Development of Hemostatic Dressings for Use in Military Operations*

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1.0   INTRODUCTION

On the battlefield, hemorrhage from wounds remains the leading cause of mortality, accounting for 50% of all deaths [1]. Hemorrhage is also the second leading cause of mortality among injured civilians, accounting for 39% of civilian trauma deaths [2-4]. The primary field-ready methods for control of hemorrhage—tourniquets, direct pressure, bandages, and clamping—have not changed greatly in several centuries [5]. These interventions, even in the hands of experts, are not always effective [6]. In Vietnam, 50% of combat deaths resulted from wounds with uncontrollable bleeding. Of these wounds, about 11% were inflicted in sites accessible for first aid treatment without need for surgical intervention [1,7]. More effective hemostatic methods could have been prevented up to one third of the deaths from exsanguination during the Vietnam War [1, 8]. This background information strongly illustrates the need to develop a better hemostatic method to improve the immediate care and survival of casualties in the field.

For the past nine years, the United States Army has worked closely with the American Red Cross (ARC) to develop a field-ready hemostatic dressing that can effectively stop arterial bleeding from major wounds. The ARC has an active program to develop fibrin sealant hemostatic agents, the focus of one of our research projects. The organization also controls 50% of collected human plasma, the current source of fibrinogen and thrombin proteins, which are the main components of fibrin sealant dressing. This article briefly reviews the history and development of fibrin sealant dressing as well as other hemostatic products (e.g., chitosan dressing) and the important role that the US Army Institute of Surgical Research (ISR) has played in developing these products.

2.0   HISTORICAL BACKGROUND

The first experiments to control bleeding with fibrin date back to 1909, when Bergel reported the hemostatic properties of fibrin powder in the operative field [9]. The first attempt to make a form of dry fibrin hemostatic product for use by trauma surgeons was during World War I, when Grey [10] and Harvey [11] produced pre-polymerized fibrin tampons and thin plaques to control bleeding in parenchymal organs. Although these

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

materials are passive hemostatic agents, incapable of polymerizing and cross-linking directly with the tissue, they worked relatively well. The combination of fibrinogen and thrombin was first used in 1944 by Cronkite et al. [12] and Tedrick et al. [13] for anchoring skin grafts, but because of poor adhesion, it was not widely accepted. During World War II, fibrin glue, fibrin sheet foam, and fibrin powder were mass-produced from pooled plasma, but were withdrawn in 1946 because they transmitted hepatitis. Subsequently, in 1977, all pooled human plasma fibrinogen products, including a commercial liquid FS preparation that was licensed in Europe, were recalled by the FDA because of the high risk of hepatitis transmission. Today, advances in viral removal (nanofilteration) and inactivation technologies (solvent-detergent, pasteurization, and dry heat treatment), combined with sophisticated donor screening, have reduced the risks of viral transmission from plasma products to extremely low levels. This decreased risk, coupled with strong clinical interest, has led to a resurgence in the development of FS products in the United States.

3.0   FIBRIN SEALANT (FS)

FS is composed of purified, virally inactivated human fibrinogen and human thrombin that combine to form a fibrin clot. FS may be used for control of bleeding, tissue gluing, and as a delivery vehicle for drugs and biologics. The hemostatic and adhesive properties of FS are important for certain types of surgical procedures and appear to be useful in treating severe trauma injuries [14].

The hemostatic function of FS mimics the final stages of the blood coagulation cascade. Once the protein components are dissolved in a fluid (e.g., saline or blood), the enzymatic activity of thrombin converts fibrinogen to fibrin monomers by cleaving small peptides (fibrinopeptides A and B) from the molecules. The fibrin monomers rapidly assemble into fibrils and fiber strands, thereby forming a three-dimensional gel network. Thrombin also converts inactive Factor XIII (FXIII), present with fibrinogen, into its active form (FXIIIa) in the presence of calcium chloride. The FXIIIa transforms the soluble fibrin gel into a dense, insoluble fibrin clot at the bleeding site [15,16]. The fibrin clot binds to the tissue by different modes (covalent, non-covalent and mechanical bonding) and physically stops the bleeding. In contrast to passive hemostatic agents (collagen, cellulose, etc.) that promote the patient's own blood clotting mechanism, FS coagulates independently from patient blood and is therefore useful even in patients with severe coagulopathy. The product is ideally suited for treating traumatic and surgical bleeding in hemophiliac patients [17].

