

Original Article

Safety and efficacy of sFilm-FS, a novel biodegradable fibrin sealant, in Göttingen minipigs

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Abstract: Bleeding during surgical procedures is a common complication. Therefore, hemostatic agents have been developed to control bleeding, and fibrin sealants have several benefits. sFilm-FS is a novel fibrin sealant that comprises a biodegradable co-polymeric film embedded with human fibrinogen and thrombin. Herein, the safety and efficacy of sFilm-FS were compared using a liver and spleen puncture model of Göttingen minipigs with those of the standard hemostatic techniques (control animals) and EVARREST[®], a reference fibrin sealant. Hemostasis and reduced blood loss were more effectively achieved with sFilm-FS than with the standard techniques in the control animals and comparable to those achieved with EVARREST[®]. No treatment-related adverse effects were observed in any of the groups. Histopathological evaluation indicated that sFilm-FS was slightly and moderately reactive at the liver puncture site and spleen, respectively, compared with the standard techniques in the control animals. These changes are expected degradation reactions of the co-polymeric film and are not considered as adverse events. No treatment-related abnormalities were noted in the other evaluated organs. Additionally, no evidence of local or systemic thromboses was noted. These results support the use of sFilm-FS for hemostasis in humans. (DOI: 10.1293/tox.2021-0030; J Toxicol Pathol 2021; 34: 319–330)

Key words: biodegradability, local tolerability, biodegradable materials, hemostasis, bleeding, animal models

Introduction

Surgical bleeding is a common complication in almost all forms of surgeries, and prompt bleeding control and appropriate hemostasis are vital for successful operation and to prevent complications, such as hematomas or excessive bleeding that can endanger the patient's life^{1–3}. Surgical bleeding can cause higher infection rates and ventilator use and prolong hospitalization and operative time^{4–6} causing considerable clinical and financial burdens^{4, 7, 8}.

The standard techniques of bleeding control include mechanical compression, suturing, and electrocautery;

however, recently, new adjunctive hemostatic agents have been introduced. These include sponge products, non-active oxidized cellulose pads, infrared-sapphire coagulation, and polyvinyl alcohol sponge^{1, 9}. One of the more advanced hemostatic agents is the active fibrin sealant, which has been recently introduced and is commercially available. It provides improved hemostasis and wound repair and enables sealing of wounded surfaces and tissues^{1, 10–13}. Such sealants possess several beneficial properties, namely quick activation, natural clotting process simulation, favorable safety profile, and reabsorbability^{3, 5, 14}. Fibrin sealants have demonstrated better results than the conventional techniques for reducing intraoperative blood loss^{5, 13, 15–17}.

Fibrin clot formation is a major component of hemostasis, and fibrinogen and thrombin are critical for this process. Additionally, thrombin participates in many additional hemostasis-related processes, including vasoconstriction, fibrin stabilization, platelet activation, and fibrinolysis control^{18–20}. Thrombin and fibrinogen can be combined in a dry form without initiating the clotting cascade, and when they are constituted on films, they can be used on bleeding sites. Upon hydration, a fibrin clot is formed that adheres the film to the tissue, providing both better hemostasis and mechani-

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cal strength to the injured tissue^{18, 21}.

sFilm-FS (Sealantium Medical, Rosh Ha'AYin, Israel) is a novel sealing and hemostatic device for the effective control of surgical bleeding and leakage despite the reduced amounts of both fibrinogen and thrombin (i.e., less than 1 mg/cm² and 1 IU/cm² of human fibrinogen and thrombin, respectively) compared with the reference fibrin sealant—EVARREST® (Omrix Biopharmaceuticals, Ltd., Ness-Ziona, Israel).

sFilm-FS is a bioabsorbable patch that consists of a copolymeric film (PECALA co-polymer, device constituent), embedded with a mixture of lyophilized human fibrinogen and thrombin powders (biological constituents) and calcium chloride. The PECALA co-polymeric film (device constituent) is a novel co-polymer containing polyethylene glycol (PEG), polycaprolactone (PCL), and polylactic acid (PLA).

On application to the injured tissue, hydration promotes an instant reaction between fibrinogen and thrombin, forming a fibrin clot that causes the film to adhere to the injured tissue, providing a sealant action in addition to hemostasis.

The PECALA co-polymeric film was tested for biocompatibility and biodegradation according to the International Organization for Standardization (ISO) 10993 standards and has passed the tests for cytotoxicity, irritation, sensitization, acute systemic toxicity, genotoxicity (Ames and mouse lymphoma assays), and hemolysis. The local tissue response was assessed following subcutaneous implantation of either the PECALA co-polymeric film or sFilm-FS in Sprague Dawley (SD) rats. Fragments of sFilm-FS were noted within the tissue envelope, and a steady-state tissue response was attained after 8 weeks of implantation. Furthermore, the safety and efficacy of sFilm-FS were evaluated using a liver and spleen puncture model of female domestic pigs over short and long durations of 1 and 3 months, respectively, after implantation. No remnants of sFilm-FS were noted at the implantation sites at 3 months after implantation.

