest that with high shear bleeding, success is significantly influenced by the capacity of the dressing to both rapidly absorb blood and promote clotting. In terms of blood loss, survival is a confounding factor; animals that survived for a longer time had more occasions to experience rebleeding with increased MAP. The lack of rebleeding was an indication of dressing efficacy. On the other hand, animals that bled excessively after 15 minutes tended to die more rapidly, thereby exhibiting a lower total blood loss.

The 5-minute compression stage is necessary to promote close contact between the dressing and the source of bleeding. This compression procedure seems key for efficacy, initiating contact and adherence soon after application; otherwise, risk of rebleeding may be high, especially for dressings that depend on adherence (eg, HC and FP-21). It is obvious that in this puncture injury model, where the vessel is not fully sectioned and always under risk of high shear-rate bleeding, full obstruction of the created orifice by natural clot formation is almost unattainable. Thus, the initial closing of the aperture relies almost solely on the firm contact between dressing and blood vessel. This might explain why, when there is not a firm contact, rebleeding occurred at the release of compression. Granular or powder

dressings are able to create a more complete seal and control bleeding better when MAP increases because they closely surrounded the vessel. In the firm-dressing category, flexible fabric-like dressings may offer a better first contact with the vessels (XS performed better than FP-21). In some cases, it seemed that a firm seal, when established for any dressing, created a pool of blood trapped in the wound underneath the dressing, which might eventually clot and act as a vascular plug.

Dressings that did not perform well in this model were still found to have interesting properties. At least one animal survived in each of the groups, suggesting that each of these test dressings may work in particular conditions. The dressings were tested as instructed by the manufacturer, but other factors could have contributed to their relatively less effective performance; their unit size could have been too small (BLS, FP-21), or the shape did not conform the wound (ie, IC, FP-21). The shape and rigidity of IC, for instance, was either extremely beneficial or extremely detrimental to its performance. The covering membrane did not easily dissolve and prevented rapid contact and absorption of blood with the hemostatic powder. In the few cases when the membrane did dissolve or break, the powder was effective in absorbing blood.

Dressings with strong adhesive properties (HC, CHI) were able to control bleeding extremely well when a strong seal was formed around the wound. A broken seal, however, became the source of leakage, leading to increased blood loss. We also observed that some granular materials were only partially soaked due to groin anatomy, placement of the dressing, or direction of bleeding. This posed problems during the removal stage, particularly for the powders (IC, CEL and WS). Removal by scooping the unabsorbed powder left a tremendous amount of residue in the wound that could be removed only with a high volume of rinse solution.

A major difference between the puncture injury model used in this study and the transection injury model used in previous protocols is that immediately after injury, the initial blood loss is lower and MAP remains high. This increases the chance to disrupt hemostasis in the puncture model and subsequently causes more blood loss and tends to lead to early death. Survival rate in the transection model was higher, despite an initially higher blood loss, likely because the animals were hypotensive at the time the hemostatic dress was applied. They also benefited from vessel retraction that occurred with this injury. Overall, the arterial puncture model is different yet relevant and very appropriate for bandage testing of severe hemorrhage observed in combat casualties.

CONCLUSIONS

WS, CEL, and XP performed similarly to ACS+ in controlling bleeding. The dressings have a comparably low incidence of rebleeding, and if rebleeding occurs, they limit blood loss and allow restoration of MAP >50 mm Hg, which ultimately leads to survival. Alternately, CHI, FP-21, HC, and BLS did not perform as well in this model of a

puncture injury with high-pressure bleeding. These dressings exhibited a high incidence of rebleeding, leading to a high volume of blood loss and a MAP <40 mm Hg, which did not support survival for the 3-hour experimental period.

AUTHOR CONTRIBUTIONS

Conception and design: AF
Analysis and interpretation: AF, KT, TT
Data collection: AF, KT, TT
Writing the article: AF
Critical revision of the article: RM, WC
Final approval of the article: AF, RM
Statistical analysis: AF
Obtained funding: RM
Overall responsibility: AF

