Effect of a Chitosan-Based Hemostatic Dressing on Blood Loss and Survival in a Model of Severe Venous Hemorrhage and Hepatic Injury in Swine

Anthony E. Pusateri, PhD, Simon J. McCarthy, PhD, Kenton W. Gregory, MD, Richard A. Harris, DVM, MS, Luis Cardenas, Albert T. McManus, PhD, and Cleon W. Goodwin, Jr., MD

Background: **Hemorrhage is a leading cause of death from trauma. An advanced hemostatic dressing could augment available hemostatic methods. We studied the effects of a new chitosan dressing on blood loss, survival, and fluid use after severe hepatic injury in swine.**

Methods: **Swine received chitosan dressings or gauze sponges. Standardized, severe liver injuries were induced. After 30 seconds, dressings were applied and resuscitation initiated. Blood loss, hemostasis, resuscitation volume, and 60-minute survival were quantified.**

Results: **Posttreatment blood loss was reduced ($p < 0.01$) in the chitosan group (264 mL; 95% confidence interval [CI], 82–852 mL) compared with the gauze group (2,879 mL; 95% CI, 788–10,513 mL). Fluid use was reduced ($p = 0.03$) in the chitosan group (1,793 mL; 95% CI, 749–4,291) compared with the gauze group (6,614 mL; 95% CI, 2,519–17,363 mL). Survival was seven of eight and two of even in the chitosan and gauze groups ($p = 0.04$), respectively. Hemostasis was improved in the chitosan group ($p = 0.03$).**

Conclusion: **A chitosan dressing reduced hemorrhage and improved survival after severe liver injury in swine. Further studies are warranted.**

Key Words: **Hemorrhage, Trauma, Hemostasis, Liver, Venous, Chitosan, Dressing, Swine.**

J Trauma. 2003;54:177–182.

Hemorrhage is the second leading cause of death after trauma in the civilian community[1–3] and the leading cause of death from battlefield trauma.[4] Hemorrhage accounts for the vast majority of operating room deaths among trauma patients.[5] In addition, incomplete hemostasis in previously recognized injuries was the leading cause resulting in reoperation for hemorrhage in trauma patients.[6] The development of new methods or devices for hemorrhage control may contribute to a future reduction in hemorrhagic deaths. The development of improved methods for hemorrhage control is currently a major emphasis within the Department of Defense Combat Casualty Care Research Program. The identification of an advanced hemostatic dressing has received particular emphasis.

Chitosan is a biodegradable, nontoxic, complex carbohydrate derivative of chitin (poly-β[1→4]-*N*-acetyl-D-glucosamine), a naturally occurring substance. Chitosan is the deacetylated form of chitin. In general, the generic term chitosan is applied when the extent of deacetylation is above 70% and the generic term chitin is used when the extent of deacetylation is insignificant, or below 20%. With less than 100% deacetylation, the chitosan polysaccharide is a linear block copolymer containing both *N*-acetyl-D-glucosamine and D-glucosamine monomer units. In its acid salt form, chitosan demonstrates mucoadhesive activity,[7] which makes it an ideal candidate for consideration as a hemostatic agent. A variety of forms of chitins and chitosans have been used to enhance hemostasis in experimental studies involving bleeding from small parenchymal defects and similar experimental insults. Liquid chitosans improved hemostasis in several animal studies.[8–11] Hemostatic activity of thin membranes of chitosan have been demonstrated in models involving peritoneal abrasions in rabbits.[12] Thin membranes of chitin have been effective for surface splenic incisions and isolated splenic capsular stripping[13,14] in animal models, and in 3-mm-deep intestinal wall incisions in human subjects.[13]

Although these materials were effective in the models used, there are currently no data available to support the use of the reported formulations for major hemorrhage from large vessels. The use of liquid hemostatic agents to achieve hemostasis when applied directly to large defects without vascular control is limited by physical considerations. Liquid hemostatic agents must be applied intraparenchymally, in conjunction with vascular control, or as part of a dressing. The data reported for chitin and chitosan in membrane form to date are limited to relatively minor bleeding and, therefore,

Submitted for publication November 5, 2001.
Accepted for publication January 19, 2002.

