

I concur with this review. I Wu 10/5/17

FOOD AND DRUG ADMINISTRATION
Center for Biologics Evaluation and Research
Office of Tissues and Advanced Therapies
Division of Clinical Evaluation and Pharmacology/Toxicology
Pharmacology/Toxicology Branch II

BLA NUMBER: STN #125640/0.0

DATE RECEIVED BY CBER: 04-NOV-2016

DATE REVIEW COMPLETED: 21-SEPT-2017

PRODUCT: VeraSeal [Fibrin Sealant (Human)]
Note: The proprietary name listed above was deemed 'Unacceptable' (03-FEB-2017). The nonproprietary name, Fibrin Sealant (Human), is used throughout the remainder of this review memo.

APPLICANT: Instituto Grifols, S.A.
 PROPOSED INDICATION: Adjunct to hemostasis for mild to moderate bleeding in adults (b) (4) undergoing surgery when control of bleeding by standard surgical techniques (such as suture, ligature, and cautery) is ineffective or impractical. Fibrin Sealant (Human) is effective in heparinized patients.
Note: In an information request sent on 03-OCT-17, the applicant was instructed to remove '(b) (4)' from the proposed indication above. The request stated that the indication may be modified via a supplemental application when supporting data are available.

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EXECUTIVE SUMMARY:

Fibrin Sealant (Human) is a combination product consisting of human plasma-derived fibrinogen and thrombin solutions, and an applicator device for drip administration. Fibrin Sealant (Human) can also be administered via an optional spray applicator, which is not supplied with the product. The applicant is proposing an indication for Fibrin Sealant (Human) to be used as an adjunct to hemostasis for mild to moderate bleeding in adults (b) (4) undergoing surgery when control of bleeding by standard surgical techniques (such as suture, ligature, and cautery) is ineffective or impractical.

The nonclinical program for Fibrin Sealant (Human) consisted of the following studies: 1) risk assessment of the excipient arginine (Arg); 2) *in vitro* proof-of-concept (POC); 3) pharmacology of drip administration in rabbit and pig models of cardiovascular surgery, and of spray application in a pig model of hepatic surgery; and 4) toxicology of systemic fibrinogen administration in healthy rodents. No animal studies were conducted to evaluate the pharmacodynamics (PD) or pharmacokinetics (PK) of Fibrin Sealant (Human). No animal studies were performed to assess the potential for carcinogenicity, *in vivo* mutagenicity, reproductive toxicity, or teratogenicity of Fibrin Sealant (Human).

The Arg content in Fibrin Sealant (Human) was similar to that of other approved fibrin sealants. Preliminary POC was demonstrated by applying Fibrin Sealant (Human) onto multiple *in vitro* test surfaces, where similar results for clotting time and surface area coverage were observed for drip and spray applicators. In pharmacology studies conducted in healthy rabbits, drip administration of Fibrin Sealant (Human) was well-tolerated in vascular surgery, with reduced surgical time and bleeding (assessed by the change in pre- vs. post-surgery wound dressing mass) compared to use of sutures alone. In additional pharmacology studies in healthy pigs, drip and spray administration of Fibrin Sealant (Human) during cardiovascular and hepatic surgery, respectively, were well-tolerated without any overt toxicities. The pharmacology results for Fibrin Sealant (Human) were similar to those seen for other approved fibrin sealants. Acute systemic toxicity studies in healthy mice and rats did not identify any product-related adverse findings following intravenous (IV) bolus administration of the fibrinogen component of Fibrin Sealant (Human). However, this is not the clinical route of administration (ROA) and only the fibrinogen component was tested. No safety concerns were identified in the resulting data from the nonclinical studies.

PHARMACOLOGY/TOXICOLOGY RECOMMENDATION:

There are no nonclinical deficiencies identified in the pharmacology/toxicology studies, and there are no outstanding requests for additional nonclinical data. The nonclinical data provided in this BLA submission support the approval of the licensure application.

Formulation and Chemistry:

Fibrin Sealant (Human) is provided as a kit (Table 1) comprised of: 1) characterized human fibrinogen 80 mg/mL; 2) characterized human thrombin 500 IU/mL; 3) two syringes (b) (4) Syringe System); 4) one syringe holder; and 5) one applicator tip (b) (4) Dual Cannula (b) (4) Applicator Tip). The biologic components, human fibrinogen and human thrombin, are manufactured from human plasma obtained from licensed plasmapheresis centers, following a fractionation and purification process. The fibrinogen and thrombin components are supplied in

the final product as frozen, sterile solutions enclosed in separate syringes and assembled on the syringe holder. The final product is available in four kit sizes corresponding to total volumes of 2, 4, 6, or 10 mL, where each kit contains an equal volume of the fibrinogen and thrombin components. Prior to administration, the final product is thawed either: 1) at room temperature (*i.e.*, 20°C - 25°C [68°F - 77°F]) for approximately 80 minutes (for the 2- and 4-mL kit sizes) or 120 minutes (for the 6- and 10-mL kit sizes); or 2) in a thermostatic water bath at a temperature of less than or equal to 37°C (99°F) for approximately 20 minutes (for the 2- and 4-mL kit sizes) or 30 minutes (for the 6- and 10-mL kit sizes). The wound bed is prepared by drying using standard techniques (*e.g.*, compresses, swabs, or suction devices). The product is administered to the wound bed via drip application through the included applicator tip or spray application using an optional spray applicator (FibriJet® Aerosol Applicator, Micromedics, Inc., 510(k) K012868) that is not supplied with the kit. The applicant claims that when combined, the fibrinogen and thrombin components generate a cross-linked fibrin clot in a process that mimics the final stage of the human coagulation cascade.

Table 1. Description of Components Included in Fibrin Sealant (Human) Kit

Component	Source (Derivation or Manufacturer)	Amount	Function
Human fibrinogen	Human plasma (licensed plasmapheresis centers)	80, 160, 240, or 400 mg	Active ingredient for generation of fibrin clot
Human thrombin	Human plasma (licensed plasmapheresis centers)	500, 1000, 1500, or 2500 IU	Active ingredient for generation of fibrin clot
Syringe	(b) (4) Syringe System (b) (4)	2 per kit	For container closure of active ingredients
Syringe holder	Laboratorios Grifols, S.A.	1 per kit	To ensure simultaneous administration of active ingredients
Applicator tip	(b) (4) Dual Cannula (b) (4) Applicator Tip (b) (4)	1 per kit	For topical drip administration of the active ingredients

Note: IU = international units

The applicant states that the dose (*i.e.*, volume) administered should be sufficient to entirely cover the wound bed in a thin, even layer, and that administration can be repeated if necessary. The applicant claims that the approximate surface area that can be covered using the various kit sizes ranges from 14 to 100 cm² (Table 2).

Table 2. Surface Area Coverage for Drip or Spray Application of Fibrin Sealant (Human)

Kit size (total volume, mL)	Wound surface area (cm ²)
2	14-20
4	28-40
6	42-60
10	70-100

Abbreviations

AA	Abdominal aorta
AAo	Ascending aorta
ABG	Arterial blood gas
AP	Arterial pressure
Arg	Arginine
BCV	Brachiocephalic vein
BW	Body weight
CBC	Complete blood count
CIFA	Centro de Investigación en Farmacología Aplicada
DART	Developmental and reproductive toxicology
ECG	Electrocardiography
GLP	Good Laboratory Practice
HPLC	High performance liquid chromatography
HR	Heart rate
IU	International unit
IV	Intravenous
LCCA	Left common carotid artery
LEJV	Left external jugular vein
LSCA	Left subclavian artery
NO	Nitric oxide
(b) (4)	(b) (4)
PD	Pharmacodynamics
PI	Pulsatility index
PK	Pharmacokinetics
POC	Proof-of-concept
PTFE	Polytetrafluoroethylene
RCCA	Right common carotid artery
RA	Right atrium
REJV	Right external jugular vein
RIJV	Right internal jugular vein
RUL	Right upper lobe
RSCA	Right subclavian artery
ROA	Route of administration
s.s.	Statistically significant
TTH	Time to hemostasis
WBC	White blood cell

Related File(s)

Master files:

CBER MF3 # (b) (4) – MASTER FILE TYPE III – (b) (4) (Sterile) and Bulk (Non-Sterile) Syringe Systems; For the manufacture of the product; Sponsor – (b) (4); **ACTIVE – letter of authorization provided**

CBER MF2 # (b) (4) – MASTER FILE TYPE II – (b) (4); Manufacture of the product; Sponsor – (b) (4); **ACTIVE – letter of authorization provided**

INDs:

IND #14988 – Fibrin Sealant; A Prospective, Single-blind, Randomized, Phase III Study to Evaluate the Safety and Efficacy of Fibrin Sealant Grifols (FS Grifols) as an Adjunct to Hemostasis During Peripheral Vascular Surgery; Sponsor – Instituto Grifols SA; **ACTIVE**

IND #14987 – Fibrin Sealant; A Prospective, Single-blind, Randomized, Phase III Study to Evaluate the Safety and Efficacy of Fibrin Sealant Grifols (FS Grifols) as an Adjunct to Hemostasis During Parenchymous Tissue Open Surgeries; Sponsor – Instituto Grifols SA; **ACTIVE**

IND #14986 – Fibrin Sealant Grifols (FS Grifols); A Prospective, Single-blind, Randomized, Phase III Study to Evaluate the Safety and Efficacy of Fibrin Sealant Grifols (FS Grifols) as an Adjunct to Hemostasis During Soft Tissue Open Surgeries; Sponsor – Instituto Grifols SA; **ACTIVE**

510(k)s:

(b) (4); Surgical Sealant Applicator; **cleared** (b) (4)

K012868 – Micromedics, Inc.; Micromedics FibriJet® Aerosol Applicator; **cleared 29-Oct-2001**

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INTRODUCTION

Management of bleeding during surgical procedures is important for maintaining a clear surgical field, preserving physiological function in the patient, and promoting wound healing.