The use of biodegradable materials is expanding throughout different medical fields, and they have also been used for hemostasis in different medical settings²². Biodegradable materials must be appropriately evaluated in pre-clinical studies; therefore, understanding the expected clinical and pathological outcomes from such studies is crucial. Although information on the expected pathological findings with the use of biodegradable materials in appropriate animal models is expanding^{23–28}, information on biodegradable materials for hemostasis is still limited.

This study aimed to compare the safety and efficacy of the application of sFilm-FS in puncture lesions in the liver and spleen of female Göttingen minipigs with those in the animals treated with standard hemostatic techniques (local mechanical pressure and cauterization) (control) and a reference fibrin sealant (EVARREST, Omrix Biopharmaceuticals, Ltd.). The liver or spleen puncture model was selected because it mimics the probable surgical bleeding complications due to iatrogenic injury or incomplete hemostasis (i.e., bleeding from a site of previous surgical repair, hemostasis,

or injuries overlooked during the primary operation). Like sFilm-FS, EVARREST is a bi-component device, which is currently in use for surgical hemostasis, with a polymeric backbone containing a fibrin sealant (8.6 mg/cm² and 37.5 IU/cm² of human fibrinogen and thrombin, respectively)²⁹.

Materials and Methods

Animal husbandry and maintenance

Fifteen female Göttingen minipigs, approximately 5–6 months of age, were obtained from Ellegaard Göttingen Minipigs A/S. Animals were housed and treated within a limited-access large animal unit (Envigo CRS Israel Ltd, Ness-Ziona, Israel) with concrete floor holding pens covered with wood shavings. For the duration of the study, the animals were provided a commercial diet and allowed free access to chlorinated and acidified drinking water. Temperature and relative humidity were maintained at 20–24 °C and 42–70%, respectively. A 12-hour light-dark cycle was maintained using an automatic timer. The study was performed in compliance with the Good Laboratory Practice standards and was approved by the National Council for Animal Experimentation (No. IL-20-2-74 and IL-20-6-262) following an application-form review, which ascertained the study's compliance with the rules and regulations set forth. Animals were handled according to the guidelines of the National Institutes of Health and the Israeli Council for Experiments on Animals.

Experimental design and surgical procedure

The animals were equally divided into three groups (n=5 females/group): the test item (sFilm-FS), reference item (EVARREST), and control groups (Table 1). Only female pigs were selected for this study as a prior subcutaneous implantation study conducted in both male and female SD rats evidenced no toxic effects. Given the same response to sFilm-FS implantation between male and female rats and animal welfare considerations, this study used only female pigs; additionally, well-designed large animal studies using a clinically relevant route of exposure have been able to offer safety data of equivalent or superior quality without requiring identical cohorts in number or sex³⁰. After appropriate administration of analgesia (subcutaneous buprenorphine), sedation (intramuscular ketamine and xylazine), and anesthesia (1–3% isoflurane administered via intermittent positive-pressure ventilation utilizing 100% O₂ at a rate of 2–4 L/min), a midline incision was established in the cranial abdominal area. The left lateral liver lobe and spleen were exteriorized, and a round incision puncture, induced by a 12-mm biopsy punch (in diameter) at a depth of 8–9 mm, was established at the center of the liver lobe or spleen (single puncture/organ). EVARREST and sFilm-FS patches (5×5 cm/patch/puncture site) were immediately applied following the punctures, covering the entire incision, in the animals from groups 2 and 3, respectively. The patches were substantially larger than the incision sites, as the main objective of this study was the assessment of safety. To deter-

Table 1. Experimental Design

Group no.	Group size	Puncturing (Bleeding induction)	Treatment (Hemostatic technique)		Scheduled termination post-surgical procedure
			Applied material	Procedure	
1	n=5	Incisional round punctures, induced by 12-mm biopsy punch to a depth of 8–9 mm, created in the liver and spleen	Not applicable (Control)	Standard hemostatic techniques by applying local mechanical pressure and cauterization	18 weeks
2	n=5		EVARREST® (Reference item)	Application of a 5×5 cm patch onto each of the induced incisions immediately following puncturing, followed by manual compression for 2–3 minutes	
3	n=5	(single puncture/organ)	sFilm-FS (Test item)		

mine a systemically viable dose of fibrinogen and thrombin in humans, patches of equal size intended for application in patients were used in this experiment. In the control group, hemostasis was achieved by the standard techniques: local application of mechanical pressure with gauze pads and conventional cauterization (36 watts using a cautery pen), performed immediately after puncturing.

Intraoperative measurements

The amount of blood loss was determined from the difference in the weights of pre-weighed dry gauze pads and blood-soaked gauze pads after model induction. Any volume of the physiological saline used during the surgical procedure was determined and subtracted from the weight of the blood-stained gauze pads, considering that the weight of 1 mL physiological saline is 1 g. The weight difference was expressed as blood loss (g) for each puncture site.

The time to hemostasis (TTH) was measured for each of the puncture sites as the time from the start of the bleeding (and patch application) until complete hemostasis. According to the manufacturers' usage instructions, a saline-moistened gauze pad was manually compressed on the sFilm-FS patch continuously for 2 minutes, while 3 minutes of continuous manual compression was required in the case of EVARREST. Each puncture site was monitored for at least 10 min to ensure complete hemostasis.