From the U.S. Army Institute of Surgical Research (A.E.P., L.C., A.T.M., C.W.G.) and Brooke Army Medical Center (R.A.H.), Fort Sam Houston, Texas, and Oregon Medical Laser Center (S.J.M., K.W.G.), Portland, Oregon.
This research was funded solely by the U.S. Army Medical Research and Materiel Command.

The opinions or assertions expressed herein are the private views of the authors and are not to be construed as official or as reflecting the views of the U.S. Department of the Army or the U.S. Department of Defense.

Address for reprints: Anthony E. Pusateri, PhD, Library Branch, U.S. Army Institute of Surgical Research, 3400 Rawley East Chambers Avenue, Fort Sam Houston, TX 78234-6315; email: anthony.pusateri@cen.amedd.army.mil.

DOI: 10.1097/01.TA.0000032512.04647.C6

are not sufficient to allow assessment of potential efficacy for severe hemorrhage.

The liver is the most commonly injured abdominal organ causing hemorrhagic death[5] and the most frequent source of uncontrolled hemorrhage requiring reoperation.[6] Grade V hepatic injuries, as classified by the Liver Injury Scale of the American Association for the Surgery of Trauma,[15] involve extensive parenchymal damage combined with major vascular laceration. Most reported mortality rates for these injuries are greater than 60%.[16] Currently, the most effective method for treating grade V liver injuries appears to be packing the abdomen with gauze sponges to achieve tamponade followed by reoperation for pack removal at a later time.[17–19] An effective hemostatic dressing could augment current methods for control of this severe form of hemorrhage. Recently, a freeze-dried chitosan-based dressing has been developed. This chitosan dressing[20] was designed to optimize the mucoadhesive surface density and structural integrity of chitosan at the site of injury. Using a model of severe hepatic trauma and vascular injury in swine, we examined the effects of a freeze-dried chitosan-based dressing on blood loss and short-term survival.

MATERIALS AND METHODS

Animals

Crossbred commercial swine were used in this study. Animals were maintained in a facility accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care International. This study was approved by the Institutional Animal Care and Use Committee of the U.S. Army Institute of Surgical Research, Fort Sam Houston, Texas. Animals received humane care in accordance with the *Guide for the Care and Use of Laboratory Animals* (National Institutes of Health publication 86-23, revised 1996).

Experimental Dressings

A high-molecular-weight, food-grade chitosan was acquired and processed to produce 10.2 × 10.2 × 0.4-cm open porous sponges of chitosan acetate salt.[20] Medical foam adhesive-coated backing (0.056 cm caliper) was attached to the top surface of the sponges to enable easy handling and uniform application of pressure in a bleeding field. Gauze sponges (10.2 × 10.2 cm) were used for the gauze control group (Johnson & Johnson Medical, Inc.).

Experimental Procedure

Animals were assigned randomly to receive either the chitosan dressings or gauze sponges. Surgical preparation consisted of the following. Animals were fasted before the surgical procedure, with water allowed ad libitum. After premedication with glycopyrrolate and a combination of tiletamine HCl and zolazepam HCl (Telazol, Fort Dodge Laboratories, Fort Dodge, IA), anesthesia was induced by mask using 5% isoflurane. The swine were intubated, placed on a ventilator, and maintained with isoflurane. Carotid arterial and jugular venous catheters were placed surgically. Laparotomy was performed and splenectomy and urinary bladder catheter placement were completed. A rectal temperature between 38.0° and 40.0°C, arterial blood pH between 7.39 and 7.41, and 15 minutes of stable mean arterial pressures (MAP) were required before further experimental procedures. Blood pressure and heart rate were recorded at 10-second intervals throughout the study period using a continuous data collection system (Micro-Med, Louisville, KY). Blood pH was monitored using a TCM 7000 blood gas monitoring system (Diametrics Medical, Inc., Roseville, MN). Baseline arterial blood samples were collected from each animal to confirm that each animal exhibited normal hematocrit, hemoglobin concentration, platelet count, prothrombin time, activated partial thromboplastin time, and plasma fibrinogen concentration.