REFERENCES

1. Blackbourne L. Combat damage control surgery. Crit Care Med 2008;36:S304-10.
2. Eastridge BJ, Malone D, Holcomb JB. Early predictors of transfusion and mortality after injury: a review of the data-based literature. J Trauma 2006;60:S20-5.
3. Eastridge BJ, Salinas J, McManus JG, Blackburn L, Bugler EM, Cooke WH, et al. Hypotension begins at 110 mm Hg: redefining "hypotension" with data. J Trauma 2007;63:291-7; discussion 297-9.
4. Mabry RL. Tourniquet use on the battlefield. Mil Med 2006;171:352-6.
5. Welling DR, Burris DG, Hutton JE, Minken SL, Rich NM. A balanced approach to tourniquet use: lessons learned and relearned. J Am Coll Surg 2006;203:106-15.
6. Thorey F, Stukenborg-Colsman C, Windhagen H, Wirth CJ : The effect of tourniquet release timing on perioperative blood loss in simultaneous bilateral cemented total knee arthroplasty: a prospective randomized study. Technol Health Care 2008;16:85-92.
7. Surgeons Aco. ATLS advanced trauma life support program for doctors. 7th edition. Chicago: American College of Surgeons; 2004.
8. Pusateri AE, Holcomb JB, Kheirabadi BS, Alam HB, Wade CE, Ryan KL. Making sense of the preclinical literature on advanced hemostatic products. J Trauma 2006;60:674-82.
9. Kozen BG, Kircher SJ, Henao J, Godinez FS, Johnson AS. An alternative hemostatic dressing: comparison of CELOX, HemCon, and QuikClot. Acad Emerg Med 2008;15:74-81.
10. Kheirabadi BS, Sieber J, Bukhari T, Rudnicka K, Murcin LA, Tuthill D. High-pressure fibrin sealant foam: an effective hemostatic agent for treating severe parenchymal hemorrhage. J Surg Res 2008;144:145-50.
11. Kheirabadi BS, Edens JW, Terrazas IB, Estep JS, Klemcke HG, Dubick MA, et al. Comparison of new hemostatic granules/powders with currently deployed hemostatic products in a lethal model of extremity arterial hemorrhage in swine. J Trauma 2009;66:316-26; discussion 327-8.
12. Arnaud F, Tomori T, Saito R, McKeague A, Prusaczyk WK, McCarron RM. Comparative efficacy of granular and bagged formulations of the hemostatic agent QuikClot. J Trauma 2007;63:775-82.
13. Sondeen JL, Pusateri AE, Coppes VG, Gaddy CE, Holcomb JB. Comparison of 10 different hemostatic dressings in an aortic injury. J Trauma 2003;54:280-5.
14. Jewelewicz DD, Cohn SM, Crookes BA, Proctor KG. Modified rapid deployment hemostat bandage reduces blood loss and mortality in coagulopathic pigs with severe liver injury. J Trauma 2003;55:275-80; discussion 280-1.
15. Wedmore I, McManus JG, Pusateri AE, Holcomb JB. A special report on the chitosan-based hemostatic dressing: experience in current combat operations. J Trauma 2006;60:655-8.
16. Rhee P, Brown C, Martin M, Salim A, Plurad D, Green D, et al. QuikClot use in trauma for hemorrhage control: case series of 103 documented uses. J Trauma 2008;64:1093-9.
17. Ward KR, Tiba MH, Holbert WH, Blocher CR, Draucker GT, Proffitt EK, et al. Comparison of a new hemostatic agent to current combat hemostatic agents in a Swine model of lethal extremity arterial hemorrhage. J Trauma 2007;63:276-83; discussion 283-4.
18. Carraway JW, Kent D, Young K, Cole A, Friedman R, Ward KR. Comparison of a new mineral based hemostatic agent to a commercially available granular zeolite agent for hemostasis in a swine model of lethal extremity arterial hemorrhage. Resuscitation 2008;78:230-5.
19. Arnaud F, Parreño-Sacdalan D, Tomori T, Delima MG, Teranishi K, Carr W, et al. Comparison of ten hemostatic dressings in a groin transection model in swine. J Trauma 2009 [in press].
20. Kheirabadi BS, Acheson EM, Deguzman R, Sondeen JL, Ryan KL, Delgado A, et al. Hemostatic efficacy of two advanced dressings in an aortic hemorrhage model in Swine. J Trauma 2005;59:25-34; discussion 34-5.
21. Alam HB, Burris D, DaCorta JA, Rhee P. Hemorrhage control in the battlefield: role of new hemostatic agents. Mil Med 2005;170:63-9.
22. Acheson EM, Kheirabadi BS, Deguzman R, Dick EJ Jr, Holcomb JB. Comparison of hemorrhage control agents applied to lethal extremity arterial hemorrhages in swine. J Trauma 2005;59:865-74; discussion 874-5.

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Appendix (online only). Characteristics of hemostatic dressings used in the puncture injury model

Hemostatic dressings by type	Advantages	Disadvantages	Ease of removal	Fitness of the dressing to the wound
Powder/granular agents				
ACS+	Easily applied, conforms to the wound, easily removed	Hemorrhage arrest depends on the amount of blood present in the wound; clotting weak	Easy	Adequate
Celox	Easily poured in the wound, conforms to all cavities in the wound; forms a uniform gel that can mix with clotted blood	Powder need to be soaked for easy removal; blood distribution is not homogeneous; quite messy to remove if not completely soaked	Messy if not soaked	Adequate
Instaclot	Very effective powder; firm clot and packing when soaked	Fragile packaging membrane breaks easily before application; packaging membrane hinders contact with blood; messy to remove	Messy if not soaked	Not pliable
WoundStat	Easily poured in the wound, conforms to all cavities in the wound; forms clay when soaked; promotes firm clot; could be remixed in the wound in case of rebleeding	Quite messy to remove if not completely soaked; heavier than other products	Messy if not soaked	Adequate
Solid (rigid) agents				
Chitoflex	Roll fits well in wound; readily dissolves in blood, easily removed	May not adhere enough to stop blood immediately	Easy	Adequate
HemCon	May seal wound very well; good ability to retain clot at tissue interface when adherent; easily removed if non adherent; lightweight	Does not always seal the wound; allows leak through unattached material; blood does not stick on the upper surface; difficult to remove when adherent	Easy	Not pliable
Polymem FP-21	Good gel; dissolves in blood; very light	Awkard fitting to wound; needs additional material	Intermediate	Not pliable
Solid (flexible) agents				
Alpha Bandage	Easy fit to wound; easily removed–lightweight	Weak absorption; weak clot formation	Easy	Adequate
BloodStop	Easy fit to wound; may form good clot with low blood flow; easily removed; very light	Product not effective for severe bleeding; insufficient sample size	Easy	Adequate
X-Sponge	Easily applied; fits wound; good clot formation; light weight	Needs initial pressure to make it wetable	Easy	Adequate