Uncontrolled bleeding can contribute to longer surgical times, increased need for blood transfusion, and other complications. The normal human coagulation cascade is characterized by a set of successive reactions that can be divided into intrinsic and extrinsic pathways based on the triggering event and the coagulation factors and cofactors involved. However, both arms culminate in a common final pathway involving activation of prothrombin to thrombin, fibrinogen to fibrin, and fibrin-stabilizing factor, which catalyzes cross-linking of fibrin molecules into an insoluble clot.

Standard surgical techniques (such as suturing, ligature, and cautery) can be used to supplement the endogenous clotting process and aid in achieving hemostasis. When these standard techniques are ineffective or impractical (*e.g.*, for a particular surgical procedure or anatomic site), additional agents such as fibrin sealants may be used as an adjunct to hemostasis. Fibrin sealants consist of coagulation factors such as fibrinogen and/or thrombin, supplied as separate solutions or embedded on an absorbable patch, and administered directly onto the bleeding site to initiate clot formation in a process similar to the final stage of the blood coagulation cascade.

Fibrin Sealant (Human), is a combination product comprised of fibrinogen and thrombin solutions derived from pooled human plasma, and an applicator device for drip administration. Fibrin Sealant (Human) can also be applied using an optional spray applicator, which is not supplied with the product.

NONCLINICAL STUDIES

PHARMACOLOGY STUDIES

Summary List of Pharmacology Studies

The following safety pharmacology studies were conducted to support the safety and rationale for administration of Fibrin Sealant (Human) to treat the proposed clinical indication.

In Vitro Studies:

Study Number	Study Title / Publication Citation	Report Number
1	<i>Bibliographic revision of the pharmacologic effect of arginine relation to fibrin sealant Grifols</i>	IG_ITEC-002062_ING
2	<i>Fibrin sealant Grifols. In vitro coagulation study.</i>	IG_ITEC-002009_ING

In Vivo Studies:

Study Number	Study Title / Publication Citation	Report Number
3	<i>Experimental study in rabbits of a new biological glue (fibrin adhesive Grifols) in vascular surgery</i>	IG_ICT-ITEC 02-2005_ING
4	<i>Experimental study performed on pigs of a new biological glue (Grifols' Fibrin-glue) in cardiac and vascular surgery</i>	IG_ICT-ITEC 04-2003_ING
5	<i>Experimental study performed on pigs of a new biological glue (Grifols' fibrin sealant) in hepatic surgery</i>	IG_USC-ITEC 02-2005_ING
6	<i>Comparative experimental study performed on rabbits of fibrin sealants in vascular surgery</i>	IG_ICT-ITEC 01-2008_ING
7	<i>Comparative experimental study performed on pigs of fibrin sealants in hepatic surgery</i>	IG_USC-ITEC 01-2007_ING
8	<i>Safety pharmacology aspects of fibrin sealant Grifols</i>	IG_ITEC-002235_ING

Note: Study No. 8 is not summarized in this review memo because it contains summaries of Study Nos. 3-7 and does not include any new nonclinical information.

Overview of Pharmacology Studies

Overview of In Vitro Studies

Study #1

The applicant performed a review of the scientific literature to assess the potential pharmacological effects of Arg, which is an excipient in the final product. The applicant also analyzed the content of Arg in Fibrin Sealant (Human) and compared it to that of other marketed comparator products.

Nine publications were cited on the potential role of Arg in wound healing, where IV and oral administration of Arg have been shown to increase generation of nitric oxide (NO) and vasodilation, increase collagen synthesis, and reduce inflammation.^{1,2,3,4,5,6,7,8,9} An *in vitro* study

¹ Naseem, K. M. (2005). "The role of nitric oxide in cardiovascular diseases." *Mol Aspects Med* 26(1-2): 33-65.

was summarized in which Arg concentrations between 0.017 mg/mL and 0.17 mg/mL were shown to reduce the activity of the sodium-potassium pump in rat aorta tissue.³ In three clinical studies, doses of 5.0 g and 11.6 g were administered via IV infusion to healthy human subjects and the higher dose was determined to be vasoactive.^{8,9,10} In one of these studies, the authors reported that the resulting blood concentration of Arg in the low-dose group was approximately eight times lower than the theoretical blood concentration¹⁰. In addition, three studies reported myorelaxation effects in subjects following external administration of Arg at concentrations between 401 mg/mL and 2003 mg/mL.^{11,12,13}

The applicant then performed an assessment to determine whether Arg is likely to contribute to the biological activity of the final product. (b) (4)

was conducted to assess the Arg content of Fibrin Sealant (Human) and three marketed comparator products. The Arg content was (b) (4) in Fibrin Sealant (Human), (b) (4) in Crosseal™, (b) (4) in Evicel®, and (b) (4) in Beriplast. The applicant concluded that, assuming a worse-case of systemic exposure for the largest kit size, the dose of Arg in Fibrin Sealant (Human) is not expected to contribute any pharmacological effect to the product.

² Miller, A. L. (2006). "The effects of a sustained-release L-Arginine formulation on blood pressure and vascular compliance in 29 healthy individuals." *Altern Med Rev* **11**(1): 23-9.

³ Akopova, O. V., O. N. Kharlamova, and G. L. Vavilova (2002). "Effect of L-arginine on Na⁺,K⁺-ATPase activity in rat aorta endothelium." *Biochemistry (Mosc)* **67**(9): 1058-61.

⁴ Loehe, F., C. J. Bruns, S. M. Nitsch, and M. K. Angele (2007). "The role of L-arginine following trauma and blood loss." *Curr Opin Clin Nutr Metab Care* **10**(1):80-7.

⁵ Shi, H. P., S. M. Wang, G. X. Zhang, Y. J. Zhang, and A. Barbul (2007). "Supplemental l-arginine enhances wound healing following trauma/hemorrhagic shock." *Wound Repair Regen* **15**(1): 66-70.

⁶ Preli, R. B., K. P. Klein, and D. M. Herrington (2002). "Vascular effects of dietary L-arginine supplementation." *Atherosclerosis* **162**(1): 1-15.

⁷ Wittmann, F., N. Prix, S. Mayr, P. Angele, M. W. Wichmann, N. K. van den Engel, T. Hernandez-Richter, I. H. Chaudry, K. W. Jauch, and M. K. Angele (2005). "L-arginine improves wound healing after trauma-hemorrhage by increasing collagen synthesis." *J Trauma* **59**(1): 162-8.

⁸ Bode-Boger, S. M., R. H. Boger, A. Creutzig, D. Tsikas, F. M. Gutzki, K. Alexander, and J. C. Frolich (1994). "L-arginine infusion decreases peripheral arterial resistance and inhibits platelet aggregation in healthy subjects." *Clin Sci (Lond)* **87**(3): 303-10.

⁹ Kanno, K., Y. Hirata, T. Emori, K. Ohta, S. Eguchi, T. Imai, and F. Marumo (1992). "L-arginine infusion induces hypotension and diuresis/natriuresis with concomitant increased urinary excretion of nitrite/nitrate and cyclic GMP in humans." *Clin Exp Pharmacol Physiol* **19**(9): 619-25.

¹⁰ Bode-Boger, S. M., R. H. Boger, A. Galland, D. Tsikas, and J. C. Frolich (1998). "L-arginine-induced vasodilation in healthy humans: pharmacokinetic-pharmacodynamic relationship." *Br J Clin Pharmacol* **46**(5): 489-97.

¹¹ Griffin, N., D. D. Zimmerman, J. W. Briel, H. J. Gruss, M. Jonas, A. G. Acheson, K. Neal, J. H. Scholefield, and W. R. Schouten (2002). "Topical L-arginine gel lowers resting anal pressure: possible treatment for anal fissure." *Dis Colon Rectum* **45**(10): 1332-6.