Liver injuries were induced as previously reported.[21] Briefly, the method included the following. The liver was retracted by manually elevating the left and right medial lobes to allow adequate exposure. Next, a specially designed clamp with two 4.5-cm sharpened tines configured in the form of an "X" was positioned with the center approximately 2 to 3 cm dorsal to the intersection of the left and right medial lobes, on the diaphragmatic surface of the liver. The base plate of the instrument was positioned beneath the quadrate lobe, on the visceral surface. The injury was induced by clamping the tines of the instrument through the parenchyma and underlying vessels of the two medial lobes so that the tines were seated in corresponding grooves in the base plate of the instrument. After the first penetration of the liver, the instrument was opened and the tines were withdrawn and repositioned laterally, such that the second application would overlap the first by 50%. After this repositioning, the liver was penetrated a second time. Documentation of the liver injury was achieved by excision and inspection of the liver at the conclusion of the experimental period. The injuries appeared as large stellate wounds with a small island of tissue in the center, and measured approximately 10 × 8 × 4 cm. The injuries were through and through, with one or more of the left medial lobar vein, right medial lobar vein, and portal hepatic vein lacerated.

Thirty seconds after injury, resuscitation was initiated with warm (39°C) lactated Ringer's solution in all animals. The goal of resuscitation was return to baseline MAP. Fluid was administered at 260 mL/min. This resuscitation regimen was continued until the desired MAP was reached and reinitiated if MAP decreased, throughout the 60-minute study period. Simultaneously with initiation of resuscitation (30 seconds postinjury), treatments were applied as follows. One dressing was applied to the surface of the quadrate lobe to cover the penetrating injury and two other dressings were applied to the injury from the diaphragmatic aspect. Compression was applied for 60 seconds in the dorsoventral direction. After 60 seconds, the injury was inspected to determine whether hemostasis was achieved. Next, the

applicator's hands were repositioned and pressure was applied for 60 seconds in the lateromedial direction, and the observation for hemostasis was repeated. This sequence was repeated for a total of four 60-second compressions. If hemostasis was complete after any compression, no further compressions were performed. Hemostasis was defined as the absence of visually detectable bleeding from the injury site.

After completion of treatment application, the abdomen was temporarily closed with size 0 monofilament suture in a continuous pattern, and the animal was monitored for 60 minutes after injury or until death, whichever came first. Death before 60 minutes was defined as a heart rate of 0. At 60 minutes, surviving animals were killed with pentobarbital.

Immediately after induction of the injury, blood was continuously suctioned from the peritoneal cavity until the start of treatment application. The volume was determined and designated as pretreatment blood loss. At the end of the study period, each abdomen was opened and the liquid and clotted intraperitoneal blood were suctioned and measured. This was designated as posttreatment blood loss. Blood in the dressings was not included in this calculation. In addition, total resuscitation fluid use was recorded. Preinjury animal blood volume was estimated as previously reported.[22]

After excision of the liver, the adherence strength of each dressing was subjectively scored. The adherence strength scoring scale consisted of a range of scores from 1 through 5. A score of 1 indicated no adherence; 2 indicated slight adherence; 3 indicated adherence adequate to cause stretching of tissue in contact with the dressing without lifting the liver from the table; 4 indicated that dressing adherence was sufficient to partially lift the liver from the table; and 5 indicated that the dressing adherence was sufficient to completely lift the liver from the table, when the dressing was grasped and lifted with forceps. For analysis, the mean score from the three dressings within each animal was treated as a single value for adherence strength.

Statistical Analysis

Body weight, estimated blood volume, number of vessels lacerated (including left medial lobar vein, right medial lobar vein, and portal hepatic vein), baseline MAP, survival time, preinjury MAP, pretreatment blood loss, and hematologic data were analyzed by analysis of variance using the GLM procedure of SAS.[23] Data are reported as means ± SEM. Data were examined for heterogeneity of variance and nonnormality. These conditions were detected for posttreatment blood loss and fluid use data. Therefore, blood loss and fluid use data were log transformed before analysis. The transformed data were analyzed by analysis of variance using the GLM procedure of SAS.[23] These data are expressed as back-transformed means and 95% confidence intervals. Pretreatment blood loss was used as a covariate for analysis of posttreatment blood loss. Categorical data, including distribution of female and male swine, hemostasis (yes or no), and survival, were analyzed by Fisher's exact test using the FREQ procedure of SAS.[23] Adhesion strength scores were analyzed by the Wilcoxon rank sum test using the NPAR1WAY procedure of SAS.[23] Adhesion scores are reported as arithmetic means and SEM. Two-sided tests were used for all comparisons.