Examinations during observation period

Animals were observed for 18 weeks. Both systemic and local reactions in all animals were clinically examined on the day of the surgery and once daily for the postoperative first week. Thereafter, the animals were observed weekly. Viability checks were performed for all animals once daily throughout the observation period and included evaluation of food intake and general animal welfare. Individual body weights of all animals were determined at the time of their obtainment and during the quarantine and acclimation period. Body weights were further determined on the day of surgery, weekly once thereafter, and finally prior to necropsy.

Blood samples were collected for clinical pathology from the animals at baseline (prior to the day of surgery); postoperative day 1 and weeks 1, 2, 4, and 12; and, finally, after the 18-week observation period. Blood samples for

pro-inflammatory cytokines (i.e., interleukin 6 [IL-6], IL-1 β , IL-8, and TNF- α) and acute phase protein levels (i.e., pig major acute phase [pig-MAP] protein, haptoglobin [HP], and C-reactive protein [CRP]) were collected from all the animals at baseline (prior to the day of surgery); postoperative day 1 and weeks 2, 4, and 12; and, finally, at the end of the 18-week observation period.

Terminal investigations

Necropsy and macroscopic examination

A full detailed necropsy and gross pathological examination was performed for all the animals following the scheduled termination. Any adverse reaction at the puncture sites in terms of the presence of hemorrhage and adhesions (i.e., adhesion to the body wall or adhesion to adjacent tissues or organs, mainly the diaphragm) was specifically considered. Adhesion surface area (adhesion area/puncture area) at the puncture sites was scored from 0 to 4 according to the following scoring system³¹: 0 = no adhesion, 1 = \leq 25% of initial puncturing area, 2 = 26–50% of initial puncturing area, 3 = 51–75% of initial puncturing area, and 4 = 76–100% of initial puncturing area. Adhesion quality and strength at the puncture and unrelated sites were scored (0–3) based on the following scoring system³²: 0 = no adhesion; 1 = film adhesion, blunt dissection; 2 = strong adhesion, sharp dissection; and 3 = very strong vascularized adhesion, sharp dissection, damage hardly preventable (adhesiolysis by traction causes organ damage). The peritoneal adhesion index (PAI) was calculated for the adhesion quality and strength. PAI is the sum of the raw scores of all the abdominal regions (a total score for the entire animal)³². Organs or tissues collected during necropsy were fixed using a 4% formaldehyde solution. The puncture sites were collected with sufficient uninjured tissue margins following photography.

Histological processing and histopathological evaluation

A histopathological evaluation was conducted by a board-certified toxicologic pathologist. No peer review was conducted. Histopathological examinations were confined to the puncture sites (i.e., a single sample of liver lobe and a single sample of spleen), brain, liver, kidney (single), spleen, lungs, heart, mesenteric lymph nodes, thymus, and femur. Puncture sites were trimmed in the middle (includ-

ing the uninjured tissue margins), processed (xylene-free), embedded in paraffin blocks, and stained with hematoxylin and eosin (H&E) for histopathological evaluation. All other organs/tissues were embedded in paraffin, sectioned, and stained with H&E.

Evaluation of puncture sites

The histopathological evaluation was based on the scoring system presented in ISO 10993-6 Annex E³³. Inflammatory cellular responses and tissue responses were scored based on a semiquantitative scale (0-4) (Tables 2 and 3). Each puncture site was assigned a total reactivity grade, calculated as follows: Average score for group = [Inflammatory cell infiltrates and necrosis parameters scores × 2 + neovascularization, fibrosis, and fatty infiltration parameters scores] / number of animals in the group. The average score for the control treatment was subtracted from the test/reference treatment average to determine the reactivity grade: 0–2.9, minimal or no reaction; 3–8.9, slight reaction; 9–15, moderate reaction; and >15.1, severe reaction. The average score for the control group was subtracted from the average of the EVARREST and sFilm-FS groups and the control group average itself, thus the reactivity score for the control group was equal to 0.

Statistical analysis

Calculations were performed using the meanSDRelative_01.2.Rnw (validated R-Script for calculations of group mean and standard deviation, Version 2) and Microsoft Excel 365. Statistical analysis was performed using MultiComp.Rnw (validated R-Script for statistical evaluation between multiple groups and/or multiple parameters between two groups). Prior to the application of the appropriate statistical method, a normality test was performed considering Gaussian distribution (Shapiro–Wilk normality test; $p < 0.01$). If the normality test was passed by all the groups, an equal-variance test was performed (*e.g.*, Bartlett test; $p < 0.01$); if the Bartlett test was passed, one-way ANOVA with Dunnett's post-test was performed, and if the test was not passed, the Kruskal–Wallis with Mann–Whitney U tests were performed. If all groups did not clear the normality tests, the Kruskal–Wallis and Mann–Whitney U tests were performed.

Results

Mortality, clinical signs, and body and organ weights

No deaths had occurred in the groups during the study period, and no adverse clinical signs were attributed to the test or reference items. At the end of the observation period, an increase in the body weight compared with that on the day of surgery was noted for all experimental groups, and

Table 2. Histological Evaluation Scoring^a - Cell Type/Response

Cell type / Response	Score				
	0	1	2	3	4
Polymorphonuclear	0				
Lymphocytes	0	Rare, 1–5/phf	5–10/phf	Heavy infiltrate	Packed
Plasma Cells	0				
Macrophages	0				
Giant Cells	0	Rare, 1–2/phf	3–5/phf		Sheets
Necrosis	0	Minimal	Mild	Moderate	Severe

phf: per high-powered (400×) field.