¹² Acheson, A. G., N. Griffin, J. H. Scholefield, and V. G. Wilson (2003). "L-arginine-induced relaxation of the internal anal sphincter is not mediated by nitric oxide." *Br J Surg* **90**(9): 1155-62.

¹³ Gosselink, M. P., M. Darby, D. D. Zimmerman, H. J. Gruss, and W. R. Schouten (2005). "Treatment of chronic anal fissure by application of L-arginine gel: a phase II study in 15 patients." *Dis Colon Rectum* **48**(4): 832-7.

Comment:

- Based on the literature review and (b) (4) analysis conducted by the applicant, it appears that the levels of Arg in Fibrin Sealant (Human) are similar to other marketed fibrin sealants, and that they are not likely to contribute to the product's mechanism of action.

Study #2

The applicant conducted an *in vitro* study to assess the clotting time of Fibrin Sealant (Human) under different thawing and storage conditions.

Three different lots of Fibrin Sealant (Human) corresponding to the minimum (2 mL), intermediate (4 mL), and maximum (10 mL) kit size volumes were analyzed for *in vitro* clotting time. Samples of each test article were thawed using two different techniques: 1) 30 minutes in a water bath at 37°C; or 2) 1 hour at room temperature. Samples were tested immediately after thawing, and after 24 hours at 5°C, 25°C, and (b) (4). The clotting time was evaluated following drip administration using an application needle with a double cannula (5.7 cm length, 20-gauge needle; (b) (4)). The mean clotting time varied from 2.5 to 3.8 seconds, and no substantial differences were observed among the thawing and storage conditions.

Comments:

- The study report contains limited details on the test article application procedure. For example, the following information was not specified in the report: 1) the total volume of each test article evaluated; 2) the technique used to apply the test article (*e.g.*, needle tip angle, timing of administration, distance from needle tip to substrate, etc.); 3) the substrate onto which the test article was administered; and 4) the method for determining clot formation (*i.e.*, visual observation, microscopic analysis, etc.).
- It is not clear if the application needle with a double cannula used in this study is identical to the final applicator tip (cannula) described in Module 3.2.P.7 of the application. The study report states that the application needle with double cannula was manufactured by (b) (4) and had a length of 5.7 cm. The applicator tip described in Module 3.2.P.7 is manufactured by (b) (4) (cleared under (b) (4)) and has a length of 5.1 cm. Both devices include a 20-gauge needle.
- It is not clear what syringe holder (if any) was used.
- The rationale for the choice of thawing times/temperatures and storage conditions was not presented in the study report.
- Although there are notable limitations concerning the design and reporting of this study, the clotting times observed appear to indicate consistent clot formation following drip application of the product. Moreover, additional *in vitro* studies were conducted using both the spray and drip applicators to evaluate clotting time and surface area coverage (IG_ITEC-002493_ING, IG_ITEC-002497_ING, IG_ITEC-002568_ING; reported in

Module 3.2.P.7 of the application). These studies support the preliminary POC of the product.

Overview of In Vivo Studies

In Vivo Studies in Healthy Animals

Study #3

Report Number		IG_ICT-ITEC 02-2005_ING
Date Report Signed		28-Jul-2008
Title		<i>Experimental study in rabbits of a new biological glue (fibrin adhesive Grifols) in vascular surgery</i>
GLP Status		No
Testing Facility		(b) (4)
Objective(s)		To evaluate the safety and POC of Fibrin Sealant (Human) for use in vascular surgery
Study Animals	Strain/Breed	(b) (4)
	Species	Rabbit
	Age	Not reported
	Body Weight (BW)	3.54 ± 0.31 kg (range: 3.16-4.36 kg)
	#/group	9
	Total #	27
Test Article		Fibrin Sealant (Human) (fibrinogen lot #307391; thrombin lot #302492) Note: The administration device consisted of a 3 mL (b) (4) syringe and a dual cannula applicator tip (length = 5.7 cm, needle = 26 gauge; (b) (4) reference (b) (4)).
Control Article		Polypropylene 8/0 sutures
ROA		Drip application onto the anastomosis site
Description of the Disease/Injury Model and Implant Procedure		All rabbits were anesthetized, and a peripheral line was placed in the ear vein for maintenance IV fluids. Animals were placed in a supine decubitus (face up) position, and a laparotomy was performed to access a 3-cm longitudinal section of the infrarenal abdominal aorta (AA). Animals were heparinized (200 U/kg/animal sodium heparin, IV). The proximal and distal AA were clamped and sectioned transversely. A termino-terminal (end-to-end) anastomosis was created, and the vessel was sealed using a four-point suture technique alone or in combination with the test article (see 'Study Groups' below). After declamping the AA, circulating heparin was neutralized. The retroperitoneum was irrigated and the laparotomy was closed using resorbable sutures. Following surgery, animals were administered analgesics (3 mg/kg/animal/day ketoprofen for three days) and antibiotics (10 mg/kg/animal enrofloxacin every 12 hours for five days). A second-look surgery was performed on day 14 post-administration to examine the anastomosis. Animals were then sacrificed to allow additional analyses.

Study Groups and Dose Levels	<p>Group A – Control article</p> <p>Group B₁ – Control article + 1 mL test article (administered <u>after</u> declamping the AA)</p> <p>Group B₂ – Control article + 1 mL test article (administered <u>before</u> declamping the AA)</p> <p>Note: Group B₂ represents the intended clinical dosing procedure for vascular surgery.</p>
Dosing Regimen	Single application
Randomization	Yes
Description of Masking	Not described
Scheduled Sacrifice Time Points	14 days post-administration

Key Evaluations and Assessments:

Study Parameter	Description
Surgical time	Assessed by the total time after anesthetic induction until closure of the laparotomy.
Bleeding	<p>Assessed by:</p> <ul style="list-style-type: none"> the number of animals that died due to bleeding of the anastomosis, or that did not achieve hemostasis within 30 min (considered ‘equivalent to death’); the difference in pre- vs. post-surgical mass of the wound dressings applied to the anastomosis; and the degree of the retroperitoneal hematoma observed during the second-look surgery on day 14, where the following scoring system was used: 0 = ‘absence of hematoma’; 1 = ‘light hematoma allowing visualization of the AA and anastomosis’; and 2 = ‘important hematoma that hides the AA and anastomosis’. <p>Note: Animals considered ‘equivalent to death’ were administered additional sutures to achieve hemostasis. It is assumed that this procedure was done for ethical reasons.</p>
Quality of anastomosis	<p>Assessed by:</p> <ul style="list-style-type: none"> palpation of pulse (binary, <i>i.e.</i>, ‘yes’ or ‘no’) distal to the anastomosis on day 0 (<i>i.e.</i>, intraoperatively post-administration) and day 14; Doppler echocardiography to determine whether flow-through anastomosis was greater than 30 cm/s (binary, <i>i.e.</i>, ‘yes’ or ‘no’) on day 0 and day 14; and macroscopic inspection of the excised AA post-mortem for evidence of occlusion, thrombosis, stenosis, and/or aneurysm following animal sacrifice, dissection, and longitudinal sectioning.
Behavior of test article	<p>Assessed by:</p> <ul style="list-style-type: none"> the persistence of the test article at the anastomosis on day 14; and the degree of retroperitoneal adherence observed post-mortem during AA dissection on day 14, where the following scoring system was used: 1 = ‘light adherence that does not hinder dissection’; 2 = ‘moderate adherence that extends the dissection period’; 3 = ‘important adherence that hinders dissection’; and 4 = ‘very important adherence’.

Key Results:

- Mortality
 - There were a total of seven unscheduled deaths in the study. Two animals in Group B₁ died during anesthesia induction and were excluded during analysis of the study results.

- Five animals died following the implantation surgery. Clinical observations and necropsy results showed a pulmonary hemorrhage in one animal (Group A), bilateral hind-limb paresis in two animals (one each in Groups A and B₂), and bilateral hind-limb with additional forelimb paresis in one animal (Group B₁).
- No deaths were attributed to the test article.
- Surgical time
 - The mean surgical time was significantly shorter ($p = 0.0001$) for animals in Group B₂ (83.6 ± 7.4 min) compared to Group A (103.8 ± 8.5 min).
- Bleeding
 - Two animals in Group A were considered 'equivalent to death' (see 'Key Evaluations and Assessments') because they required supplementary sutures to achieve hemostasis.
 - The mean change in wound dressing mass was significantly reduced ($p = 0.001$) for animals in Group B₂ (0.72 ± 0.83 g) compared to Group A (4.86 ± 2.73 g).
 - Two animals, one each in Groups A and B₁, received a score of 1 during the second-look surgery on day 14, indicating a light hematoma.
- Quality of anastomosis
 - The results for palpation of pulse and Doppler echocardiography of flow appeared to be associated with each other, and no substantial differences in these parameters were noted between the study groups.
 - Stenosis or occlusion was observed during macroscopic examination of the AA post-mortem in four animals, two each in Groups A and B₂. These findings were confirmed by the lack of a palpable pulse and a flow less than 30 cm/s on Doppler imaging results in these animals. In all cases, the findings were attributed to the suturing technique and not to the test or control articles.
- Behavior of test article
 - The test article was not visible post-mortem in any animals.
 - The degree of retroperitoneal adherence observed during AA dissection was significantly ($p = 0.0001$) increased in Groups B₁ (3.29 ± 0.76) and B₂ (3.33 ± 0.50) compared to Group A (1.71 ± 0.49).