Table 1 Preinjury Animal Characteristics

Variable	Gauze Sponge Control Group	Chitosan Group	*p* Value of Difference
No.	7	8	N/A
Body weight (kg)	39.1 ± 1.2	38.7 ± 1.1	0.82
Estimated blood volume (mL)	2,819 ± 66	2,800 ± 61	0.83
Female/male	5/2	6/2	0.88
Baseline MAP (mm Hg)	71.3 ± 3.6	68.8 ± 3.3	0.50
Preinjury MAP (mm Hg)	69.1 ± 4.8	69.3 ± 4.4	0.98
Hematocrit (%)	32.2 ± 1.1	32.6 ± 1.0	0.79
Hemoglobin (g/dL)	11.2 ± 0.4	11.3 ± 0.3	0.80
Platelets (1,000/⧗L)	567 ± 28	502 ± 25	0.11
PT (s)	10.7 ± 0.2	10.6 ± 0.2	0.70
aPTT (s)	15.7 ± 0.9	16.5 ± 0.9	0.56
Fibrinogen (g/dL)	159 ± 9	180 ± 8	0.10

PT, prothrombin time; aPTT, activated partial thromboplastin time.

RESULTS

There were no differences among treatment groups in animal body weight, estimated blood volume, distribution of animal sexes, baseline MAP, preinjury MAP, number of major vessels lacerated within the liver injury, pretreatment blood loss, or baseline hematologic values (Tables 1 and 2). Posttreatment data are depicted in Table 3. Posttreatment blood loss was reduced in the chitosan group, compared with the gauze sponge group ($p < 0.01$). Fluid use was also reduced in the chitosan group ($p = 0.03$). Survival percentage was increased in the chitosan group ($p = 0.04$). Hemostasis occurred more frequently in the chitosan group at 3 and 4 minutes postinjury ($p = 0.03$). Survival times could not be statistically compared because all but one animal survived in the chitosan group. Dressing adherence strength scores were higher in the chitosan group (3.4 ± 0.4) than in the gauze group (2.0 ± 0.2; $p = 0.03$).

DISCUSSION

This animal model involves extensive parenchymal and vascular damage. The vascular structures damaged are approximately 1 cm in diameter. In human trauma, injury to major abdominal veins, such as the portal and hepatic veins, is associated with significant hemorrhage and mortality.[24] We believe that the animal model reported here is relevant not only to hepatic trauma but also to severe venous hemorrhage in general. We have previously reported data from animals that were either untreated or were treated with perihepatic packing using this model.[21] These negative and "gold standard" controls were not considered necessary for inclusion in the present study.

Table 2 **Injury Characteristics**

Variable	Gauze Sponge Control Group	Chitosan Group	*p* Value of Difference
No. of vessels lacerated	1.86 ± 0.29	1.88 ± 0.27	0.96
Pretreatment blood loss (mL)	296.1 ± 55.4	291.1 ± 55.4	0.95
Pretreatment blood loss (mL/kg body weight)	10.6 ± 2.0	10.3 ± 2.0	0.94

Posttreatment blood loss was significantly reduced ($p < 0.01$) in the chitosan dressing group. In previous studies using chitosan as a hemostatic agent, topically applied liquid chitosan reduced lingual bleeding time in rabbits with normal hemostatic function,[9] with impaired platelet function,[10] and with impaired coagulation function.[11] Liquid chitosan was also used to control diffuse capillary bleeding in brain tissue in a feline model[8] and to treat esophageal varices in dogs.[25] Microcrystalline chitosan has been used for sealing arterial puncture sites after catheterization.[26] In another study, liquid chitosan failed to reduce blood loss in a model of hepatic hemorrhage in rats.[27]

Other chitosans and chitins have been formed into thin sheets and used for hemostasis. An 80% deacetylated chitosan membrane reduced bleeding after visceral and parietal peritoneal abrasion in a rabbit model.[12] More recently, fully acetylated chitin sheets shortened time to hemostasis, compared with commercially available absorbable collagen and oxidized cellulose hemostatic products, in a model involving 3-mm-deep surface lacerations of the spleen and isolated splenic capsular stripping in swine.[13] Hemostatic activity was also reported for this product when used for 3-mm-deep lacerations of the small intestine in human subjects.[13] In another study of splenic surface lacerations, a fully-acetylated *N*-acetylglucosamine sheet reduced time to hemostasis compared with liquid fibrin glue in swine.[14] This chitin sheet also reduced time to hemostasis compared with absorbable collagen in a model of capillary splenic hemorrhage in heparinized swine.[14]

