A totally recombinant human fibrin sealant

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\textbf{Abstract}

\textbf{Background:} Applications of plasma-derived human fibrin sealants (pdhFS) have been limited because of cost, limited supply of pathogen-screened plasma, the need for bioengineering improvements, and regulatory issues associated with federal approval. We describe a totally recombinant human fibrin sealant (rhFS), which may engender an abundant, safe, and cost-effective supply of efficacious fibrin sealant.

\textbf{Materials and methods:} A first-generation rhFS made from recombinant human fibrinogen (rhFI; produced in the milk of transgenic cows), activated recombinant human factor XIII (rhFXIIIa; produced in yeast), and recombinant human thrombin (rhFIIa; purchased, made in animal cell culture) was formulated using thromboelastography (TEG). The hemostatic efficacy of rhFS versus commercial pdhFS was compared in a nonlethal porcine hepatic wedge excision model.

\textbf{Results:} The maximal clot strength of rhFS measured in vitro by TEG was not statistically different than that of pdhFS. TEG analysis also showed that the rhFS gained strength more quickly as reflected by a steeper angle; however, the rhFS achieved this clot strength with a 5-fold lower factor I content than the pdhFS. When these fibrin sealants were studied in a porcine hepatic wedge excision model, the hemostatic scores of the rhFS were equivalent or better than that of the pdhFS.

\textbf{Conclusions:} The bioengineered rhFS had equivalent or better hemostatic efficacy than the pdhFS in a nonlethal hemorrhage model, despite the factor I concentration in the rhFS being about one-fifth that in the pdhFS. Because the rhFS is amenable to large-scale production, the rhFS has the potential to be more economical and abundant than the pdhFS, while having a decreased risk of blood-borne pathogen transmission.

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1. Introduction

The use of dried plasma as a topical hemostatic aid was documented in 1909 \cite{1}. The combination of relatively pure fibrinogen (factor I or FI) with thrombin to make fibrin glue or foam was described in 1944 \cite{2}, but it was not until improved purification technology became available that fibrin sealants (FS) became commercially available in the 1970s \cite{1}. Since that time, the efficacy of FS products as a topical hemostat or tissue adhesive has been demonstrated in numerous elective clinical...
scenarios, including peripheral vascular procedures [3], total knee arthroplasty [4], reoperative cardiac procedures [5], pulmonary resection [6], bleeding duodenal ulcer [7], and partial nephrectomy [8], FS alone was not useful during hepatectomy [9], but FS combined with a collagen matrix applied during liver resection reduced blood loss and/or postoperative drainage compared with standard operative care [10]. Examples of currently available FS formulations that use plasma-derived fibrinogen include Evicel (Ethicon, Inc; Somerville, NJ) and Tisseel (Baxter Healthcare; Deerfield, IL).

The United States Department of Defense has maintained an interest in the development of hemostatic devices using FS for control of traumatic hemorrhage [11]. There has been particular interest in hemostatic FS devices for use under coagulopathic conditions [12]. Topical hemostatic treatments that incorporate FS have been successfully used in porcine trauma models, including femoral vessel injury [13], aortic injury [14], and hepatic injury [15]. One notable FS-containing device for traumatic hemorrhage was the Dry Fibrin Sealant Dressing, produced by the American Red Cross [16]. The Dry Fibrin Sealant Dressing was efficacious in porcine models of lethal hemorrhage [12,17] and was anecdotally successful in military trauma but was discontinued due to fragility and cost issues [18]. The availability of a relatively abundant FS might increase innovation into FS-based hemostatic devices for the treatment of severe hemorrhage. The essential components of FS are: FI, the biomonomer from which fibrin polymer is made [19]; activated thrombin (factor IIa or FIIa), which catalyzes the formation of soluble fibrin from FI and also activates factor XIIIa [20]; and activated factor XIII (FXIIIa), which cross-links the fibrin polymer to itself (rendering it insoluble) and to the wound surface [21]. One abundant source for these clotting factors can be large-scale recombinant protein production. The complexity of FI and FIIa necessitates that recombinant versions of these proteins be made in animal cells [22]. We recently reported the production of recombinant human FI (rhFI) made at high concentrations in the milk of dairy cows [23]. Recombinant human FIIa (rhFIIa) already is commercially available (Recothrom; ZymoGenetics, Inc, Seattle, WA) and has been approved for topical hemostatic therapy in the United States and in Europe [24]. In contrast to FI and FIIa, FXIIIA2 is less complex; its core catalytic unit (FXIIIA2a), which is kinetically faster than the more complex tetrameric plasma-borne FXIII [21], has been produced at large scale in yeast. FXIII nomenclature and specific activity are summarized in Table 1. Recombinant FXIIIA2 (rFXIIIA2) currently is in clinical studies of FXIII replacement therapy [25]. In the present study, we used a porcine hepatic wedge resection model to compare the hemostatic efficacy of a fully recombinant human FS (rhFS), containing rhFI, rhFIIa, and recombinant human FXIIa (rFXIIa), against a commercially available, plasma-derived human FS (pdhFS) used for each group in the two-group comparison of rhFS versus pdhFS (with hemostatic score as the outcome measurement) was determined with a statistical power analysis [26], using Δ/σ (Cohen δ, in which Δ is the desired difference in means set by the observer and σ is the estimated standard deviation) = 2.0, false-positive rate (α) = 0.05, false-negative rate (β) = 0.2, and power (π, or 1 – β) = 0.8.