Comment:

- It appears that Fibrin Sealant (Human) is well-tolerated and contributes to improved hemostasis when administered via drip application as an adjunct to sutures in a healthy rabbit model of vascular surgery.

Study #4

Report Number		IG_ICT-ITEC 04-2003_ING
Date Report Signed		25-Aug-2003
Title		<i>Experimental study performed on pigs of a new biological glue (Grifols' Fibrin-glue) in cardiac and vascular surgery</i>
GLP Status		No
Testing Facility		(b) (4)
Objective(s)		To evaluate the safety and POC of Fibrin Sealant (Human) for use as an adjunct to sutures in vascular surgery
Study Animals	Strain/Breed	(b) (4)
	Species	Minipig
	Age	Not reported
	BW	59.3 ± 3.5 kg (range: 26-120 kg)
	#/group	14 Note: Each animal was administered both the control and test article and therefore served as its own control.
	Total #	14
Test Article		Fibrin Sealant (Human) (fibrinogen lot #220090; thrombin lot #219390) Note: The administration device consisted of a 3 mL (b) (4) syringe and a dual cannula applicator tip (length = 5.7 cm, needle = 26 gauge; (b) (4) reference (b) (4)).
Control Article(s)		Polypropylene 6/0 sutures
ROA		Drip application onto the anastomosis site

Description of the Disease/Injury Model and Implant Procedure	<p>Animals were anesthetized, intubated, and heparinized. Vascular injuries were created in cervical (n = 8 animals), thoracic (n = 3 animals), or both regions (n = 3 animals). In each region, the vessels/tissues were exposed, clamped, cut, and dosed (1 mL/group/injury/animal) according to the sequences below.</p> <p>For <u>cervical</u> injuries, target vessels included the left (L) and right (R) common carotid arteries (LCCA, RCCA), the L and R subclavian arteries (LSCA, RSCA), the L and R external jugular veins (LEJV, REJV), and the R internal jugular vein (RIJV). Injuries were created and dosed in the following order:</p> <ul style="list-style-type: none">• bilateral transverse incisions in the LEJV and REJV;• LCCA-RCCA bypass using a polytetrafluoroethylene (PTFE) graft;• two longitudinal incisions in the middle third of the LCCA;• two transverse incisions in the proximal third of the LSCA; and• RCCA-RSCA bypass using an autologous RIJV graft. <p>For <u>thoracic</u> injuries, target vessels and cardiac tissues included the brachiocephalic vein (BCV), the ascending aorta (AAo), the right atrium (RA) of the heart, and the right upper lobe (RUL) of the lung. Injuries were created and dosed in the following order:</p> <ul style="list-style-type: none">• two transverse incisions in the BCV;• two longitudinal incisions in the AAo;• two longitudinal incisions in the external wall of RA; and• one incision in the RUL. <p>Note: For the RUL injury site, no control article was used due to the risk of postoperative pneumothorax.</p>																														
Study Groups and Dose Levels	<p>Group A – Control article + test article Group B – Control article</p> <p>Note: As shown in the summary table below, different quantities of control article sutures were used for each study group at the various injury sites. No rationales were provided to support the number of sutures used for each injury site and study group.</p> <table><tr><th>Injury site</th><th>Group A sutures</th><th>Group B sutures</th></tr><tr><td>LEJV and REJV</td><td>8</td><td>12</td></tr><tr><td>LCCA-RCCA bypass</td><td>12</td><td>16</td></tr><tr><td>LCCA</td><td>8</td><td>12</td></tr><tr><td>LSCA</td><td>8</td><td>12</td></tr><tr><td>RCCA-RSCA bypass</td><td>8</td><td>12</td></tr><tr><td>BCV</td><td>8</td><td>12</td></tr><tr><td>AAo</td><td>6</td><td>8</td></tr><tr><td>RA</td><td>5</td><td>8</td></tr><tr><td>RUL</td><td>N/A</td><td>N/A</td></tr></table>	Injury site	Group A sutures	Group B sutures	LEJV and REJV	8	12	LCCA-RCCA bypass	12	16	LCCA	8	12	LSCA	8	12	RCCA-RSCA bypass	8	12	BCV	8	12	AAo	6	8	RA	5	8	RUL	N/A	N/A
Injury site	Group A sutures	Group B sutures																													
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LSCA	8	12																													
RCCA-RSCA bypass	8	12																													
BCV	8	12																													
AAo	6	8																													
RA	5	8																													
RUL	N/A	N/A																													
Dosing Regimen	Single application																														
Randomization	No; All surgeries were performed by same investigator, who alternated dosing at each injury site to avoid training bias.																														
Description of Masking	Nurses were masked to study groups when recording suture time.																														
Scheduled Sacrifice Time Points	4-6 weeks post-administration (34.6 ± 5.9 days)																														

Key Evaluations and Assessments:

- Hemostasis, as assessed by the compression time and the need for supplementary sutures
- Vital signs, assessed during the first 15 minutes post-administration by arterial blood gas (ABG) testing, monitoring of heart rate (HR), arterial pressure (AP), arrhythmia (if any), and electrocardiography (ECG)
- Complete blood count (CBC), assessed prior to the surgeries on day 0 and at sacrifice
- Hemodynamics, assessed intraoperatively on day 0 and at sacrifice using a flow probe to measure flow and pulsatility index (PI, where $PI = [\text{maximum flow} - \text{minimum flow}] / \text{mean flow}$, and values below 4 and 5 are considered normal)
- Echocardiography, performed intraoperatively on day 0 and prior to sacrifice for assessment of patency in cervical target vessels
- Suture time, assessed intraoperatively by a nurse
- Incidence of thrombosis, aneurysm, and/or pneumothorax
- Histopathology of administration sites, assessed by a pathologist

Key Results:

- One animal was euthanized in the operating room because it did not awake within four hours after surgery. The compromised physical condition resulting in the decision to euthanize was attributed to low animal BW (26 kg) and prolonged clamping of the neck arteries.
- Hemostasis
 - Fewer supplementary sutures were required to achieve hemostasis in Group A compared to Group B at all administration sites.
 - Group A required less compression time than Group B to achieve hemostasis. Notably, Group B in the LCCA-RCCA bypass procedure required compression for 3-10 min to achieve hemostasis; this strategy was not required for Group A.
- Vital signs
 - No substantial changes were noted in the animals.
- CBC
 - A slight decrease (trend, not statistically significant [s.s.]) in the mean white blood cell (WBC) count was observed between day 0 (8700 ± 2787 WBC/ μ L) and sacrifice (7514 ± 2774 WBC/ μ L).
- Hemodynamics

- There were no significant differences in mean flow or PI between Groups A and B at either time point for the bilateral EJV injuries and the LCCA incisions.
- On day 0 post-administration, the PI was greater than 5 in four animals in the LSCA, and in one animal in the LCCA-RCCA bypass PTFE graft.
- On the day of sacrifice, the PI was greater than 5 in the LSCA in four animals.
- **Note:** Flow and PI were not compared between study groups for the LSCA injuries and the bypass procedures because the administration sites for each group were downstream from each other in the same vessel/graft.
- Echocardiography
 - All vessels and grafts in all animals appeared patent on day 0 post-administration.
 - On the day of scheduled sacrifice, four animals had PTFE graft occlusions. These findings were attributed to the inherent thrombotic potential of the grafts.
- Suture time
 - The suture time was significantly shorter for Group A compared to Group B at three of the five cervical and at one of the three thoracic administration sites, where the results for the remaining sites followed the same trend (not s.s.).
- No animals showed signs of aneurysm development or pneumothorax.
- Histopathology
 - The test article was not visible in samples taken from 10/13 Group A animals that survived to their scheduled sacrifice. In the other three animals, the test article was visible at one discrete administration site per animal, and it appeared to be accompanied by the presence of microscopic cavities containing necrotic material and polymorphonuclear leukocytes.
 - Inflammation was visible around the suture sites in all animals. Using a three-point histological scoring system, there appeared to be a slight trend (not s.s.) of increased eosinophilia in the inflammatory cell infiltrate around sutures in Group A compared to Group B.
 - Out of a total of 136 total sutures evaluated histologically, 16 in Group A and 13 in Group B had dystrophic calcifications around the suture. No differences in the severity of these calcifications were found using a four-point scoring system.
 - In animals showing PTFE graft occlusions (see 'Echocardiography' above), there were complementary findings of thrombi on histopathology slides in the native vessel around the anastomosis (both study groups).