Although hemostatic efficacy has been previously demonstrated with chitosan and chitin, this is the first study in which hemorrhage control has been demonstrated with a chitosan hemostat in a model of severe, rapidly flowing hemorrhage from large, low-pressure vascular structures. The chitosan dressing reported here reduced blood loss ($p < 0.01$) compared with gauze sponges (264 mL and 2,879 mL, respectively). Hemostasis was attained more frequently in the chitosan group after 3 minutes. In addition, fluid use was decreased and survival was increased in the chitosan group. These results are similar to those reported for a fibrin hemostatic dressing, using this same model.[21] In that study, blood loss in the fibrin hemostatic dressing group was 544 mL, whereas that in the placebo dressing group was 4,222 mL, in similarly sized animals. Survival was increased in the fibrin hemostatic dressing group compared with the untreated controls but was not different from the placebo dressing group. Fluid use was reduced compared with the untreated control group but not compared with the placebo dressing group. Others have reported reduced blood loss with the fibrin hemostatic dressing after severe liver injury, both with[28] and without coagulopathy.[22]

The chitosan-based dressing used in this study is lightweight and flexible, and requires no refrigeration or other special storage conditions. Chitin is an abundant and inexpensive material that can be converted to chitosan by relatively simple processing procedures. This may offer an attractive option to fibrinogen-based hemostatic agents, which are potentially infectious and expensive, because of the scarcity of fibrinogen and the extensive processing required.

The adherence scores combined with the blood loss results suggest that the adhesiveness and tensile strength are adequate for the control of severe hemorrhage. However, the long-term stability of a vascular seal formed by this chitosan-based dressing is unknown and must be studied. The use of a chitosan-based dressing for the treatment of experimental liver trauma also raises the important questions of absorb-

Table 3 **Posttreatment Blood Loss, Fluid Use, Hemostasis, and Survival**

Variable	Gauze Sponge Control Group	Chitosan Group	*p* Value of Difference
Posttreatment blood loss (mL)	2,879 (95% CI, 788–10,513)	264 (95% CI, 82–852)	<0.01
Posttreatment blood loss (mL/kg body weight)	102.4 (95% CI, 28.2–371.8)	9.4 (95% CI, 2.9–30.3)	<0.01
Fluid use (mL)	6614 (95% CI, 2,519–17,363)	1793 (95% CI, 749–4,291)	0.03
Survival (%)	2/7 (28.6)	7/8 (87.5)	0.04
Survival time (min: nonsurvivors only)	38.4 ± 5.8 (n = 5)	10.0 (n = 1)	N/A
Hemostasis at 1 minute (%)	0	50	0.08
Hemostasis at 2 minutes (%)	0	50	0.08
Hemostasis at 3 minutes (%)	0	62	0.03
Hemostasis at 4 minutes (%)	0	62	0.03

CI, confidence interval.

ability and possible adverse tissue or systemic reactions. None of these questions has been examined for the formulation used in the present study.

The safety of chitin and chitosan for oral[29–32] and topical[13,33] applications has been established in a number of animal and human studies. However, there is no prior experience of chitosan in human implant applications. A number of reports suggest that chitosan may activate chemotactic and phagocytotic responses.[34–40] It has been suggested[34,36] that phagocytes have binding receptors for the *N*-acetylglucosamine and glucosamine functional groups, which are apparently also present in the proteoglycan cell wall of certain types of bacteria.[41–44] Increased levels of interleukin-8, interleukin-1, gamma-interferon, and C3 have been associated with these responses.[35,45–48] These phenomena may play a role in the reported effect of chitosan to enhance wound healing.[49]

There have been numerous reports of hemostatic agents capable of controlling capillary bleeding. Although diffuse bleeding in coagulopathic patients can be a lethal problem,[6] it does not constitute the major cause of acute exsanguination from trauma. The more important problem is bleeding from larger vessels.[5,24] Challenging animal models are necessary to identify hemostatic agents that may have potential for use in the most severe hemorrhage. A hemostatic agent that is effective for hemorrhage involving significant parenchymal and vascular damage would also be expected to be effective in capillary bleeding. The converse is not necessarily true. The physical properties necessary for a hemostatic dressing to control bleeding from large vascular structures may be very different from the properties necessary for the control of diffuse bleeding or bleeding from small vessels. Careful attention should be paid to the ability of hemostatic agents to control major hemorrhage from large blood vessels.