3.1   Liquid Fibrin Sealant

Commercial liquid preparations of fibrin sealant are highly effective when they are used in a conventional setting (e.g., a pre-scheduled operation); however, they do suffer from several limitations if they are considered for trauma application. For example, liquid FS is difficult and time-consuming to prepare because it involves hydrating two lyophilized products, which requires warming and prolonged agitation. The liquid mixture can successfully control a majority of surgical oozing that is of low volume and pressure, but it cannot treat high volume venous or high-pressure arterial hemorrhages, as it is diluted and washed away by the high volume of blood flowing out of lacerated large vessels. In order to address these limitations and broaden the spectrum of the injuries that can be treated with FS, a new physical form of fibrin sealant in the shape of a dry dressing was developed.

3.2   Dry Fibrin Sealant Dressing (DFSD)

DFSD technology was developed for both field medical (pre-hospital) and conventional surgical applications. The dressings are simple to use and designed for self-application, buddy-application or administration by an

emergency medical technician. The victim or caregiver only needs to open the packaging, remove the product, and press the dressing onto the bleeding wound for approximately two to three minutes to treat superficial or extremity injuries that may result in significant blood loss. DFSD may also be used in a hospital setting to control severe parenchymal hemorrhages that either cannot be controlled by conventional methods or require a meticulous operating procedure with the risk of prolonged ischemic time and organ failure.

3.2.1   Early Development of DFSD

The development of DFSD involved several formulation and structural design changes before the final product was ready for manufacturing and distribution. The first prototype of DFSD applicable to trauma surgery was developed at the Letterman Army Institute of Research in the early 1990s [18]. The aim was to deliver a large amount of fibrinogen and thrombin to a wound, producing a fibrin clot with greater density and strength than forms naturally, binding tightly to underlying tissues. The first *in vivo* test of the prototype dressing, which was composed of powdered fibrinogen and thrombin spread on gauze, was performed at the Letterman Army Institute of Research in 1993. The results, published in 1995, convincingly demonstrated that application of DFSD on an arterial laceration (pig femoral artery) could minimize the bleeding and maintain arterial blood pressure at a normal level [14].

The original ARC dressing (1st generation) produced with advice from the Letterman team in 1995 had a similar composition; i.e., a mixture of dry powdered fibrinogen and thrombin was pressed on a silicon backing material. The backing was intended for handling and application of DFSD onto the wound until hemostasis was achieved, after which it would be removed, leaving behind only the reabsorbable fibrin clot. This prototype was tested *in vivo* in two severe hemorrhage models including a defined femoral arteriotomy [19] and a complex ballistic injury [20] in the extremity of large animals. The dressings produced hemostasis after application and reduced the overall bleeding by 83% (123 ± 48 ml) and 77% (124 ± 64 ml) in both conditions, as compared to the control treatments (standard gauze or placebo dressing) with blood loss of 734 ± 134 ml and 377 ± 64 ml, respectively. The mean arterial pressure was at least 25 mmHg higher in the dressings treated animals than those in the controls. Hemostasis was achieved in the arteriotomy model without compromising the arterial blood flow to the hind leg of the animals [19].