^aExample of scoring system presented in the ISO 10993-6 Annex E.

Table 3. Histological Evaluation Scoring^a – Tissue Response

Response	Score				
	0	1	2	3	4
Neovascularisation	0	Minimal capillary proliferation, focal, 1 to 3 buds	Groups of 4 to 7 capillaries with supporting fibroblastic structures	Broad band of capillaries with supporting fibroblastic structures	Extensive band of capillaries with supporting fibroblastic structures
Fibrosis	0	Narrow band	Moderately thick band	Thick band	Extensive band
Fatty infiltrate	0	Minimal amount of fat associated with fibrosis	Several layers of fat and fibrosis	Elongated and broad accumulation of fat cells about the incision site	Extensive fat completely surrounding the incision site
Foreign debris	0	Minimal	Mild	Moderate	Severe

^aExample of scoring system presented in the ISO 10993-6 Annex E.

differences during the entire observation period between the groups were nonsignificant. No treatment-related changes were noted in the organ weight or organ weight to body weight ratio values between the groups.

Blood loss

The mean blood loss was significantly lower in the sFilm-FS-treated group compared with that in the control group for both liver lobe (5.5 g vs. 33.6 g, respectively, $p < 0.05$) and spleen (6.6 g vs. 98.3 g, respectively, $p < 0.01$) puncture sites. Similarly, the EVARREST group had significantly lower mean blood loss ($p < 0.01$) compared to that in the control group. Differences between the sFilm-FS and EVARREST groups were not significant (Table 4).

Time to hemostasis

The TTH values in the sFilm-FS-treated group were significantly lower than those in the control in both the liver lobe (02:33 vs. 17:19, respectively, $p < 0.01$) and spleen (04:23 vs. 41:04, respectively, $p < 0.01$) puncture sites. Similarly, the EVARREST group had significantly lower mean blood loss ($p < 0.05$ for the spleen and $p < 0.01$ for the liver) than did the control group. Additionally, the mean TTH value ($p < 0.05$) was significantly lower in the sFilm-FS-treated group than that in the EVARREST group (02:33 vs. 03:30, respectively) following puncturing of the liver lobe (Table 4).

Clinical pathology and pro-inflammatory cytokine and acute phase protein levels

Application of sFilm-FS or EVARREST did not alter the hematology, biochemistry, or coagulation parameters. A few changes in the clinical pathology parameters were evident on the first postoperative day, which were attributable to the surgical procedure or increased blood loss. A slight decrease in the red blood cell count and hemoglobin, and hematocrit levels was noted only in the control group. These values were within the baseline range or slightly lower than the minimal value of the range. A slight prolongation of the prothrombin time compared with that at the baseline levels was evident in all groups. A slight increase in the white blood cell count above the baseline levels accompanied by

an increase in neutrophils and a decrease in lymphocyte levels was noted in the EVARREST and control groups. In the sFilm-FS group, only an increase in the neutrophils and a decrease in the lymphocytes were observed. Increased alkaline phosphatase values above baseline levels were noted in all groups, which may be stress-related. An increase in the creatine phosphokinase level above the baseline was noted for all groups that may reflect muscle damage due to surgery.

None of the treatments influenced pro-inflammatory cytokines (IL-6, IL-1 β , IL-8, and TNF- α) or acute phase proteins (pig-MAP, HP, and CRP), tested in the porcine serum over postoperative 18 weeks. None of the treatments resulted in signs of inflammation.

Macroscopic findings

All macroscopic findings observed during necropsy were confined to the liver and spleen (Figs. 1 and 2). Adhesions of these organs to the body wall and diaphragm, including or excluding the puncture site, were observed in almost all animals from all experimental groups. No remnants of sFilm-FS were noted at the puncture sites.

A granular whitish tissue, covering both spleen and liver lobe puncture sites, was observed in all the animals from the sFilm-FS-treated group, and the liver and spleen puncture sites of all the animals from the EVARREST[®] group were covered by a smooth whitish tissue. In the control group, the spleen puncture site was identified as a round indented area in three of the five animals, whereas in the other animals, the puncture site was not visible (covered by adhesion to the body wall). The puncture site in the liver lobe was not apparent in four of the five animals (covered by adhesion to the diaphragm), whereas the puncture site was identified as a round indented area in only one of the animals.