Combined hepatic parenchymal and major venous injuries have a high mortality rate.[16] The most frequently used method for the control of hemorrhage from severe liver injuries is gauze packing. The method of hemostasis described here appears to be rapid and effective, and may provide a useful alternative or adjunct treatment in the future. This chitosan hemostatic dressing may also be useful for other forms of severe hemorrhage. Of particular interest to the military is the potential for use by personnel with little medical training for treatment of hemorrhage that is accessible for compression, such as that of the extremities. Although not a significant problem in civilian trauma, compressible hemorrhage is a significant problem in combat casualties.[50] Considering the simplicity of use and minimal storage requirements, this dressing appears promising as a potential new method for hemorrhage control in the field.

We have demonstrated that a freeze-dried chitosan-based hemostatic dressing is capable of controlling severe parenchymal and large venous hemorrhage in a swine model of severe liver injury. Although these findings are promising, further questions must be answered. Future studies will address the issues of longer term hemostasis, absorbability, and potential toxicity. This chitosan formulation may provide an additional valuable tool for hemostasis that may be useful to surgeons and emergency medical personnel.

ACKNOWLEDGMENTS

We thank Raul Martinez, Douglas Cortez, John Uscilowicz, and Jennifer Butcher for their outstanding technical support.

REFERENCES

1. Acosta JA, Yang JC, Winchell RJ, et al. Lethal injuries and time to death in a level one trauma center. *J Am Coll Surg.* 1998;186:528–533.
2. Sauaia A, Moore FA, Moore EE, et al. Epidemiology of trauma deaths: a reassessment. *J Trauma.* 1995;38:185–193.
3. Shackford SR, Mackersie RC, Holbrook TL, et al. The epidemiology of traumatic death: a population-based analysis. *Arch Surg.* 1993; 128:571–575.
4. Bellamy RF. Causes of death in conventional warfare. *Mil Med.* 1984;149:55–62.
5. Hoyt DB, Bulger EM, Knudson MM, et al. Death in the operating room: an analysis of a multicenter experience. *J Trauma.* 1994; 37:426–432.
6. Hirshberg A, Wall MJ, Ramchandani MK, Mattox KL. Reoperation for bleeding in trauma. *Arch Surg.* 1993;128:1163–1167.
7. Evans EE, Kent SP. The use of basic polysaccharides in histochemistry and cytochemistry: IV—precipitation and agglutination of biological materials by aspergillus polysaccharide and deacetylated chitin. *J Histochem Cytochem.* 1962;10:24–28.
8. Brandenberg G, Leibrock LG, Shuman R, Malette WG, Quigley H. Chitosan: a new topical hemostatic agent for diffuse capillary bleeding in brain tissue. *Neurosurgery.* 1984;15:9–13.
9. Klollevold PR, Lew DS, Ellis DG, Bertolami CN. Effect of chitosan on lingual hemostasis in rabbits. *J Oral Maxillofac Surg.* 1991; 49:858–863.
10. Klokkevold PR, Subar P, Fukayama H, Bertolami CN. Effect of chitosan on lingual hemostasis in rabbits with platelet dysfunction induced by epoprostenol. *J Oral Maxillofac Surg.* 1992;50:41–45.
11. Klokkevold PR, Fukayama H, Sung EC, Bertolami CN. The effect of chitosan (poly-N-acetyl glucosamine) on lingual hemostasis in heparinized rabbits. *J Oral Maxillofac Surg.* 1999;57:49–52.
12. Fukasawa M, Abe H, Masaoka T, et al. The hemostatic effect of deacylated chitin membrane on peritoneal injury in a rabbit model. *Surg Today.* 1992;22:333–338.
13. Cole DJ, Connolly RJ, Chan MW, et al. A pilot study evaluating the efficacy of a fully acetylated poly-N-acetyl glucosamine membrane formulation as a topical hemostatic agent. *J Surg.* 1999;126:510–517.
14. Chan MW, Schwaitzberg SD, Demcheva M, Vournakis J, Finkielsztein S, Connolly RJ. Comparison of poly-N-acetyl glucosamine (P-GlcNAc) with absorbable collagen (Actifoam), and fibrin sealant (Bolheal) for achieving hemostasis in a swine model of splenic hemorrhage. *J Trauma.* 2000;48:454–458.
15. Moore EE, Cogbill TH, Jurkovich GJ, Shackford SR, Malangoni MA, Champion HR. Organ injury scaling: spleen and liver (1994 revision). *J Trauma.* 1995;38:323–324.
16. Boone DC, Federle M, Billiar TR, Udekwu AO, Peitzman AB. Evolution of management of major hepatic trauma: identification of patterns of injury. *J Trauma.* 1995;39:344–350.
17. Cue JI, Cryer HG, Miller FB, Richardson JD, Polk HC. Packing and planned reexploration for hepatic and retroperitoneal hemorrhage: critical refinements of a useful technique. *J Trauma.* 1990;30:1007–1013.