The first generation of DFSD was later abandoned because of the instability of the powdered materials, which frequently fell off, and the non-absorbable nature of the cotton and silicon backings. An alternative dressing with absorbable backing was developed (2nd generation) that had additional patient benefits. It could be placed in the wound safely and be reabsorbed entirely by the body without the need for removing the backing sheet and disturbing the hemostatic clot. The other necessary change in these dressings was a new method for incorporating fibrinogen and thrombin into the dressing. In the new product, fibrinogen and thrombin were layered (one layer of fibrinogen over one layer of thrombin) on top of an absorbable backing material (Vicryl TM or Dexon TM mesh) and freeze-dried into one sheet. The underlying protein layer was attached to the supporting material by the addition of a thin layer of sucrose that embedded the absorbable mesh. However, the *ex vivo* laboratory testing, which measured the adhesiveness of the dressing to vascular tissue, revealed very poor adhesive strength of these dressings regardless of which component was the top layer [21]. The reason appeared to be that as soon as saline was added to dissolve the dressing, a thin layer of fibrin clot was formed at the interface of the two proteins and prevented the proper mixing of fibrinogen and thrombin. The result was the formation of a non-homogeneous clot with low adhesive properties and poor hemostatic efficacy. The structural design of the dressings therefore had to be modified.

In the new design (3rd generation), the thrombin layer was placed between two layers of fibrinogen in a "sandwich" configuration. The new design allowed better mixing of active components and complete

polymerization of the fibrinogen into a uniform fibrin clot that showed superior adhesive properties [21]. The concentration of fibrinogen, which is the critical factor in determining the adhesive strength of the dressing, was optimized (15 mg/cm^2) based on the results of a study using large animals that was conducted at William Beaumont Army Medical Center, in El Paso, TX [22].

The final laboratory-produced DFSD (Fig. 1) was tested extensively in a variety of innovative hemorrhage models developed by ISR scientists to determine the efficacy and potential benefit in military operations. For example, a model of severe liver injury was developed in large animals (pigs) using a custom-designed clamp with two 4.5 cm sharpened blades that lacerated major hepatic veins and liver tissue in a manner similar to a gunshot injury [23].

DFSD   CD

Figure 1.

Figure 1. Photographs of the hemostatic dressings that were successfully tested and are currently distributed in far-forward military operation overseas. DFSD: dry fibrin sealant dressing, composed of human fibrinogen and thrombin with an absorbable backing material, is made by the American Red Cross. CD: chitosan dressing derived, from a natural polysaccharide known as chitin found abundantly in shellfish, is manufactured by HemCon, Inc.

Hemorrhage in this model is so severe that it often results in exsanguination of the animal if not treated (Fig. 2). The same injury was also produced in animals in which their natural blood clotting capacity was severely diminished by blood dilution and hypothermia (coagulopathy syndrome), similar to conditions that develop in trauma patients or in battlefield casualties. In these circumstances, the bleeding is more persistent, harder to control and more likely to be fatal if not treated promptly. DFSD controlled these life-threatening hemorrhages in both models and offered a simple and effective method of hemostasis that was superior to standard care (gauze packing) practiced in hospitals [24]. The long-term effects of the dressings were also evaluated in specialized urological operations performed in large animals. For example, application of DFSD dressing on the prostatic bed following prostatectomy (removal of the prostate gland) reduced blood loss by 25 to 30% and shortened the operation time to half that required for other groups [25]. Similarly, when DFSD

was used to treat bleeding from the kidney after a partial nephrectomy operation [26] or a stab wound injury [27], the hemorrhage was stopped more rapidly (less blood loss) and the surgery was completed in a shorter time resulting in less ischemic injury compared with standard surgical techniques. The secondary bleeding and leakage of urine from the injured portion of the kidney were also prevented during the six-week post-operative recovery period, during which time the organ healed properly. These long-term studies were conducted at Brook Army Medical Center in San Antonio, TX.
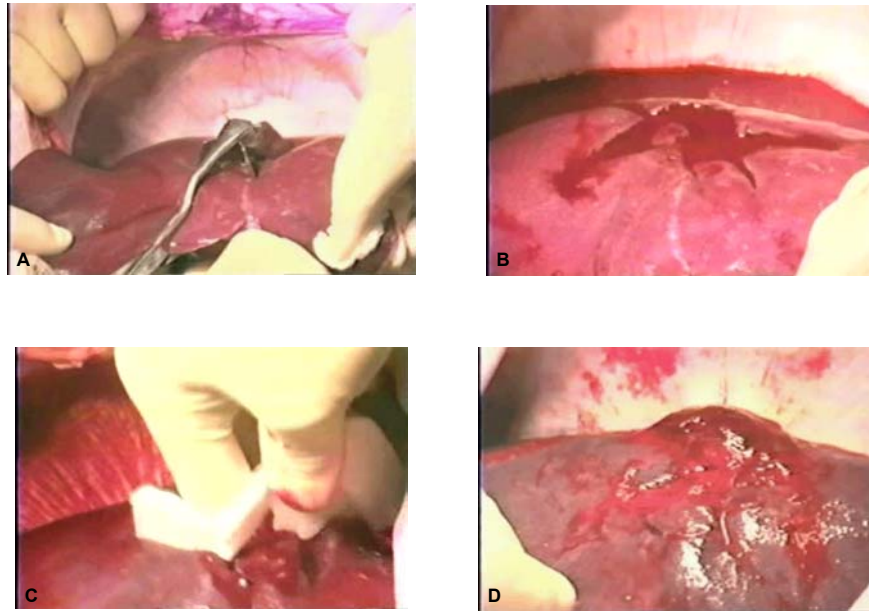