2.2. Clotting factor sources

rhFI was produced in the milk of transgenic cows by inserting the primary sequence of the human transgenes for the α-, β-, and γ-chains of fibrinogen into the cow genome by nuclear transfer [23]. Southern blot analysis confirmed the presence of the three transgenes. The rhFI expressed in the milk of the transgenic cows was characterized by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), Western blot, γ and γ'-chain content, fibrinopeptide phosphorylation, glycosylation, thrombin-catalyzed activation, thrombin-catalyzed protofibril formation, factor XIIIa-catalyzed molecular crosslinking, viscoelasticity, scanning electron microscopy, and tissue sealant function [23]. The main differences between rhFI made in transgenic cow milk and plasma-derived fibrinogen (pdFI) was the γ'-chain content [23].

The human FXIIIA1 gene was expressed in Pichia pastoris [23,27]. The expressed rhFXIIa was characterized by SDS-PAGE, Western blot, Pefakit FXIII incorporation assay, FXIIIA1-catalyzed molecular cross-linking of fibrin, and viscoelasticity [27]. Human rhFIIa (Recothrom) was purchased from ZymoGenetics, Inc. Human pdFI depleted of plasminogen, von Willebrand factor, and fibrinectin was purchased from Enzyme Research Laboratories (South Bend, IN). Commercial human pdhFS (Tisseel, unless otherwise specified) was purchased from Baxter BioSurgery (Deerfield, IL).

2.3. Determination of clotting factor concentration and activity

The concentrations of the purified stocks of rhFI, pdFI, and rFXIIa were determined by OD280 and the bicinchoninic acid

Table 1 – Sources of factor XIII activity.

<table>
<thead>
<tr>
<th>Factor XIII species</th>
<th>Abbreviation</th>
<th>Activity (U/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma-derived tetrameric factor XIII</td>
<td>FXIII</td>
<td>40; 6–8</td>
</tr>
<tr>
<td>Plasma-derived, dimeric, catalytic subunit factor XIII</td>
<td>FXIIIa2</td>
<td>N/A</td>
</tr>
<tr>
<td>Recombinant dimeric catalytic subunit factor XIII</td>
<td>rFXIIIa2</td>
<td>140</td>
</tr>
<tr>
<td>Plasma-derived, activated, dimeric factor XIII</td>
<td>FXIIIa2a</td>
<td>N/A</td>
</tr>
<tr>
<td>Recombinant human, activated factor XIII</td>
<td>rhFXIIIa</td>
<td>7000</td>
</tr>
</tbody>
</table>

N/A = not applicable.

Activity based on normal plasma pool, which by definition is 1 U/mL.