Comments:

- It appears that drip application of Fibrin Sealant (Human) is well-tolerated across a variety of cardiovascular surgery applications in healthy minipigs.
- There are limited efficacy data presented in this study. Although suture time was significantly reduced in Group A compared to Group B for some of the injury sites, this finding was not consistent. It is unclear whether this result is related to a lack of statistical

power or other factor(s). Moreover, the interpretability of the study results – that Group A required fewer supplementary sutures and less compression compared to Group B – is limited because individual animal data and statistical information for these parameters were not presented in the study report.

Study #5

Report Number		IG_USC-ITEC 02-2005_ING
Date Report Signed		30-Sept-2005
Title		<i>Experimental study performed on pigs of a new biological glue (Grifols' fibrin sealant) in hepatic surgery</i>
GLP Status		No
Testing Facility		(b) (4)
Objective(s)		To evaluate the safety and efficacy of Fibrin Sealant (Human) for use in hepatic surgery
Study Animals	Strain/Breed	Not specified
	Species	Pig
	Age	50 days old
	BW	25 ± 2 kg at the beginning of the study
	#/group	15
	Total #	15
Test Article		Fibrin Sealant (Human) (fibrinogen lot #0420490; thrombin lot #232593) Note: The administration device consisted of a 3 mL (b) (4) syringe, a spray applicator (Micromedics reference SA-6105), and a compressed air regulator ((b) (4) reference (b) (4)).
Control Article(s)		N/A
ROA		Spray application on the site of liver injury
Description of the Disease/Injury Model and Implant Procedure		Animals were anesthetized, and active hemorrhaging was induced by resecting a 3 cm long x 0.5 cm thick portion of the liver. The test article was sprayed onto the injury using an external O ₂ source. No other interventions were used to achieve hemostasis.
Study Groups and Dose Levels		Group 1 – 5 mL test article
Dosing Regimen		Single application
Randomization		No
Description of Masking		N/A
Scheduled Sacrifice Time Points		1 month post-administration

Key Evaluations and Assessments:

- Hemodynamics, assessed intraoperatively by using a cardiovascular monitor to measure HR and the systolic, diastolic, and mean AP
- Respiration, assessed intraoperatively by using a respiratory monitor to measure the final exhaling fraction of CO₂, inhaling and exhaling fraction of O₂, respiratory rate, and O₂ saturation

- Time to hemostasis (TTH), assessed intraoperatively
- Post-operative hemorrhaging, assessed as the volume of drainage from the surgical site during the first 24 hours post-administration
- Test article reabsorption, assessed visually during collection of liver tissue samples for histopathology
- Histopathology, assessed at sacrifice using tissue samples collected from the administration site and from another area in the liver where no test article had been applied

Key Results: There were no overt toxicities observed in the study. All animals survived to scheduled sacrifice, and no hemodynamic or respiratory changes were observed during the surgeries compared to baseline (at anesthetic induction). The only complications included one case each of an infection and an abdominal hernia. The test article was effective in achieving hemostasis without other interventions. Drainage was minimal during the first 24 hours post-administration, where none was observed in 10 animals, and a combination of hematic drainage and peritoneal exudate was noted in the remaining animals (2 mL in 3 animals, and 3 mL in 2 animals). The mean TTH was 56 ± 6 sec. Remnants of the test article were still visible in a 1-3 cm long region at the administration site during the liver biopsy procedures. Gross inspection of the liver biopsies showed apparent granuloma formation in areas dosed with the test article. These findings were confirmed on histopathology slides by the presence of an inflammatory cell infiltrate containing eosinophils, other polymorphonuclear leukocytes, and multinucleated giant cells, surrounded by peripheral fibrous tissue. The internal hepatic tissue underneath the administration was characterized by inflammatory cells that appeared to infiltrate the liver connective tissue. These findings were attributed to a foreign body reaction.

Comment:

- It appears that spray application of Fibrin Sealant (Human) is well-tolerated and effective in achieving hemostasis in a porcine model of hepatic surgery. However, no individual animal data were provided in the study report, so these findings could not be independently verified.

Study #6

Report Number		IG_ICT-ITEC 01-2008_ING																		
Date Report Signed		19-Jun-2009																		
Title		<i>Comparative experimental study performed on rabbits of fibrin sealants in vascular surgery</i>																		
GLP Status		No																		
Testing Facility		(b) (4)																		
Objective(s)		To compare Fibrin Sealant (Human) to three marketed comparator products when used in vascular surgery in terms of the: 1) number of sutures required; 2) amount of bleeding; 3) surgical time; 4) risk of stenosis; 5) tolerability; 6) risk of aneurysm formation; and 7) product reabsorption.																		
Study Animals	Strain/Breed	(b) (4)																		
	Species	Rabbit																		
	Age	Not reported																		
	BW	3.69 kg (range: 2.67 - 4.54 kg)																		
	#/group	10																		
	Total #	40																		
Test and Reference Articles		<table border="1"> <thead> <tr> <th>Test article</th><th>Manufacturer</th><th>Lot</th></tr> </thead> <tbody> <tr> <td>Fibrin Sealant (Human)</td><td>Instituto Grifols, S.A.</td><td>IBNC6A5ST1</td></tr> <tr> <th>Reference articles</th><th>Manufacturer</th><th>Lot</th></tr> <tr> <td>Tisseel VH</td><td>Baxter Healthcare Corp.</td><td>VNT3A044</td></tr> <tr> <td>Evicel®</td><td>OMRIX Biopharmaceuticals, Ltd.</td><td>K36F150</td></tr> <tr> <td>Crosseal™</td><td>OMRIX Biopharmaceuticals, Ltd.</td><td>K09Q170</td></tr> </tbody> </table> <p>Note: The administration device used with each test/reference article was not specified in the study report.</p>	Test article	Manufacturer	Lot	Fibrin Sealant (Human)	Instituto Grifols, S.A.	IBNC6A5ST1	Reference articles	Manufacturer	Lot	Tisseel VH	Baxter Healthcare Corp.	VNT3A044	Evicel®	OMRIX Biopharmaceuticals, Ltd.	K36F150	Crosseal™	OMRIX Biopharmaceuticals, Ltd.	K09Q170
Test article	Manufacturer	Lot																		
Fibrin Sealant (Human)	Instituto Grifols, S.A.	IBNC6A5ST1																		
Reference articles	Manufacturer	Lot																		
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Evicel®	OMRIX Biopharmaceuticals, Ltd.	K36F150																		
Crosseal™	OMRIX Biopharmaceuticals, Ltd.	K09Q170																		
Control Article		N/A																		
ROA		Drip application onto the anastomosis site																		
Description of the Disease/Injury Model and Implant Procedure		Refer to Study #3.																		
Study Groups and Dose Levels		Group A – Fibrin Sealant (Human) Group B – Tisseel VH Group C – Evicel® Group D – Crosseal™ Note: The volume of each test/reference article applied to the anastomosis was not specified in the study report.																		
Dosing Regimen		Single application																		
Randomization		Yes																		
Description of Masking		Not described																		
Scheduled Sacrifice Time Points		Days 14 and 28 post-administration																		

Key Evaluations and Assessments:

Study Parameter	Description
Surgical time	Assessed by the total time after anesthetic induction until closure of the laparotomy.
Bleeding	<p>Assessed by:</p> <ul style="list-style-type: none"> the number of animals that died due to bleeding of the anastomosis, or that did not achieve hemostasis within 30 min (considered 'equivalent to death'); the difference in pre- vs. post-surgical mass of the wound dressings applied to the anastomosis; and the degree of retroperitoneal hematoma observed during the second-look surgery on day 14, where the following scoring system was used: 0 = 'absence of hematoma'; 1 = 'light hematoma allowing visualization of the AA and anastomosis'; and 2 = 'important hematoma that hides the AA and anastomosis'. <p>Note: Animals considered 'equivalent to death' were administered additional sutures to achieve hemostasis. It is assumed that this procedure was done for ethical reasons.</p>
Quality of anastomosis	<p>Assessed by:</p> <ul style="list-style-type: none"> palpation of pulse (binary, <i>i.e.</i>, 'yes' or 'no') distal to the anastomosis on day 0 (<i>i.e.</i>, intraoperatively post-administration) and day 14; Doppler echocardiography to determine whether flow through anastomosis was greater than 30 cm/s (binary, <i>i.e.</i>, 'yes' or 'no') on day 0 and day 14; and macroscopic inspection of the excised AA post-mortem for evidence of occlusion, thrombosis, stenosis, and/or aneurysm following animal sacrifice, dissection, and longitudinal sectioning.
Behavior of test article	<p>Assessed by:</p> <ul style="list-style-type: none"> the persistence of the test article at the anastomosis on day 14; and the degree of retroperitoneal adherence observed post-mortem during AA dissection on day 14, where the following scoring system was used: 1 = 'light adherence that does not hinder dissection'; 2 = 'moderate adherence that extends the dissection period'; 3 = 'important adherence that hinders dissection'; and 4 = 'very important adherence'.