18. Feliciano DV, Mattox KL, Burch JM, Bitondo CG, Jordan GL. Packing for control of hepatic hemorrhage. *J Trauma.* 1986;26:738–743.
19. Hirshberg A, Walden R. Damage control for abdominal trauma. *Surg Clin North Am.* 1997;77:813–820.
20. McCarthy SJ. Hemorrhage wound dressing for control of severe bleeding and methods of producing the same. U.S. Patent Application. 2001.
21. Holcomb JB, Pusateri AE, Harris RA, et al. Effect of dry fibrin sealant dressings vs gauze packing on blood loss in grade V liver injuries in resuscitated swine. *J Trauma.* 1999;46:49–57.
22. Pusateri AE, Holcomb JB, Harris RA, et al. Effect of fibrin bandage fibrinogen concentration on blood loss after grade V liver injury in swine. *Mil Med.* 2001;166:217–222.
23. SAS Institute, Inc. *SAS/STAT User's Guide*. 4th ed. Cary, NC: SAS Institute, Inc; 1990.
24. Tyburski JG, Wilson RF, Dente C, Steffes C, Carlin AM. Factors affecting mortality rates in patients with abdominal vascular injuries. *J Trauma.* 2001;50:1020–1026.
25. Kulling D, Vournakis JN, Woo S, et al. Endoscopic injection of bleeding esophageal varices with a poly-N-acetyl glucosamine gel formulation in the canine portal hypertension model. *Gastrointest Endosc.* 1999;49:764–771.
26. Hoekstra A, Struszczyk H, Kivekas O. Percutaneous microcrystalline chitosan application for sealing arterial puncture sites. *Biomaterials.* 1998;19:1467–1471.
27. Kind GM, Bines SD, Staren ED, Templeton AJ, Economou SG. Chitosan: evaluation of a new hemostatic agent. *Curr Surg.* 1990; 47:37–39.
28. Holcomb JB, Pusateri AE, Harris RA, et al. Dry fibrin sealant dressings reduce blood loss, resuscitation volume, and improve survival in hypothermic coagulopathic swine with grade V liver injuries. *J Trauma.* 1999;47:233–242.
29. Hirano S, Seino H, Akiyama Y, Nonaka I. Biocompatibility of chitosan by oral and intravenous administration. *Polym Eng Sci.* 1988;59:897–901.
30. Jing SB, Li L, Ji D, Takiguchi Y, Yamaguchi T. Effect of chitosan on renal function in patients with chronic renal failure. *J Pharm Pharmacol.* 1997;49:721–723.
31. Pittler MH, Abbot NC, Harkness EF, Ernst E. Randomized, double blind trial of chitosan for body weight reduction. *Eur J Clin Nutr.* 1999;53:379–381.
32. Wuolijoki E, Hirvela T, Ylitalo P. Decrease in serum LDL cholesterol with microcrystalline chitosan source. *Methods Find Exp Clin Pharmacol.* 1999;21:357–361.
33. Peh KK, Khan TA, Ch'ng HS. Mechanical, bioadhesive strength and biological evaluation of chitosan films for wound dressing. *J Pharm Pharm Sci.* 2000;3:303–311.
34. Peluso G, Petillo O, Ranieri M, et al. Chitosan mediated stimulation of macrophage function. *Biomaterials.* 1994;15:1215–1220.
35. Shibata Y, Foster WY, Metzger LA, Myrvik QN. Alveolar macrophage priming by intravenous administration of chitin particles, polymers of N-acetyl-D-glucosamine, in mice. *Infect Immun.* 1997;65:1734–1741.