Figure 2.

Figure 2. Photographs of the severe liver injury and profuse venous bleeding model in swine. A. The liver tissue and underlying major veins of the two medial lobes are lacerated twice using a custom-designed clamp with sharp blades (X shape). B. The result appears as a large stellate wound (approx. 10 X 8 X 4 cm) similar to a gunshot injury. C. The wound is packed with three dressings and held for four minutes (or shorter if hemostasis is achieved), after which the animal is closed, resuscitated and monitored for one hour. D. Treatment with DFSD produced hemostasis during the four-minute compression period and bleeding is completely stopped.

4.0   SELECTION OF BEST HEMOSTATIC DRESSING FOR MILITARY USE

In addition to DFSD, ISR investigated a number of other hemostatic dressing products with potential benefit to soldier care. Some of these agents were already approved (e.g., Surgicel, Avitene) and some were under development to be licensed by the FDA and used in a variety of clinical settings. The U.S. Army wants to select the most effective hemostatic dressing that meet the far-forward needs of military use. The ideal hemostatic dressing, as defined by the US Special Operation Command, should meet the following conditions:

1. Able to stop large-vessel arterial and venous bleedings within 2 minutes after application on the wound.
2. Ready to use; no mixing or special preparation.
3. Simple to apply by the wounded personnel, his buddy or medics without any training.

4. Stable at room temperature for at least two years and in extreme temperatures (between 40 and -10° C) for several weeks or longer.
5. Safe to use and pose no risk of bacteria or viral transmission.

Companies and organizations were solicited to submit their products for vigorous testing to see which would meet the above criteria. A total of nine hemostatic dressings, including two fibrin sealant dressings (one made by the ARC and the other by Nycomed in Austria), were submitted for evaluation. Except for the fibrin sealant dressings, the remaining dressings are considered passive hemostats, promoting the clotting mechanisms of the patient's own blood as a means to stop the bleeding. In general, passive hemostats act by enhancing platelet aggregation, accelerating intrinsic and extrinsic pathways of clot formation and protecting the blood clot from degradation. In some cases, they were claimed to cause a local vasoconstriction (e.g., the Marine algae polymer dressing) and thereby reduce bleeding from the vessels. The reduced efficacy of passive hemostats in trauma patients with decreased blood clotting capacity (coagulopathic patient) has been acknowledged.

The dressing candidates were subjected to two severe hemorrhage tests in large animals. In the first study [28], the dressings were applied to Grade V liver injury in swine, which represents high volume venous bleeding. The efficacy of each dressing was compared with a control treatment in which cotton gauze was used to stop the hemorrhage. The ARC fibrin sealant was the only product that significantly increased hemostasis and reduced bleeding when compared with control treatments. In the second study [29], the dressings were tested in an even more challenging model that involved severe high-pressure arterial bleeding that produced 100% fatality within 10 minutes after injury. The hemorrhage was produced by making a 4.4 mm diameter hole in the abdominal aorta of the pig, which was allowed to bleed freely for 6 seconds. While bleeding continued, dressings were placed through a pool of blood over the injury site and pressure held for 4 minutes. Hemostasis was determined following removal of manual compression. For controls, either the Army standard gauze dressing was used (negative control) or the vessel was clamped and properly sutured (positive control). The only dressing that effectively sealed the vascular injury and prevented further blood loss and the death of the animals was the fibrin sealant dressing made by ARC (Fig. 3). The efficacy of this dressing during a 1-hour observation period (short-term) equalled that of the standard suturing technique. Animals treated with other hemostatic dressings exsanguinated during the observation period.