Key Results:

- **Mortality**
 - There was a total of five unscheduled deaths. One animal in Group A died during the first surgery before applying the test article, and four animals (two each in Groups C and D) were euthanized prior to their scheduled sacrifice time point due to the presence of hind-limb paralysis. None of these deaths were attributed to the test or control articles.
- **Surgical time**
 - There were no significant differences in surgical time between the study groups.
- **Bleeding**
 - Groups A and B showed similar results for the study parameters related to bleeding. Two animals in Group A and three in Group B were considered 'equivalent to death' during the initial surgery. In addition, one animal in each of these two study groups received a score of '1' on day 14 corresponding to a light

hematoma. On the other hand, four animals in Group A and three in Group B experienced no bleeding as measured by no change in wound dressing mass.

- The remaining animals across the four study groups showed various changes in wound dressing mass, ranging from: 1-14 g in Group A; 1-6 g in Group B; 1-31 g in Group C; and 1-13 g in Group D.
- Quality of anastomosis
 - All animals had a palpable pulse in the AA distal to the anastomosis, as well as normal flow curve morphology and velocity via echocardiography.
 - There was no macroscopic evidence of stenosis, occlusion, or aneurysm formation in any animals.
- Behavior of test/reference article
 - Neither the test article nor the reference articles were visible during necropsy (for the four animals that were euthanized due to hind-limb paralysis) or the second-look surgery on days 14 and 28 post-administration.
 - Three animals (one in Group A and two in Group D) received a score of '2' corresponding to moderate adherences.

Comment:

- Fibrin Sealant (Human) appears to be well-tolerated and performs similarly to other approved comparator products when applied via topical drip administration as an adjunct to sutures in a healthy rabbit model of vascular surgery.

Study #7

Report Number	IG_USC-ITEC 01-2007_ING																			
Date Report Signed	10-Jun-2009																			
Title	<i>Comparative experimental study performed on pigs of fibrin sealants in hepatic surgery</i>																			
GLP Status	No																			
Testing Facility	(b) (4)																			
Objective(s)	To compare the safety and efficacy of Fibrin Sealant (Human) to three approved comparator products for use in hepatic surgery																			
Study Animals	Strain/Breed	Not specified																		
	Species	Pig																		
	Age	45 days old																		
	BW	25 ± 5 kg at the beginning of the study																		
	#/group	5																		
	Total #	20																		
Test and Reference Articles	<table border="1"> <thead> <tr> <th>Test article</th><th>Manufacturer</th><th>Lot</th></tr> </thead> <tbody> <tr> <td>Fibrin Sealant (Human)</td><td>Instituto Grifols, S.A.</td><td>IBNC6A5ST1</td></tr> <tr> <th>Reference articles</th><th>Manufacturer</th><th>Lot</th></tr> <tr> <td>Tisseel®</td><td>Baxter Healthcare Corp.</td><td>VNT3F009</td></tr> <tr> <td>Evicel®</td><td>OMRIX Biopharmaceuticals, Ltd.</td><td>K30F12K</td></tr> <tr> <td>Crosseal™</td><td>OMRIX Biopharmaceuticals, Ltd.</td><td>K11Q20K</td></tr> </tbody> </table> <p>Note: The administration device for each reference article was provided by the manufacturer. For the test article, the study report lists the spray applicator as S-6105 from Micromedics. It is assumed that this is a typographical error, and that SA-6105 was used.</p>		Test article	Manufacturer	Lot	Fibrin Sealant (Human)	Instituto Grifols, S.A.	IBNC6A5ST1	Reference articles	Manufacturer	Lot	Tisseel®	Baxter Healthcare Corp.	VNT3F009	Evicel®	OMRIX Biopharmaceuticals, Ltd.	K30F12K	Crosseal™	OMRIX Biopharmaceuticals, Ltd.	K11Q20K
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Control Article(s)	N/A																			
ROA	Spray application on the site of liver injury																			
Description of the Disease/Injury Model and Implant Procedure	Animals were anesthetized, and active hemorrhaging was induced by resecting a 3 cm long x 0.5 cm thick portion of the liver. The test article was sprayed onto the injury using an external O ₂ source. No other interventions were used to achieve hemostasis.																			
Study Groups and Dose Levels	Group 1 – 3 mL Fibrin Sealant (Human) Group 2 – 3 mL Tisseel® Group 3 – 3 mL Evicel® Group 4 – 3 mL Crosseal™																			
Dosing Regimen	Single application																			
Randomization	No																			
Description of Masking	N/A																			
Scheduled Sacrifice Time Points	4 weeks post-administration																			

Key Evaluations and Assessments:

- Hemodynamics, assessed intraoperatively by using a cardiovascular monitor to measure HR and the systolic, diastolic, and mean AP
- Respiration, assessed intraoperatively by using a respiratory monitor to measure the final exhaling fraction of CO₂, inhaling and exhaling fraction of O₂, respiratory rate, and O₂ saturation
- Time to hemostasis (TTH), assessed intraoperatively
- Post-operative hemorrhaging, assessed as the volume of drainage from the surgical site during the first 24 hours post-administration
- Test article reabsorption, assessed visually during liver tissue biopsy and on histopathology slides
- Histopathology, assessed at sacrifice using tissue samples collected from the administration site and from another area in the liver where no test article had been applied

Key Results: There were no overt toxicities observed in the study. All animals survived to their scheduled sacrifice, and no changes in hemodynamic or respiratory parameters were observed during the surgeries compared to baseline (at anesthetic induction). Five animals developed an infection at the surgical site (one per group for Groups 1, 2, and 3; two in Group 4). Three animals developed an asymptomatic abdominal hernia (the distribution of these cases was not described but was stated as similar among the study groups). All animals achieved hemostasis at the hepatic injury site without requiring other interventions. The mean TTH was 50 ± 3 sec in Group A, 47 ± 4 sec in Group 2, 51 ± 6 sec in Group 3, and 49 ± 6 sec in Group 4. There were no differences observed in the amount of post-surgical drainage between the study groups. No residual hematomas were found in any animals during the second (biopsy) surgery performed 1 month post-administration. The test and reference articles were still visible at the administration site in most animals in Groups 1-3 during the liver biopsy procedure; however, no reference article was observed histologically in three of the five animals in Group 4.

Macroscopic inspection of the liver biopsies showed apparent foreign body granulomas at the administration site. A qualitative scoring system was used to assess histopathology slides for: 1) the presence of fibrosis; 2) changes in the adjacent tissue; 3) inflammatory cells; and 4) material residues. The results for each category ranged from '-' to '+++'; however, the study report does not include a description corresponding to each score. Nevertheless, it appears that the hepatic samples from all study groups showed similar levels of fibrosis ('+' to '+++') and inflammatory cells ('+/-' to '+++'), with minimal changes in the adjacent tissue ('-' to '+'). Results for the material residues appeared to be consistent with the visual assessments conducted during the biopsy procedure. The summary of the histopathological data describes granulomatous formations containing numerous polymorphonuclear cells surrounded on the periphery by lymphocytes, histiocytes, multinucleated giant cells, mononuclear inflammatory cells, and

fibrous tissue. The internal hepatic tissue underneath the administration site was characterized by inflammatory cells that infiltrated the liver connective tissue. These findings were consistent between the study groups and were attributed to a foreign body reaction against the test/reference articles.

Comment:

- It appears that Fibrin Sealant (Human) performs similarly to other approved comparator products when applied via topical spray administration in a healthy porcine model of hepatic surgery.

SAFETY PHARMACOLOGY STUDIES

No safety pharmacology studies were conducted.

PHARMACOKINETIC STUDIES

Per the applicant, no PK studies were conducted because the product is intended for local administration and a local effect. The applicant states that the product is metabolized in the same way as endogenous fibrin (*i.e.*, by fibrinolysis and phagocytosis). In Study #4 evaluating drip application in a porcine model of vascular surgery, Fibrin Sealant (Human) was not visible at the administration site in most animals during the histopathological analysis performed at 4-6 weeks post-administration. However, these findings differ from the results of Study #5 and #7, in which Fibrin Sealant (Human) was still visible one month after spray administration in a porcine model of hepatic surgery. Fibrin Sealant (Human) appears to have a similar degradation profile to other approved comparator products (Study #6 and #7).

