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Case Series

Hemostasis and Safety of a Novel Fibrin Dressing Versus Standard Gauze in Bleeding Cancellous Bone in a Caprine Spine Surgery Model

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Abstract

Background: Decorticated bone is a significant source of blood loss in scoliosis surgery. Current hemostatic methods include packed gauze (GS), physical barriers such as bone wax, and xenograft collagen-based materials. We assessed the safety and efficacy of a novel fibrin dressing (dextran-thrombin-fibrinogen [DTF]) compared to GS. This dressing comprises lyophilized thrombin and fibrinogen embedded in an elastic electrospun nanofiber dextran matrix.

Purpose: The study tests the hypothesis that DTF is more efficacious than GS in control of bleeding from cancellous bone.

Study Design: A preclinical Good Laboratory Practices (GLP) study.

Methods: We enrolled 10 goats that were followed for 28 ± 1 days. Each animal was randomly assigned to the test or control group. Both test and control animals had 4 cancellous bone injuries. Test animal injuries were treated with DTF, whereas standard GS was used to control bleeding in the control animals. Bleeding at the bone injury site was characterized as either none, oozing, flowing, or pulsatile and was assessed at 4 and 8 minutes after dressing application. Goats were survived 28 ± 1 days and then necropsied.

Results: Application of the fibrin dressing to bleeding cancellous bone, both posterior spinal lamina, and iliac crest graft sites, resulted in control of bleeding within 4 minutes at all injury sites. Eighty percent of control injury sites continued to bleed after 8 minutes and required application of bone wax to control bleeding. There were no differences in prothrombin time, partial thromboplastin time, or fibrinogen levels between test and control animals at 1 or 28 days. We observed no adverse histologic reactions at 28 days.

Conclusion: The fibrin dressing is an efficacious and safe method of controlling blood loss from cancellous bone in a spine surgery model.

Keywords: Hemostasis; Fibrin dressing; Bleeding cancellous bone

Introduction

Spine deformity surgery generally involves decortication and exposure of large surfaces of cancellous bone that can lead to significant blood loss. Various strategies have been developed to minimize blood loss and associated morbidities including hypotensive anesthesia [1], injection of epinephrine into the soft tissues, and preoperative administration of tranexamic acid [2]. Blood loss from bone can be reduced by direct physical application of topical hemostatic agents (eg, Gelfoam and FloSeal) [3-5], bone wax, and gauze.

Currently available topical hemostatic agents are not ideal for use in bleeding cancellous bone, and package inserts specifically require their removal prior to wound closure. Porcine or bovine collagen/gelatin products, whether in sheets or in a slurry form with thrombin, interfere with bone healing. Allografts can elicit an inflammatory response. Although the material can temporarily control bleeding, it cannot be left in the fusion bed for continued hemostasis. Further, the large bleeding surface areas exposed in deformity surgery make use of agents such as FloSeal impractical because of cost. Bone wax, while effective, also can interfere with bone healing or successful arthrodesis. Bone wax can be removed from the surface, but

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not from cancellous spaces without further resection of bone.

Gauze sponges, packed into areas of bleeding cancellous bone temporarily tamponade the bleeding, but must be removed prior to wound closure. Sponge packing is the most common strategy used to control hemostasis during spine surgery, but it does not allow for continued hemostasis after surgery.

Fibrin sealants are another strategy to control blood loss and are available either in a frozen liquid form or as lyophilized proteins combined with a backing dressing generally derived from mammalian collagen. Liquid fibrin does not reduce blood loss from decorticated transverse processes during spine fusion surgery [6]. Collagen-backed fibrin sealants have not been tested on bleeding bone and may impede healing [7].

A novel fibrin dressing that stops arterial bleeding [8-10] also may be useful in control of hemorrhage from other tissues, including bone. This dressing comprises lyophilized thrombin and fibrinogen embedded in a fully absorbable matrix of electrospun dextran nanofibers. Prototype dressings used salmon proteins, whereas the current dressing uses human proteins. The solid physical state of the dressing provides initial hemostasis by tamponade [11]. Further contact with fluid (eg, blood) completely dissolves the electrospun dextran carrier and solubilizes the proteins, which then interact to form a fibrin seal.

Given the various strategies to control bleeding, we wanted to test the new fibrin dressing against the most commonly used hemostatic method. We tested the hypothesis that this dextran-thrombin-fibrinogen (DTF) dressing would be more efficacious than packed gauze sponges (GSs) at achieving hemostasis in bleeding cancellous bone in a posterior lumbar fusion and iliac crest graft model.