1. Reported activity of plasma FXIII [37].
2. Reported activity of FXIIIA2 made in Saccharomyces cerevisiae [25].
3. Reported activity of rFXIIa made in Pichia pastoris [23].
2.4. Thromboelastography

The effect of rhFI concentration on clot formation kinetics and strength (Fig. 1) was determined with thromboelastography (TEG), using a TEG 5000 Thromboelastograph (Haemonetics Corp, Braintree, MA). rhFI (0–12 mg/mL) was incubated at 37°C with rhFIIa (53 U/mL), rFXIIIa (2400 U/mL), and CaCl2 (12 mM). The thromboelastograph was calibrated each day of use; each point of each analysis was run in triplicate. TEG Analytical Software (version 4.2.2; Haemonetics Corporation, Braintree, MA) was used to calculate the time to clot initiation (R), time to clot firmness of 20 mm (K), alpha angle (α), maximal clot strength (maximal amplitude [MA], which was directly related to the shear elastic modulus strength, G), and percent lysis 60 min after MA (LY60) [29]. A single-factor analysis of variance was performed to compare the effect of plasmin on pdhFS and rhFS. The Student t-test (two-tailed, with unequal variances) was used to compare rhFS and pdhFS, with alpha set at 0.05.

2.5. Immunoblotting

Immunoblotting was used to estimate the mass concentrations of FXIII in the commercial pdhFS (Tisseel). Reduced samples were resolved with SDS-PAGE on 4%–12% NuPage Bis-Tris gels (Invitrogen, Carlsbad, CA) and then transferred onto polyvinylidene fluoride membranes (Millipore, Billerica, MA). Blots were probed with polyclonal antibodies for FXIII (F0019-46; United States Biological, Swampscott, MA) or FIIa (T5045-10B; United States Biological).

2.6. Swine hepatic injury models

Domestic swine (castrated males, aged 3 mo, weight 33–36 kg) were purchased from the Agricultural Research and Development Center (Mead, NE) of the University of Nebraska–Lincoln. Each subject was fasted for 12 h before surgery, but with free access to water. Each subject was premedicated with Telazol (4.4 mg/kg; Zoetis, Madison, NJ), ketamine (2.2 mg/kg), and xylazine (2.2 mg/kg) as a single intramuscular injection. An intravenous line was established in an auricular vein, oral endotracheal intubation was performed, and anesthesia was maintained with 0.5%–1.5% isoflurane using a Matrx VMS veterinary anesthesia machine (Midmark Corp, Versailles, OH). Mechanical ventilation was maintained at 12–15 breaths/min with a tidal volume of 10–15 mL/kg, to keep the end-tidal pCO2 at 30–35 mm Hg. A heating pad was under each subject to support body temperature. A carotid arterial catheter was placed for pressure monitoring and blood sampling, and a jugular venous catheter was placed for isotonic fluid and medication administration, all via a surgical cutdown in the left neck. Arterial pressure, end-tidal pCO2, rectal temperature, cardiac electrical activity, and pulse oximetry were continuously recorded with a Bionet BMS Veterinary Monitor (Bionet America, Inc, Tustin, CA) interfaced to a laptop computer. Each swine subject was maintained under an appropriate level of isoflurane anesthesia (indicated by the absence of corneal reflex) for the duration of the experiment; before euthanasia, the isoflurane was increased (see the following sections).

The porcine normothermic nondilutional stellate liver laceration model was adapted from a previous description [17]. After the above initial setup, a ventral midline incision was made, splenectomy was performed, and a transabdominal cystostomy tube was placed. Splenectomy generally has been performed in severe porcine hemorrhage models to eliminate the confounding effects of splenic autotransfusion [30]. A liver laceration was created with a custom-built liver injury clamp (Fig. 2A, inset), which consisted of tines in an X-configuration (5 cm diameter) on one arm of the clamp, and a base plate on the other arm onto which the tines seat. The base plate was placed on the inferior surface of the liver against the quadrate lobe, between the cystic duct and the portal vein. The tines were positioned over the liver dome, 4–5 cm anterior to the vena cava at the base of the left medial hepatic segment. The clamp then was closed, forcing the tines through the liver dome and onto the base plate. The test sealant (10 mL) then was applied immediately into the laceration, and the edges of the laceration were held together with manual compression for 5 min. Only one clamp application per subject was performed. No other hemostatic maneuver (e.g., cotton gauze, laparotomy packs, Pringle maneuver) was used other than sealant with manual compression. This model was intended for preliminary qualitative observation of sealant efficacy only. Quantitative data (blood loss volume, mean arterial pressure, and so forth) was not recorded for the stellate laceration model.