TOXICOLOGY STUDIES

Summary List of Toxicology Studies

The following toxicology studies were conducted to evaluate the safety of Fibrin Sealant (Human) following administration in various animal species.

Toxicology Studies:

Study Number	Study Title / Publication Citation	Report Number
9	<i>Preliminary study of the acute toxicity of the fibrinogen component of fibrin-glue Grifols by intravenous route in mouse</i>	IG_CIFA-ITEC 016-02_ING
10	<i>Study of the acute toxicity of the fibrinogen component of fibrin-glue Grifols by intravenous route in mouse</i>	IG_CIFA-ITEC 018-02_ING
11	<i>Study of the acute toxicity of the fibrinogen double inactivated (FG-DI) component of fibrin-glue Grifols by intravenous route in mouse</i>	IG_CIFA-ITEC 007-03_ING
12	<i>Preliminary study of the acute toxicity of the fibrinogen component of fibrin-glue Grifols by intravenous route in rat</i>	IG_CIFA-ITEC 015-02_ING
13	<i>Study of the acute toxicity of the fibrinogen double inactivated (FG-DI) component of fibrin-glue Grifols by intravenous route in rat</i>	IG_CIFA-ITEC 008-03_ING
14	<i>Study of the acute toxicity of the fibrinogen component of fibrin-glue Grifols by intravenous route in rat</i>	IG_CIFA-ITEC 019-02_ING

Note: All listed studies are summarized in this review memo under ‘Overview of Toxicology Studies.’ The utility of these data for evaluating the safety of Fibrin Sealant (Human) is limited because the clinical ROA was not used (the IV ROA is contraindicated in the label) and the final product was not tested. Even in a worst-case scenario in which inadvertent systemic exposure occurs, the fibrinogen component is unlikely to be administered alone.

Developmental and Reproductive Toxicology Studies:

Per the applicant, studies were not conducted to evaluate this safety endpoint because the product is composed of human plasma proteins. As Fibrin Sealant (Human) is administered via drip or spray application onto the wound site, systemic exposure or distribution to other organs or tissues is not expected.

Genotoxicity Studies:

Per the applicant, studies were not conducted to evaluate this safety endpoint because the product is composed of human plasma proteins. Direct interaction between peptides/proteins and deoxyribonucleic acid (DNA) or other chromosomal material is not expected.

Carcinogenicity/Tumorigenicity Studies:

Per the applicant, studies in animals were not conducted to evaluate this safety endpoint because the product is composed of human plasma proteins.

Other Safety/Toxicology Studies

No other safety/toxicology studies were conducted.

Overview of Toxicology Studies

Study #9

Report Number		IG_CIFA-ITEC 016-02_ING
Date Report Signed		21-Oct-2010
Title		<i>Preliminary study of the acute toxicity of the fibrinogen component of fibrin-glue Grifols by intravenous route in mouse</i>
GLP Status		Yes; compliant with (b) (4) of July 19
Testing Facility		(b) (4)
Objective(s)		To perform an initial evaluation of the toxicity of the fibrinogen component of Fibrin Sealant (Human) following single-dose IV administration in mice
Study Animals	Strain/Breed	(b) (4)
	Species	Mouse
	Age	8 weeks old
	BW	Males: 32.2-36.2 g; Females: 29.2-30.2 g at the beginning of the study
	#/sex/group	2
Total #		4
Test Article(s)		Fibrinogen component of Fibrin Sealant (Human) (fibrinogen lot #219890)
Control Article(s)		None
ROA		IV bolus
Description of the Disease/Injury Model and Implant Procedure		One female was dosed on day 0 via IV bolus into the tail vein using a maximum volume of 1 mL/100 g BW. Subsequent animals were dosed on day 2 (one female and one male) and day 4 (one male).
Study Groups and Dose Levels		Group 1 – 360 mg/kg
Dosing Regimen		Single
Randomization		No
Description of Masking		N/A
Scheduled Sacrifice Time Points		14 days post-administration
Study Parameters	Mortality	Assessed daily
	Clinical examinations	Assessed by the Irwin test at 30 minutes, 60 minutes, 2 hours, 4 hours, and 8 hours post-administration, then daily
	BW	Assessed weekly
	Macroscopic pathology	Assessed post-mortem
	Organ weights	Assessed post-mortem for the spleen, heart, liver, kidneys, and testicles/ ovaries

Key Results:

- There were no unscheduled deaths or abnormal clinical examinations, macroscopic pathology, or organ weight findings.
- A slight decrease in BW (approximately 0.9% loss) was observed in one female between the first and second weeks post-administration. Given the small number of animals and the small magnitude of weight loss, this finding may be attributed to individual variation.

Comment:

- Clinical pathology and histopathology were not conducted for this study.

Study #10

Report Number		IG_CIFA-ITEC 018-02_ING
Date Report Signed		29-Apr-2015
Title		<i>Study of the acute toxicity of the fibrinogen component of fibrin-glue Grifols by intravenous route in mouse</i>
GLP Status		Yes; compliant with (b) (4) of July 19
Testing Facility		(b) (4)
Objective(s)		To evaluate the toxicity of the fibrinogen component of Fibrin Sealant (Human) following single-dose IV administration in mice
Study Animals	Strain/Breed	(b) (4)
	Species	Mouse
	Age	8 weeks old
	BW	Males: 36.1 ± 1.9 g (range: 34.0-38.0 g) at the beginning of the study Females: 30.6 ± 2.2 g (range: 29.4-33.8 g) at the beginning of the study
	#/sex/group	5
	Total #	10
Test Article(s)		Fibrinogen component of Fibrin Sealant (Human) (fibrinogen lot #220090) Note: This lot was manufactured using a different purification process (<i>i.e.</i> , different nanofiltration system) compared to the final product.
Control Article(s)		None
ROA		IV bolus
Description of the Disease/Injury Model and Implant Procedure		One female and one male were dosed on day 0 via IV bolus into the tail vein using a maximum volume of 1 mL/100 g BW. Subsequent animals were dosed on day 2 (two females and two males) and day 4 (two females and two males).
Study Groups and Dose Levels		Group 1 – 360 mg/kg
Dosing Regimen		Single
Randomization		No
Description of Masking		N/A
Scheduled Sacrifice Time Points		14 days post-administration
Study Parameters	Mortality	Assessed daily
	Clinical examinations	Assessed by the Irwin test at 30 minutes, 60 minutes, 2 hours, 4 hours, and 8 hours post-administration, then daily
	BW	Assessed weekly
	Macroscopic pathology	Assessed post-mortem
	Organ weights	Assessed post-mortem for the spleen, heart, liver, kidneys, and testicles/ovaries

Key Results:

- No unscheduled deaths or abnormal clinical examinations, macroscopic pathology, or organ weight findings were reported.
- Slight decreases in BW were observed in three animals (one male [0.8% loss] and two females [0.3% and 1.9% loss]) during the first week post-administration and in two animals (one male [1.3% loss] and one female [2.6% loss]) between the first and second weeks post-administration. Given the small number of animals, the low magnitude of weight loss, and the fact that the losses recorded at the earlier time point did not persist to the later time point, this finding may be attributed to individual variation.

Comment:

- Clinical pathology and histopathology were not conducted for this study.

Study #11

Report Number		IG_CIFA-ITEC 007-03_ING
Date Report Signed		30-Apr-2015
Title		<i>Study of the acute toxicity of the fibrinogen double inactivated (FG-DI) component of fibrin-glue Grifols by intravenous route in mouse</i>
GLP Status		Yes; compliant with (b) (4) of July 19
Testing Facility		(b) (4)
Objective(s)		To evaluate the safety of the fibrinogen component of Fibrin Sealant (Human) following single-dose IV administration in mice
Study Animals	Strain/Breed	(b) (4)
	Species	Mouse
	Age	8 weeks old
	BW	Males: 37.0 ± 1.7 g (range: 34.7-38.8 g) at the beginning of the study Females: 28.2 ± 0.7 g (range: 27.3-28.8 g) at the beginning of the study
	#/sex/group	5
	Total #	10
Test Article(s)		Fibrinogen component of Fibrin Sealant (Human) (fibrinogen lot #307990)
Control Article(s)		None
ROA		IV bolus
Description of the Disease/Injury Model and Implant Procedure		One animal per sex was dosed on day 0 via IV bolus into the tail vein using a maximum volume of 1 mL/100 g BW. Subsequent animals were dosed on day 2 (one animal per sex) and day 3 (three animals per sex).
Study Groups and Dose Levels		Group 1 – 360 mg/kg
Dosing Regimen		Single
Randomization		No
Description of Masking		N/A
Scheduled Sacrifice Time Points		14 days post-administration
Study Parameters	Mortality	Assessed daily
	Clinical examinations	Assessed by the Irwin test at 30 minutes, 60 minutes, 2 hours, 4 hours, and 8 hours post-administration, then daily
	BW	Assessed weekly
	Macroscopic pathology	Assessed post-mortem
	Organ weights	Assessed post-mortem for the spleen, heart, liver, kidneys, and testicles/ovaries

Key Results:

- Two females experienced slight to moderate decreases in BW during the study, where one female showed a 1.1% loss during the first week post-administration and another displayed a 7.7% loss between the first and second weeks post-administration. There were no unscheduled deaths, abnormal clinical observations, or signs of toxicity noted in the post-mortem parameters; consequently, this finding may be attributed to individual variation.