Materials and Methods

Ten dairy-bred goats (5 castrated male, 5 female) weighing 35.5 to 58.0 kg were used in this study. Ages ranged between 14 and 29 months. The animals were housed and cared for in a USDA registered facility in accordance with standard criteria (facility Registration Number 41-R-0084) [12]. The study was conducted in accordance with FDA Regulations on Good Laboratory Practices (GLP) for Nonclinical Laboratory Studies CFR Title 21 Part 58 [13]. The original protocol and all changes to the protocol were approved by the NAMSA Institutional Animal Care and Use Committee (IACUC). The number of experimental animals was kept to the minimum but adequate to ensure statistical significance [14].

The test article, DTF, was provided by the manufacturer (St. Teresa Medical, Inc., Eagan, MN). These were finished (packaged, sterilized) dressings with lyophilized human thrombin and fibrinogen embedded in a 7 × 7 cm sheet of electrospun USP-grade dextran. The control article, 10 × 10 cm sterile cotton gauze sponges (GS), was procured independently from the manufacturer (Merit Medical, Chester, VA).

A physical examination of each animal was performed by the facility veterinarian before proceeding with the study. Animals were anesthetized with atropine (0.005−0.02 mg/kg, intramuscular), buprenorphine (0.01 mg/kg, intramuscular), ketamine (2.2−5.5 mg/kg, intravenous), and diazepam (0.3−0.5 mg/kg, intravenous). On occasion, propofol (1−8 mg/kg, intravenous) was used to aid induction. Anesthesia was maintained using isoflurane (0.5% to 5.0%, inhalant). Each animal received penicillin G (30,000 U/kg, intramuscular) preoperatively. Blood pressure was monitored via an intra-arterial catheter and transducer.

Strict sterile technique, including gown, gloves, and drapes, was used for the surgical procedures. A posterior approach to the lumbar spine was performed, as well as separate skin and fascial incisions over both iliac crests, creating 3 separate skin wounds exposing 4 bone test sites per animal. Hemostasis of the muscle was obtained with electrocautery. The lamina of two lumbar vertebrae and the iliac crests were decorticated with a chisel. To avoid cross-contamination, the right L4 lamina and the left L5 lamina were decorticated with the spinous processes and interspinous ligament left intact. The cancellous bone bleeding was graded as either pulsatile, flowing, oozing, or none. The animals had been randomized to either control or test article which the surgeon learned at this stage of the procedure.

The randomized dressing (gauze only or test article plus gauze) was applied directly to the bleeding surface. A cotton gauze backing was used behind the DTF dressings because the test article dissolved after application. Light manual pressure to keep the dressing in place was maintained by the surgeon for 4 minutes. After 4 minutes, the gauze was removed and bone bleeding was again graded by the surgeon. If bleeding continued, a second identical dressing was applied with 4 minutes of compression, and then the bone was observed and bleeding was graded. If bleeding persisted after the second dressing application, the surgeon sealed the surface with bone wax.

All gauze dressings were collected, weighed, and compared to an equal number of unused gauzes to estimate blood loss. Results were analyzed using Student *t* test.

Performance of the test article was assessed by presence of controlled bleeding within 4 minutes, blood loss, and the need for dressing reapplication. Four data points were obtained from each animal: right L4 lamina, left L5 lamina, and the right and left iliac crests. Results were analyzed statistically using a Student *t* test (blood loss) and a simple chi-squared test (dressing performance).

Each animal was then recovered, survived, and maintained in the FDA-registered facility for 28 days, at the end of which time they were again anesthetized in a similar manner and then euthanized by barbiturate overdose.

Safety was assessed directly by histopathology and indirectly by measurement of coagulation factors as an indication of immunologic reaction to the proteins. Three sections were obtained from each injury site, decalcified, embedded in paraffin, and processed for histopathologic examination using hematoxylin and eosin dye. Each site was examined microscopically for soft tissue reaction as well as the response of the bone to the injury. The histopathologist was blinded with regard to which dressing each animal received until after the tissue had been examined.

Coagulation studies were obtained before the procedure and then again on the 1st and 28th postoperative days to determine the potential of cross-reacting antibodies to the thrombin proteins. These consisted of prothrombin time (PT), activated partial thromboplastin time (aPTT), and fibrinogen levels.

Results

All animals survived the 28-day period of the study. Animals were similar with regard to weight of controls (45.5 ± 5.5 kg) and test subjects (46.6 ± 8.1 kg), p = .80. All animals were healthy at the onset of the study and tolerated the surgical procedures well. All had a similar degree of mild hind limb lameness for the duration of the study.

Three wound complications developed in the five control animals (3/15 wounds) compared with none (0/15) in the test animals (p = .068, chi-squared test). One was a wound dehiscence over the left iliac crest, without exudate, that did not heal prior to the end of the study. Another animal had a wound dehiscence over the right iliac crest with purulent exudate, which also remained open through the study. The third animal developed a dehiscence and purulent exudate over the midline spinal incision, which did not heal before the end of the study period.

Complete hemostasis was obtained at all injury sites in all test animals after a single 4-minute dressing application. Bleeding prior to treatment was graded as oozing in 12 of the injury sites and flowing in 8 injury sites.