5.0 MANUFACTURING OF DFSD

Although none of the nine dressings tested met all of the desired criteria for military use, the ARC DFSD dressing met the more important requirements including those for high efficacy, ready-to-use, stability at room temperature, and biological safety of the product. The promising outcomes of the animal studies, along with a well-planned proposal submitted by the ARC and a manufacturing facility (CSL Bioplasma, Victoria, Australia) prompted substantial financial support by the US Army Medical Research and Material Command to advance the production of this dressing from the laboratory bench to a large-scaled manufacturing facility in 2001.

One of the shortcomings of the ARC DFSD, noticed during testing, was the fragile nature of the fibrinogen sheets, which break down easily and slough off when the dressings were handled. One reason for this problem was the incorporation of thrombin as a thin layer between the two fibrinogen sheets in a sandwich configuration. Because of the differences in protein and buffer composition between fibrinogen and thrombin, the crystallized thrombin layer acted as a weak interface between the dry fibrinogen sheets and reduced the firm attachment of the fibrinogen layers. As a result, the upper layer of fibrinogen was frequently delaminated

and easily flicked off during handling or rough transportation. To minimize this problem, two modifications were made at the manufacturing level. First, the dressings were made, stored, and transported in rigid plastic containers so that they would be protected from some inevitable hits and damage during transportation. Second, the design for incorporating thrombin into the dressing was changed. This modification required further experimentation to prove the equivalency of the scaled-up production dressing with the laboratory-made dressings that were tested earlier.

Figure 3.

Figure 3. Photograph of the aortic injury and fatal arterial hemorrhage model in swine. A 4.4 mm hole in the abdominal aorta is sealed and hemorrhage is prevented with the use of a DFSD. The dressing completely adheres to the blood vessel and surrounding tissue (From 29; Reprinted with permission from Lipincott Williams & Wilkins).

In the newly designed dressing, thrombin was placed as minute aliquots (~100 dots,1 cm apart), spread evenly over the first layer of fibrinogen and covered with the second layer of the fibrinogen. This " polka dot" arrangement allowed better attachment of fibrinogen layers, particularly on the area void of thrombin, and easier way toward total automation of dressing production and robotic application of the components. Initial *in vitro* tests performed at the manufacturing facility did not show any significant difference between the new dressing design and the prototype in which thrombin was sprayed as a continuous layer. This result, however, had to be confirmed in a challenging *in vivo* study to ensure equal efficacy of the dressings.

The equivalency study was performed at the ISR and the dressings (prototype vs. first polka dot design) were tested in the liver hemorrhage model (described earlier) in normal swine [30]. Hemostasis achieved with each dressing was compared with that of a control group in which the liver injury was treated with standard gauze. Although the polka dot design reduced the hemorrhaging, it appeared to be less effective in reducing blood loss (31% larger blood loss than the prototype design), and achievement of hemostasis was not substantially better than with the gauze. This result clearly indicated that, despite the favorable *in vitro* test results by the

manufacturer, the *in vivo* efficacy of the newly designed dressings was not equal to the prototype. The lower efficacy of the polka dot design appeared to be due to inadequate mixing of thrombin and fibrinogen and therefore incomplete polymerization of fibrinogen across the dressing once it was dissolved in blood.