For the wedge excision model, a ventral midline incision was made and a cystostomy tube was placed. The left lobe of
the liver was exteriorized, and sites for wedge-shaped (“pie-slice”) hepatic excisions were lightly scored with electrocautery on the liver capsule (Fig. 3A) along the anterior edge of both the left medial and left lateral segment (two separate but identical series of wedge excisions per subject). The base of each excision (i.e., distance along the lobar edge) was maintained constant at 1 cm. The excision depth (i.e., distance from lobar edge to apex of excision; Fig. 3A) of each series ranged from 0.5 to 3.0 cm, in crescendo fashion using 0.5 cm increments, for a total of six excisions per series, all cut with scissors (Supplementary Video File). Two such series of excisions were performed per subject, for a total of 12 excisions per subject. Only one type of sealant (either the rhFS or pdhFS) was used in any given subject.

Each excision was treated immediately with up to 1 mL of FS without digital or gauze compression or other adjunct (Supplementary Video File). pdhFS (Tisseel) was administered with the double-barrel common-channel syringe system (Duploject, Baxter Healthcare; Fig. 3C) provided by the manufacturer, as per the user instructions. The rhFS (as defined in Table 2) was administered using two 1 mL syringes taped together, with 30 gauge needles bent into convergence (Fig. 3D). One syringe contained the rhFI and rFXIIIa, and the other syringe contained the rhFIa and calcium.

Hemostasis 30 s after treatment of each excision was scored as follows: 0 = failure/minimal hemostasis; 1 = decreased but steady bleeding; 2 = oozing; and 3 = hemostasis. Quantification of the small amount of blood loss, which occurred during the treatment phase, did not produce reliable data, so the above visual analog score of hemostasis was used. If an excision was not hemostatic 30 s after application of 1 mL of the test sealant, then the excision was packed with cotton gauze before performing the next excision, to minimize the ongoing blood loss. Excisions were performed during a single nonsurvival anesthetic, which lasted for 30–45 min. After completion of the two series of excisions, each subject was administered 5% isoflurane for 3 min, the supradiaphragmatic inferior vena cava then was transected, and each subject was allowed to expire from exsanguination while under deep isoflurane anesthesia.

2.7. Fibrinogen immunohistochemistry

Liver specimens were fixed in 10% neutral-buffered formalin, dehydrated, and then embedded in paraffin blocks. Paraffin sections (5 μm) underwent antigen retrieval with Target Retrieval Solution (Dako North America, Carpinteria, CA) as per the manufacturer’s instructions. The presence of both human and swine fibrinogen were detected by sequential dual-immunohistochemical staining followed by a hematoxylin counterstain. Signal detection was performed using the Dako EnVision G/2 Doublestain System (Dako North America, Inc, Carpinteria, CA), as per the manufacturer’s instructions. Briefly, endogenous peroxidase and alkaline phosphatase activity present in the tissue were blocked and then the swine fibrinogen antibody (Kamiya Biomedical Company, Seattle, WA) was applied and visualized using the horseradish peroxidase/3,3’-diaminobenzidine tetrahydrochloride reagents from the Dako system (Dako North America, Inc). A second blocking step was performed before incubation with the human fibrinogen antibody (Abcam, Inc, Cambridge, MA). The human fibrinogen antibody was visualized using alkaline phosphatase–based Permanent Red (Dako North America), as per the manufacturer’s instructions.

3. Results

3.1. Effect of FI on clot formation

The effect of rhFI concentration (0–12 mg/mL) on the kinetics and strength of clot formation with rhFS was analyzed by TEG (Fig. 3). The speed of clot formation, as measured by clot initiation time (R), time to clot firmness (K), and angle (α), increased as the rhFI concentration was increased to 6 mg/mL but remained constant >6 mg/mL of rhFI (R = 17 s, K = 50 s, α = 82°). The maximal clot strength (MA) continued to increase with each increase of rhFI concentration (up to 80 mm with rhFI = 12 mg/mL). For further in vivo studies, we targeted an rhFI concentration of 9 mg/mL in the rhFS, which generated...
MA >50 mm. At constant rhFI concentration, the calcium concentration (3–15 mM) did not affect the thromboelastographic parameters of the rhFS (data not shown).

3.2. Thromboelastography of pdhFS versus rhFS

The formulation of the first-generation rhFS formulation is compared with commercial pdhFS (Tisseel) in Table 2. The concentration of fibrinogen was about 5-fold greater in the pdhFS with respect to the rhFS. Furthermore, the pdhFS contained ~2-fold higher level of FIIa than the rhFS. The rhFS, however, had ~50-fold higher FXIII activity compared with the pdhFS. When evaluated by TEG (Table 3), the rhFS had significantly faster clotting kinetics compared with pdhFS, as measured by the angle of clot development (α) (Table 3); the maximal clot strength (MA) was not statistically different (P = 0.059) nor were the R and K values.