Comment:

- Clinical pathology and histopathology were not conducted in this study.

Study #12

Report Number		IG_CIFA-ITEC 015-02_ING
Date Report Signed		21-Oct-2010
Title		<i>Preliminary study of the acute toxicity of the fibrinogen component of fibrin-glue Grifols by intravenous route in rat</i>
GLP Status		Yes; compliant with (b) (4) of July 19
Testing Facility		(b) (4)
Objective(s)		To conduct a preliminary study on the safety of the fibrinogen component of Fibrin Sealant (Human) following single-dose IV administration in rats
Study Animals	Strain/Breed	(b) (4)
	Species	Rat
	Age	8 weeks old
	BW	Males: range = 204.3-211.4 g at the beginning of the study Females: range = 156.8-161.6 g at the beginning of the study
	#/sex/group	2
Total #		4
Test Article(s)		Fibrinogen component of Fibrin Sealant (Human) (fibrinogen lot #219890)
Control Article(s)		None
Route of Administration		IV bolus
Description of the Disease/Injury Model and Implant Procedure		One animal per sex was dosed on day 0 via IV bolus into the tail vein using a maximum volume of 0.5 mL/100 g BW. The remaining two animals were dosed on day 2 following a 48-hour staggering period.
Study Groups and Dose Levels		Group 1 – 360 mg/kg
Dosing Regimen		Single
Randomization		No
Description of Masking		N/A
Scheduled Sacrifice Time Points		14 days post-administration
Study Parameters	Mortality	Assessed daily
	Clinical examinations	Assessed by the Irwin test at 30 minutes, 60 minutes, 2 hours, 4 hours, and 8 hours post-administration, then daily
	BW	Assessed weekly
	Macroscopic pathology	Assessed post-mortem
	Organ weights	Assessed post-mortem for the spleen, heart, liver, kidneys, and testicles/ovaries

Key Result:

- No abnormalities were observed in any of the study parameters.

Comment:

- Clinical pathology and histopathology were not conducted in this study.

Study #13

Report Number		IG_CIFA-ITEC 019-02_ING
Date Report Signed		29-Apr-2015
Title		<i>Study of the acute toxicity of the fibrinogen component of fibrin-glue Grifols by intravenous route in rat</i>
GLP Status		Yes; compliant with (b) (4) of July 19
Testing Facility		(b) (4)
Objective(s)		To evaluate the safety of the fibrinogen component of Fibrin Sealant (Human) following IV administration in rats
Study Animals	Strain/Breed	(b) (4)
	Species	Rat
	Age	8 weeks old
	BW	Males: 214.7 ± 9.0 g (range: 199.8-222.0 g) at the beginning of the study Females: 155.2 ± 5.4 g (range: 149.5-162.2 g) at the beginning of the study
	#/sex/group	5
	Total #	10
Test Article(s)		Fibrinogen component of Fibrin Sealant (Human) (fibrinogen lot #220090) Note: This lot was manufactured using a different purification process (<i>i.e.</i> , different nanofiltration system) compared to the final product.
Control Article(s)		None
ROA		IV bolus
Description of the Disease/Injury Model and Implant Procedure		One animal per sex was dosed on day 0 via IV bolus into the tail vein using a maximum volume of 0.5 mL/100 g BW. Subsequent animals were dosed on day 2 (two animals per sex) and day 3 (two animals per sex).
Study Groups and Dose Levels		Group 1 – 360 mg/kg
Dosing Regimen		Single
Randomization		No
Description of Masking		N/A
Scheduled Sacrifice Time Points		14 days post-administration
Study Parameters	Mortality	Assessed daily
	Clinical examinations	Assessed by the Irwin test at 30 minutes, 60 minutes, 2 hours, 4 hours, and 8 hours post-administration, then daily
	BW	Assessed weekly
	Macroscopic pathology	Assessed post-mortem
	Organ weights	Assessed post-mortem for the spleen, heart, liver, kidneys, and testicles/ ovaries
	Histopathology	Additional histopathology was performed using sections taken from the liver and kidney of one animal

Key Results:

- There were no unscheduled deaths or abnormalities reported in the clinical observations and organ weights.
- One male experienced a slight decrease in BW (2.5% loss) between the first and second weeks post-administration. No other signs of toxicity were noted for this animal, so this finding may be attributed to individual variation.
- Macroscopic pathology revealed a group of white points (approximately 1 x 0.25 cm) on the edge of one of the hepatic lobes in one male. Histopathological results for this animal showed the presence of multiple dystrophic calcifications. These focal lesions were surrounded by giant multinucleated cells with osteoclastic activity and inflammatory cells (lymphocytes and mastocytes). In these regions, there was a disappearance of the liver's normal architecture with accompanying fibrosis, diffuse inflammation, a loss of hepatocytes, and biliary duct hyperplasia. Although this finding may be indicative of an ischemic event, this outcome is not likely due to the absence of renal lesions in the histopathology results. The report states that the cause of the hepatic lesions may be attributed to a parasite or a neonatal umbilical infection.

Comment:

- The report states that blood samples were collected from 8/10 animals for potential analysis of liver function, but these samples were not tested.

Study #14

Report Number		IG_CIFA-ITEC 008-03_ING
Date Report Signed		29-Apr-2015
Title		<i>Study of the acute toxicity of the fibrinogen double inactivated (FG-DI) component of fibrin-glue Grifols by intravenous route in rat</i>
GLP Status		Yes; compliant with (b) (4) of July 19
Testing Facility		(b) (4)
Objective(s)		To evaluate the safety of the fibrinogen component of Fibrin Sealant (Human) following IV administration in rats
Study Animals	Strain/Breed	(b) (4)
	Species	Rat
	Age	8 weeks old
	BW	Males: 205.4 ± 5.7 g (range: 198.6-211.7 g) at the beginning of the study Females: 149.2 ± 2.4 g (range: 147.4-152.5 g) at the beginning of the study
	#/sex/group	5
	Total #	10
Test Article(s)		Fibrinogen component of Fibrin Sealant (Human) (fibrinogen lot #307990)
Control Article(s)		None
ROA		IV bolus
Description of the Disease/Injury Model and Implant Procedure		One animal per sex was dosed on day 0 via IV bolus into the tail vein using a maximum volume of 0.5 mL/100 g BW. Subsequent animals were dosed on day 2 (two animals per sex) and day 3 (two animals per sex).
Study Groups and Dose Levels		Group 1 – 360 mg/kg
Dosing Regimen		Single
Randomization		No
Description of Masking		N/A
Scheduled Sacrifice Time Points		14 days post-administration
Study Parameters	Mortality	Assessed daily
	Clinical examinations	Assessed by the Irwin test at 30 minutes, 60 minutes, 2 hours, 4 hours, and 8 hours post-administration, then daily
	BW	Assessed weekly
	Macroscopic pathology	Assessed post-mortem
	Organ weights	Assessed post-mortem for the spleen, heart, liver, kidneys, and testicles/ ovaries
Histopathology		Additional histopathology was performed using sections taken from the liver and kidney of one animal

Key Results:

- There were no unscheduled deaths or abnormalities reported in the clinical observations.
- One male showed decreased size and weight in one testicle located in the abdominal cavity. Histopathological analysis of this testicle revealed germinal epithelium atrophy, a lack of spermatogenesis, a slight increase in the number of Leydig cells, and areas of slight fibrosis. This finding was attributed to a congenital cryptorchidism defect.

Comment:

- Clinical pathology was not conducted in this study.

APPLICANT'S PROPOSED LABEL

Section 8 ('Use in Specific Populations') was revised to comply with 21 CFR 201.56(d)(1), 201.57(c)(9), and 201.57(c)(14).

Section 13.2 ('Animal Toxicology and/or Pharmacology') was removed because the nonclinical toxicology studies did not contribute information necessary for the safe and effective use of the final product that was not incorporated in other sections of the labeling.

CONCLUSION OF NONCLINICAL STUDIES

Review of the nonclinical studies did not identify any safety findings. The nonclinical data support approval of the license application.

KEY WORDS/TERMS

Fibrin Sealant (Human), fibrin sealant, fibrinogen, thrombin, hemostasis,