Adhesion scores

Differences in the mean scores of the adhesion surface area and adhesion quality and strength at any of the puncture sites of the sFilm-FS or EVARREST[®] groups were nonsignificant compared with those of the control group, al-

Table 4. Blood Loss and Time to Hemostasis (TTH)

Group no. & Sex	Group size	Treatment		Blood loss (g) on surgery day		TTH (mm:ss) on surgery day	
				Liver lobe	Spleen	Liver lobe	Spleen
1F	n=5	Control	Mean	33.6	98.3	17:19	41:04:00
			\pm SD	(n=5) 28.7	(n=5) 83.97	(n=5) 10:16	(n=5) 27:24:00
2F	n=5	EVARREST [®] (Reference item)	Mean	3.7 $\blacklozenge\downarrow$	6.1 $\blacklozenge\downarrow$	03:30 $\blacklozenge\downarrow$	03:50 $\blacklozenge\downarrow$
			\pm SD	(n=5) 2.34	(n=5) 1.47	(n=5) 0:29	(n=5) 0:37
3F	n=5	sFilm-FS (Test item)	Mean	5.5 $\blacklozenge\downarrow$	6.6 $\blacklozenge\downarrow$	02:33 $\blacklozenge\downarrow$	04:23 $\blacklozenge\downarrow$
			\pm SD	(n=5) 2.38	(n=5) 2.26	(n=5) 0:28	(n=5) 2:42

$\blacklozenge\downarrow$ p 0.05 vs. Control (Kruskal-Wallis, Mann-Whitney U test).
 $\blacklozenge\blacklozenge\downarrow$ p<0.01 vs. Control (Kruskal-Wallis, Mann-Whitney U test).

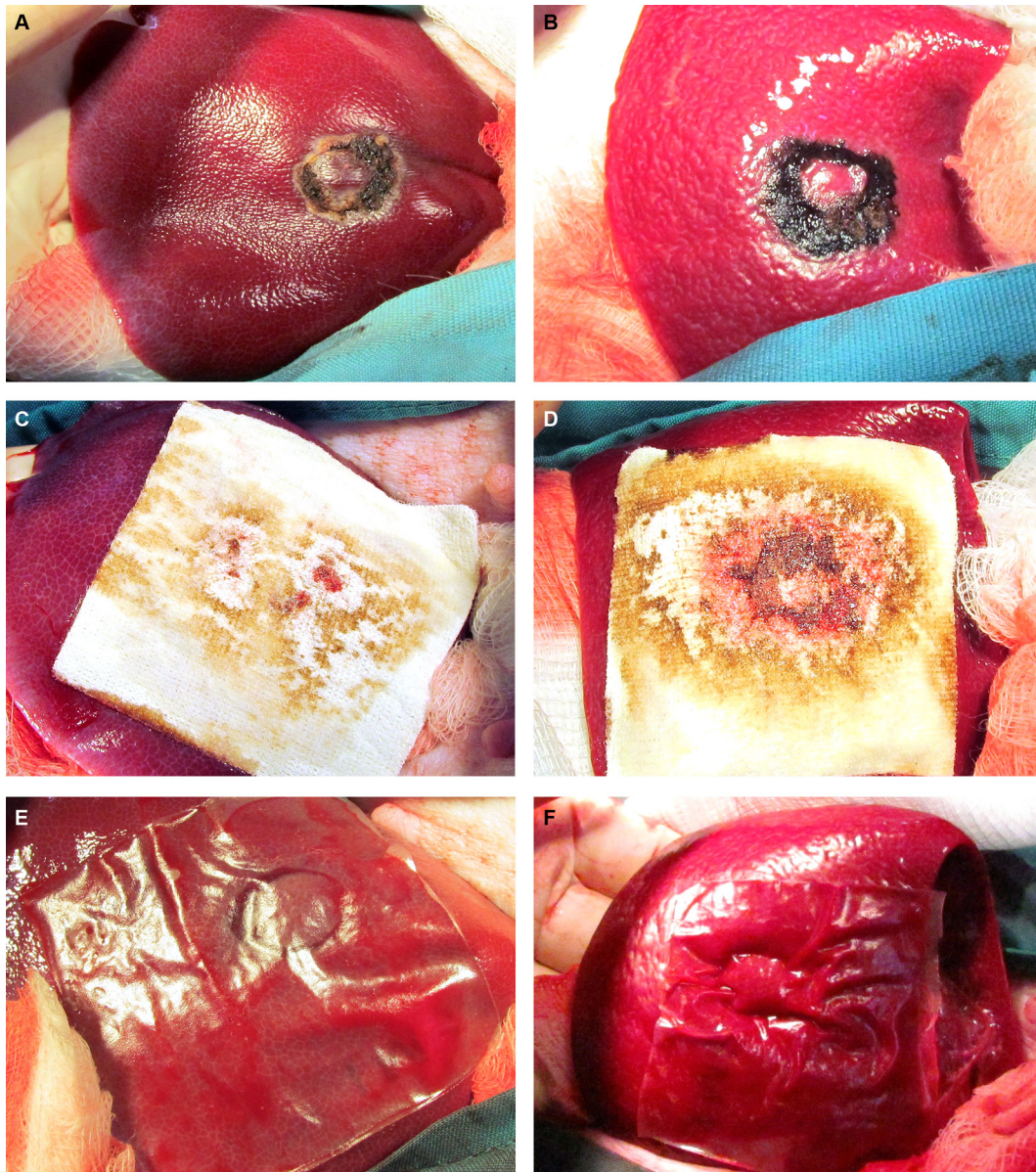


Fig. 1. Photographs of the puncture sites on day 0.