3.3. Treatment of stellate liver laceration with rhFS versus pdhFS

Preliminary experiments with the sealants in a porcine liver laceration model suggested that the rhFS had better hemostatic efficacy compared with the commercial pdhFS.
Injection of 10 mL of pdhFS into a stellate laceration of the liver dome followed by 5 min of manual compression resulted in persistent hemorrhage (Fig. 2A); on the other hand, hemostasis was achieved with the rhFS treatment (Fig. 2B; N = 2 swine per sealant). Subjectively, the white clot generated by the rhFS set up quicker, appeared more opaque, and felt more adherent than the clot generated by the pdhFS. Postmortem examination of the liver ex vivo demonstrated that one large hepatic vein was injured in each subject; there were no major portal, biliary, or hepatic artery injuries.

### 3.4. rhFS versus pdhFS in the hepatic wedge excision model

To determine whether there were differences in hemostatic efficacy between rhFS and pdhFS, the hemostasis assay in Figure 3, involving small hepatic wedge excisions, was devised expressly for this study. The primary determinant in this assay was the hemostatic action of the sealant alone; that is, there would be no external compression of the bleeding surfaces by the surgeon. In brief, wedges cut were made along the edge of a liver lobe (Fig. 3A), treated with a defined amount of sealant, and then a visual analog hemostasis score was assigned. Preliminary work with this model demonstrated that bleeding from an excision with a 0.5-cm cut depth was easy to control with sealant alone, whereas bleeding from 3-cm excision was quite difficult to control. So a range of excisions with a stepwise increase in cut depth (0.5–3 cm) was chosen as a discriminator of hemostatic efficacy. This range (or series) of excisions was performed twice in each subject, on separate liver lobes during the same anesthetic. Each subject tolerated these procedures well with <150 mL blood loss and minimal to no perturbation in vital signs (Fig. 3B). TEG of blood drawn immediately before versus after procedure completion did not demonstrate any deterioration of clotting during the excisions (done in all subjects; data not shown).

The intended technique of FS delivery was to use a double-chamber single-channel syringe (a proprietary device for delivery of the pdhFS; Fig. 3C) in all subjects. This device keeps the fibrinogen and the activated thrombin in separate chambers; when the operator depresses the linked syringe plungers, the protein solutions are mixed in a common channel and then ejected from the syringe tip. Use of this device with the proprietary pdhFS produced a reasonably consistent stream of sealant with occasional clogging of the tip. Initial attempts at delivery of the rhFS with this proprietary double-chamber single-channel syringe, however, resulted in tip clogging before adequate rhFS could be delivered to the wound. To deliver the rhFS to the wound, the improvised delivery system shown in Figure 3D was used. Two tuberculin-type syringes were taped together, and the needles were bent to produce a convergent stream. This syringe setup moved the mixing of the rhFS components from inside the syringe assembly to outside on the wound surface, which enabled rhFS delivery at a volume and flow subjectively equivalent to that obtained with the pdhFS.

The subjective impression from treating hepatic wedge excisions with rhFS versus pdhFS confirmed the earlier observation from the hepatic laceration model, that is, the clot produced by the rhFS set up quicker, was more opaque, and was more tenacious/adherent than the clot produced by the pdhFS (Fig. 3A,F,G). A comparative analysis of hemostatic efficacy of these two sealants in the hepatic wedge excision model is shown in Figure 3H. Overall, the hemostatic scores of the rhFS were equivalent or better than those of the pdhFS. At cut depths of 1.0 and 1.5 cm, the rhFS had greater hemostatic efficacy than the pdhFS; there was a nonsignificant trend of greater efficacy at the 2.0 cm depth. At cut depth >2.5 cm, the hemorrhage mostly was too brisk to control with either sealant in the absence of any extrinsic compression (Fig. 3H).