In contrast, complete hemostasis at 4 minutes was obtained in only one of the control animal injury sites (p < .001). Bleeding prior to initial treatment was graded as oozing in 12 of the injury sites and flowing in 8 injury sites. A second dressing application resulted in complete hemostasis in an additional 5 animals. Therefore, hemostasis was achieved within 8 minutes using gauze alone in 30% of control animal injury sites, significantly less than one dressing application of the test article (p < .001).

Average blood loss at the injury sites in the gauze sponges for all 20 injuries in test animals was 1.1 ± 0.77 g, compared with 2.4 ± 1.02 g in control animals (p < .0001). These data are summarized in the Table.

Histopathologic examination of the injury sites showed normal early response to bone injury and healing. The test article was associated with a slightly higher inflammatory response at the iliac crest sites, but not at the vertebral injury sites, consistent with the resorption of a foreign material.

Table
Results comparing hemostasis and estimated blood loss (EBL) in control versus test articles.

Group	Hemostasis at 4 minutes	Hemostasis at 8 minutes	EBL (g)
Control (GS)	1/20	6/20	1.1 ± 0.77
Test (DTF)	20/20	20/20	2.4 ± 1.05
p value	<.001	<.001	.00031

DTF, dextran-thrombin-fibrinogen; GS, gauze sponge.

We found no differences between control and test group animals with respect to PT, aPTT, or fibrinogen levels either preoperatively or at either of the postoperative test points.

Discussion

Our results prove the hypothesis that the DTF dressing is more efficacious than standard gauze sponges at providing hemostasis on decorticated bleeding cancellous bone in a caprine spine surgery model. A single application of the DTF dressing stopped all blood flow within 4 minutes 100% of the time, whereas a second application of GS was required in 19 of 20 control animal injuries. Even after the second dressing application, hemostasis was controlled in only 30% of control animal injuries.

The blood lost to the gauze sponges was significantly less in the DTF-treated injuries compared with the GS-treated injuries. Granted, the injury sites were small, and young goat cancellous bone does not bleed profusely, but the finding was very clear both to observation as well as to statistical analysis. The fact that three wounds dehisced over GS-treated injuries but none over the DTF-treated injuries suggests that the dressing could reduce wound complications, possibly by decreased postoperative bleeding.

Furthermore, the DTF dressing appears to be safe because we found no evidence of immunologic response to the human proteins manifested by changes in the coagulation studies, and the dressing did not interfere with or retard normal bone response to injury. The safety of this dressing has been demonstrated in other studies using salmon proteins [15,16] and a 56-day GLP study of resorption in rats using human proteins [17].

Essentially, the DTF dressing works initially by providing tamponade to retard the blood loss [11]. As the dextran solubilizes and disappears, the proteins solubilize and interact with each other to seal the injury. The residual material consists of a thin layer of clot that seals the tissue to prevent further bleeding.

If these findings are extrapolated clinically to surgeries that involve large surfaces of bleeding cancellous bone, such as spine reconstruction or pelvic osteotomy, the implications carry great importance. The DTF dressing

conceivably could reduce time spent controlling bleeding and could reduce total blood loss. This could lead to shorter operative times, fewer complications, fewer transfusions, better recoveries, and shorter hospitalizations.

This dressing already has been shown to reduce blood loss and increase survival in arterial bleeding models. The dressing resulted in a 90% survival of anemic and coagulopathic swine with a lethal femoral artery injury in a US Army combat shock/trauma model [9,10,18]. The sealant properties of the DTF dressing in this model resulted in restoration of blood flow to the lower limb in 78% of animals despite a 6-mm arteriotomy in the common femoral artery. The dressing also resulted in 100% survival of swine with 4.4-mm-diameter aortic injuries [16].

It is not surprising that the DTF dressing outperformed GS dressings in this model, since gauze sponges are poor hemostatic agents. However, a dearth of good alternatives is available to the spine surgeon. Most topical agents consist primarily of mammalian collagen and should be removed prior to wound closure. Fibrin sealants are available either in a frozen liquid form, which is not effective against cancellous bone bleeding, or with an equine collagen backing that has not been tested in bleeding bone. We chose gauze as the control because it is the most common topical method used to control bleeding during spinal deformity surgery [19].

The dressing has been used successfully in other preclinical studies including swine hepatic injury, vertebral corpectomy, and canine nail and pad injuries [unpublished data]. Several studies have demonstrated its safety, and the safety of a prototype that used salmon proteins, with regard to immunologic response as well as histopathologic findings [9,10,15-17]. Although the costs of fibrin products are higher than gauze or bone wax, they can be produced at a similar cost to other currently available hemostatic agents and at the same time have greater benefits. We feel that this dressing could potentially reduce the blood loss and complications associated with spine reconstruction surgery.

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