Representative photographs of the puncture site taken on day 0 (treatment day) from the liver and spleen of the control group (A and B, respectively), from the EVARREST-treated group (C and D, respectively), and sFilm-FS-treated group (E and F, respectively).

though the mean scores in the control group were the lowest (Table 5 and Supplementary Table 1). The mean scores of the sFilm-FS and EVARREST® groups were either identical or remarkably similar. Mean PAI values were calculated according to adhesion quality and strength scoring system and were not significantly different between the sFilm-FS and EVARREST® groups or compared with those of the control group (Table 6 and Supplementary Table 2).

Histopathological findings

No treatment-related changes were noted in any of the examined organs in any of the three groups, except for the changes at the puncture sites (Fig. 3).

Control and EVARREST puncture sites: The liver and spleen puncture sites were covered by a relatively thin, linear, mature connective tissue (Fig. 3A–E). Only minor inflammatory infiltrations were identified.

sFilm-FS puncture sites: The liver and splenic puncture sites were covered by multinodular structures (i.e., correlating with the granular surface that was observed macroscopically), composed of relatively thick external mature connective tissue and surrounding cavities that previously contained the sFilm-FS (Fig. 3F–I). Within these nodular structures, the inner layer (i.e., interfacing the cavity) was characterized by a relatively thin, minimal foreign body reaction (composed of mixed mononuclear cells, such as

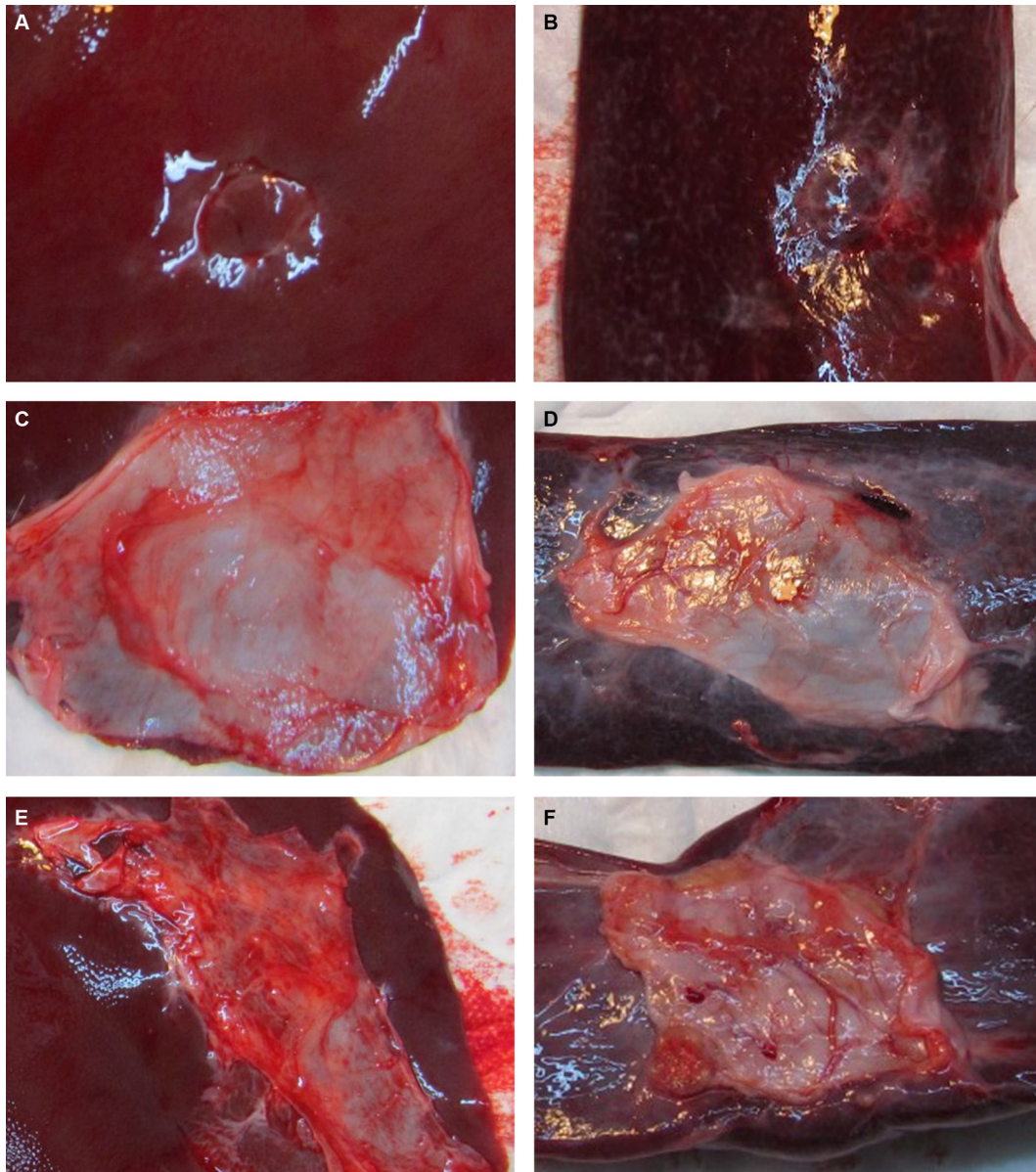


Fig. 2. Photographs of the puncture sites on termination day.