36. Usami Y, Minami S, Okamato Y, Matsuhashi A, Shigemasa Y. Influence of chain length of N-acetyl-D-glucosamine and D-glucosamine residues on direct and complement mediated chemotactic activities for canine polymorphonuclear cells. *Carbohydr Polym.* 1997;32:115–122.
37. Suzuki K, Tokoro A, Okawa Y, Suzuki S, Suzuki M. Effect of N-acetylchito-oligiosaccharides on activation of phagocytes. *Microbiol Immunol.* 1986;30:777–787.
38. Kosaka T, Kaneko Y, Nakada Y, Matsuura M, Tanaka S. Effect of chitosan implantation on activation of canine macrophages and polymorphonuclear cells after surgical stress. *J Vet Med Sci.* 1996; 58:963–967.
39. Tokura S, Tamura H, Azuma I. Immunological aspects of chitin and chitin derivatives administered to animals. In: Jolles P, Muzzarelli RA, eds. *Chitin and Chitinases*. Basel, Switzerland: Birkhauser Verlag; 1999:279–292.
40. Schmidt RJ, Chung LY, Andrews AM, Spyratou O, Turner TD. Biocompatibility of wound management products: a study of the effects of various polysaccharides on murine L929 fibroblast proliferation and macrophage respiratory burst. *J Pharm Pharmacol.* 1993;45:508–513.
41. Komlos L, Ben-Efraim S, Lewis NJ, Hart J, Halbrecht I. Synergistic effect of N-acetyl-D-glucosamine (NAG) on mitogenic, antigenic and allogenic stimulation of normal lymphocytes. *Int J Immunopharmacol.* 1984;6:593–599.
42. Schlesinger PH, Rodman JS, Doebber TW, et al. The role of extra-hepatic tissues in the receptor-mediated plasma clearance of glycoproteins terminated by mannose or N-acetyl-glucosamine. *Biochem J.* 1980;192:597–606.
43. Shepherd VL, Campbell EJ, Senior RM, Stahl PD. Characterization of the mannose/fucose receptor on human mononuclear phagocytes. *J Reticuloendothel Soc.* 1982;32:423–431.
44. Warr GA. A macrophage receptor for (mannose/glucose-amine)-glycoproteins of potential importance in phagocytic activity. *Biochim Biophys Res Commun.* 1980;93:737–745.
45. Minami S, Masuda M, Suzuki H, Okamato Y, Kato K, Shigemasa Y. Subcutaneous injected chitosan induces systemic activation in dogs. *Carbohydr Polym.* 1997;285:285–294.
46. Minami S, Suzuki H, Okamato Y, Fujinaga T, Shigemasa Y. Chitin and chitosan activate complement via alternative pathway. *Carbohydr Polym.* 1998;36:151–155.
47. Mori T, Okumura M, Matsura M, et al. Effects of chitin and its derivatives on the proliferation and cytokine production of fibroblasts in vitro. *Biomaterials.* 1997;18:947–951.
48. Tanigawa T, Yoshinori T, Tomita K, et al. Effect of chitin on the production of interleukin-1b in human blood monocytes. *Yonago Acta Med.* 1992;35:147–150.
49. Muzzarelli RA, Mattioli-Belmonte M, Pugnaloni A, Biagini G. Biochemistry, histology and clinical uses of chitins and chitosans in wound healing. In: Jolles P, Muzzarelli RA, eds. *Chitin and Chitinases*. Basel, Switzerland: Birkhauser Verlag; 1999:251–264.
50. Joint Technical Reporting Group for Munitions Effectiveness. *Evaluation of Wound Data and Munitions Effectiveness in Vietnam (WMDEV)*. Vol I of III. Final Report. Alexandria, VA: Defense Technical Information Center (AD879516); December 1970.