### 3.5. Immunohistochemistry

Double immunohistochemistry of porcine and human FI in treated wedge excisions is shown in Figure 4. The fibrin cap over the hepatic wound surface typically was thicker in the rhFS sections compared with the pdhFS sections. These double-stained sections also demonstrated the mixing of the native porcine fibrin (brown color) with human (plasma-derived or recombinant) fibrin (red color) over the surface of the wound. In both the pdhFS- and rhFS-treated samples, the predominant FI in the clot appeared to be endogenous in origin (i.e., porcine). Additional images of hepatic wedge excisions treated with rhFS have been supplied in Supplementary Figure S1.
4. Discussion

Our intention in the design of this first-generation rhFS was to achieve clotting speed and strength comparable with an available pdhFS formulation, as determined by TEG. The commercial pdhFS used in this report had a clot onset of <10 s and maximal clot strength of 50–70 mm displacement. To obtain a clot onset of <10 s, 106 U/mL of rhFIIa was used (somewhat less than the level of FIIa contained in the pdhFS; Table 2). To obtain a maximal clot strength of >50 mm, 2500 U/mL of rFXIIIa were used. We believe that by providing a relatively large amount of rFXIII in its activated form, we produced higher rates of cross-linked fibrin formation and focused rhFIIa activity on conversion of fibrinogen to fibrin.

We were able to decrease the fibrinogen content of rhFS (9 mg rhFI/mL) relative to pdhFS (>50 mg pdFI/mL), while achieving the same in vitro clotting speed and strength. A typical dose of pdhFS applied to the stellate liver laceration model contained >340 mg of pdFI, whereas the typical dose of rhFS contained 90 mg of rhFI. The rhFS contained 3.6 mg per dose of rFXIIIa, whereas pdhFS contained a constitutive level of <1 mg FXIII. Importantly, when the rhFS and pdhFS were studied in the porcine hepatic wedge resection model, the rhFS had equivalent or greater hemostatic efficacy than commercial pdhFS, despite the fibrinogen concentration in the rhFS being only one-fifth that in the pdhFS.

The hemostatic potential of FS has been shown to be dependent on the degree of cross-linking achieved by FXIII [21]. Prior studies reported that depletion of FXIII in a commercial FS (Beriplast P; Aventis Behring, Marburg, Germany) decreased the ability of that sealant to control hemorrhage in porcine vascular procedures [31] and that the relative hemostatic efficacy of FS was dependent on the concentration of FXIII [32]. These previous data suggested that the optimal FXIII concentration in pdhFS was in the range of 40–80 U/mL when the pdFI concentration was 50–100 mg/mL. The data from the present study suggested that the use of rFXIIIa could produce an rhFS that had equivalent or better hemostatic properties than the pdhFS, even when the fibrinogen content of the former was relatively low.

For posttranslationally complex proteins, the signature of the host cells on the recombinant human protein potentially can cause immunologic and biologic activity issues. In the case of rhFI, the N-linked glycosylation added to the human protein sequence of the recombinant fibrinogen was the only notable posttranslational difference between pdhFS and rhFS [22]. We have seen no issues with the biologic activity of any of the recombinant proteins used in the rhFS. Definitive studies of immunologic effects would require human clinical trials.

We did not intend our hepatic wedge excision model to be a surrogate for more lethal models of traumatic hemorrhage [18], and we have not implied that rhFS or any other sealant might be useful as a stand-alone treatment for severe traumatic hemorrhage. The wedge excision model developed for this study was intended to quantify and compare the hemostatic efficacy of sealants acting without any other adjunct (such as manual compression) at the wound surface. We wanted to minimize variables that might have influenced or confounded the hemostatic activity of a sealant acting alone. We also did not intend to analyze the tissue fixation (welding or “glue”) properties of the sealants, for example, for skin
graffing [33], reinforcement of gastrointestinal anastomosis [34], or sutureless anchorage of prosthetic mesh [35].

We previously estimated that it would require approximately 300 transgenic cows to produce 1 metric ton of purified rhFS per year [23]. An abundant source of rhFS might lead to approximately 300 transgenic cows to produce 1 metric ton of purified rhFS for medicinal purposes planned.

Further studies on this condition using a hypothermic, hemodiluted swine model [12] are underway. An abundant source of recombinant fibrinogen for intravenous administration also could impact the treatment of hypofibrinogenemic conditions, such as the dilutional/consumptional state that can occur during resuscitation from traumatic hemorrhage [36]. Further studies on this condition using a hypothermic, hemodiluted swine model [12] are planned.

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Appendix. Supplementary data
Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jss.2013.09.039.

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