Representative photographs of the puncture site taken on the termination day, 18 weeks post-surgery. A and B: Puncture site of the liver and spleen, respectively, of the control group; C and D: Puncture site of the liver and spleen, respectively, from the EVARREST-treated group. Note the presence of smooth whitish tissue, covering both liver and spleen puncture sites; E and F: Puncture site of the liver and spleen, respectively, of the control sFilm-FS-treated group. Note the presence of a granular whitish tissue, covering both the liver and spleen puncture sites. The granular surface correlated with the reported microscopic multinodular structures (see Fig. 3). The adhesions in the two treated groups seem more extensive due to the large surface area of material adhered to normal tissue surrounding the puncture sites.

polymorphonuclear cells, lymphocytes, macrophages, and giant cells). The composition of the minimal foreign body reaction reflects an ongoing minor chronic active inflammation. The cavity contained minimal-to-mild mineralized granules, but no traces of sFilm-FS were identified (residual score of 0).

Total reactivity grading: The EVARREST had the lowest score for both the liver and spleen puncture sites, followed by the control group (Table 7 and Supplementary Tables 3 and 4). The highest scores were observed in the

sFilm-FS group.

No treatment-related microscopic changes were noted in the other evaluated organs for both the sFilm-FS and EVARREST® groups. Additionally, no evidence of local or systemic thrombosis was noted.

Discussion

In the current study, sFilm-FS showed excellent hemostatic properties, with significantly lower mean blood loss

Table 5. Adhesion Scores

Group no.	No. of animals	Treatment	Adhesion surface area		Adhesion quality & strength		
			Liver lobe	Spleen	Liver lobe	Spleen	
1	n=5	Control	Mean	3.2 (n=5)	1.6 (n=5)	1.6 (n=5)	0.8 (n=5)
			± SD	1.79	2.19	0.89	1.10
			Median	4	0	2	0
			Incidence	4/5	2/5	4/5	2/5
2	n=5	EVARREST® (Reference item)	Mean	4.0 (n=5)	4.0 (n=5)	2.0 (n=5)	1.8 (n=5)
			± SD	0.00	0.00	0.00	0.45
			Median	4	4	2	2
			Incidence	5/5	5/5	5/5	5/5
3	n=5	sFilm-FS (Test item)	Mean	4.0 (n=5)	4.0 (n=5)	2.0 (n=5)	1.8 (n=5)
			± SD	0.00	0.00	0.00	0.00
			Median	4	4	2	2
			Incidence	5/5	5/5	5/5	5/5

Table 6. Peritoneal Adhesion Index (PAI)

Group no.	Group size	Treatment	PAI	
1F	n=5	Control	Mean	5.2 (n=5)
			± SD	1.92
2F	n=5	EVARREST® (Reference item)	Mean	5.4 (n=5)
			± SD	0.89
3F	n=5	sFilm-FS (Test item)	Mean	5.6 (n=5)
			± SD	0.89

PAI was calculated for adhesion quality and strength, and is the sum of the raw scores in all abdominal regions (a single total score for each animal).

and TTH values compared to those with the standard hemostatic techniques. These results were comparable with those obtained with the reference sealant–EVARREST. sFilm-FS demonstrated significantly better TTH than EVARREST in the liver lobe. EVARREST contains 8.6 mg/cm² of fibrinogen and 37.5 IU/cm² of thrombin³⁴. By contrast, sFilm-FS contained less than 1 mg/cm² fibrinogen and 1 IU/cm² thrombin. The comparable hemostatic properties of sFilm-FS are therefore attributable to the additional sealant action (i.e., the formed fibrin clot causes adherence of the film to the injured tissue), thus providing sealant properties in addition to hemostasis, facilitating faster and better hemostatic effects.

The macroscopic findings of the sFilm-FS group included a granular whitish tissue enveloping both the spleen and liver lobe puncture sites. This is in contrast with the puncture sites in the reference group that exhibited whitish smooth tissue. The differences in the macroscopic findings are postulated to stem from the fragmentation properties of the sFilm-FS, as noted 8 weeks after subcutaneous implantation, leading to a multinodular presentation.

This study shows that the application of sFilm-FS in

spleen and liver puncture sites in female Göttingen minipigs is safe and does not affect systemic or local adverse events. Furthermore, its use did not result in increased inflammatory markers, as was evident from the normal levels of several serum cytokines and acute phase protein markers.

Changes in the clinical pathology parameters related to increased blood loss were observed only in the control group, while all groups exhibited changes in clinical pathology parameters attributable to the surgical procedure.