A new pattern with better distribution of thrombin throughout the dressing seemed to be a logical approach to improve the mixing and consequently the hemostatic function of the product. This was achieved by dividing the same amount of thrombin into smaller aliquots and evenly distributing them between the fibrinogen layers. This design retained the advantage of preventing the delamination of fibrinogen layers but allowed more direct contact of the thrombin with fibrinogen and better mixing upon dissolution. The new polka dot designed dressings, along with the prototypes, were subjected to the same *in vivo* testing by the ISR staff, using the severe liver hemorrhage model in swine [30]. The results showed significant improvement in hemostatic function of the new product. The efficacy of scaled-up production was equal to the prototype dressings produced in the laboratory setting. Last year, this dressing received preliminary approval by FDA in the military arena for treating external injuries and is currently distributed among Special Operations Forces under an Investigational New Drug (IND) protocol.

6.0   CHITOSAN HEMOSTATIC DRESSING

Recently, a new hemostatic dressing has been developed by the Oregon Medical Laser Center with potential utility to control severe extremity hemorrhages (figure 1). The dressings are made of chitosan, a derivative of a natural polysaccharide known as chitin found abundantly in shellfish (e.g., shrimps). Various forms of chitin and chitosan have been shown to enhance hemostasis in experimental studies. The chitosan dressings offer several advantages over DFSD with regard to the durability and ease of application, particularly in the far-forward military arena. Application of these dressings over wounds does not require any special precautions or use of non-adhesive gloves, which may be necessary for DFSD. They also seem to maintain their adhesive function even if they become wet before being placed on the wounds. Because of their chemical structure (very similar to cellulose), these dressings are more stable and tolerant to prolonged exposure to extreme temperatures   (-50° to +140° F). The chitosan dressing was not available when the hemostatic efficacies of the other nine dry dressings were compared; however, the encouraging preliminary data provided by the manufacturer prompted evaluation of a chitosan dressing (prototype) in the standard liver hemorrhage model in swine [31]. The results of this study at the ISR showed that chitosan dressing treatment could significantly reduce blood loss, mortality rate, and enhance hemostasis, when compared with a control treatment (cotton gauze).

More recently, the manufacturing company (HemCon, Inc) received FDA approval for the use of the chitosan dressing to treat external bleeding. This led to large-scale manufacturing and production of hundreds of dressings ready for shipment to the battlefield. The limited *in vitro* and *in vivo* tests carried out by the company showed no significant differences between the scaled-up production and the prototype versions previously tested successfully at ISR. However, the experience with the DFSD development obligated ISR and the Army to thoroughly test the product before recommending its shipment and distribution among the troops.

Samples of production dressings were subjected to a standardized model of liver hemorrhage in swine. Data were compared with results obtained using the prototype chitosan dressing under identical study conditions. The production version of the dressing did not improve initial hemostasis, nor change the overall bleeding or survival rate, compared with gauze used as control dressings. These results clearly indicated that the efficacy of the mass-produced dressings was inferior to the prototype version and not suitable for release in the military arena.

This conclusion led to rapid improvements in the manufacturing process of the chitosan dressings by HemCon, Inc and production of a new version that was more flexible and absorbant with better adhesive properties than the previous product. The new dressings were expeditiously (in one day) tested in the standard hemorrhage model with participation of nearly the entire ISR staff. Once the hemostatic efficacy of these dressings was confirmed in the large animal study, shipment of the final product was recommended for possible treatment of combat casualties in Iraq and Afghanistan.

7.0 SUMMARY

The results of nearly a decade of laboratory and animal research are the development and production of two highly effective hemostatic dressings with potentially life-saving properties. These dressings are presently distributed among the soldiers in far-forward military operations overseas. Each product has its own unique advantages and may be more suitable for use in special circumstances. The chitosan dressings are more stable, durable, easy to use and less expensive. They are more likely to be utilized in the first aid stage for temporary control of bleeding. On the other hand, DFSD's are more flexible (after contact with blood) and better able to conform and adhere to a complex injury, with proven efficacy against the most aggressive and life-threatening hemorrhages. The required careful application process of the DFSD is a potential limiting factor for widespread use of this product.

This report summarizes the important role that the United State Army has played in the development of hemostatic products relevant to military needs and treatment of combat casualties. The invention and employment of challenging hemorrhage models in large animals has been essential in identifying the most promising hemostatic dressings and guiding these products through manufacturing and optimization processes to the extent that they are now available for use in our Armed Forces.

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