Comparing the control group with the group treated with EVARREST, histopathological evaluation of the puncture sites revealed increased reaction scores with sFilm-FS (Table 7). sFilm-FS was found to be slightly reactive when applied to the liver lobe puncture site and moderately reactive at the spleen puncture site, while the EVARREST patch was found to be non-reactive at both puncture sites. The different tissue reactions to sFilm-FS and EVARREST® are attributable to the components in each of the fibrin sealants and not to the biological components. EVARREST® is a fibrin sealant patch comprising human fibrinogen and thrombin embedded in a cellulose matrix that degrades after 8 weeks of use³⁵. The higher scores observed with sFilm-FS

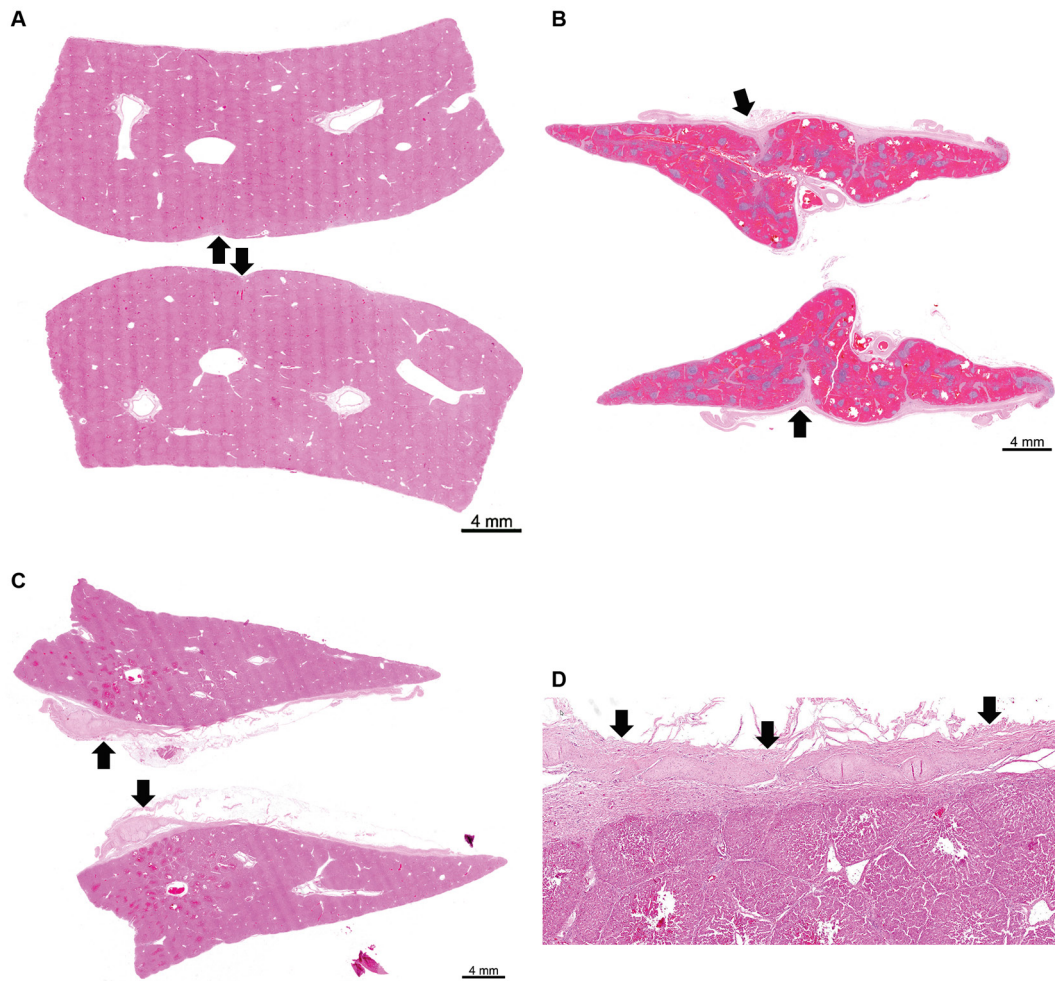


Fig. 3. Histopathology photographs of puncture sites.

A: The liver puncture site from a control animal. The puncture site is covered by a relatively thin, linear mature connective tissue (arrows). H&E. B: Spleen puncture site from a control animal. The puncture site is covered by a relatively thin, linear mature connective tissue (arrows). H&E. C: Liver puncture site from an animal treated with EVARREST. The puncture site is covered by a relatively thin, linear, mature connective tissue (arrows). H&E. D: Higher magnification of Figure 3C. The puncture site is covered by a relatively thick linear mature connective tissue (arrows) H&E.

were expected and are explained by the presence of a degradation reaction, characteristic of biodegradable polymeric materials^{24, 36, 37}. Such reactive changes in response to the absorption of biodegradable materials are not considered as adverse^{38–40}. After 18 weeks of follow-up, no macroscopic remnants of sFilm-FS were observed, and complete degradation (*i.e.*, Grade 4) with complete absorption was observed as expected.

At the end of the absorption cycle, certain absorbable implants may leave trace amounts of process residue, such as mineralized debris, that become sequestered within giant cells or within a fibrous capsule or trapped within bone trabeculae, and assessed as innocuous²⁴.

Absorbable implants confront the evaluating pathologist with a unique challenge in that the tissue response is composed of two processes: inflammation and absorption, that involve identical or overlapping cell populations. Re-

gardless of the process (absorption or inflammation), the cell types recruited at the implant site are inflammatory in nature (*i.e.*, primarily macrophages, multinucleated giant cells, granulocytes, lymphocytes). Macrophages can be found both during the first weeks after implantation and at the final stage of the bioresorption process given their role in tissue repair and healing³⁷.

The device constituents of sFilm-FS include PEG, PCL, and PLA, which are naturally degraded products, are biocompatible, and have a low toxicity profile. Medical devices based on polymers containing lactide have a long history since their first use in 1970 as implantable sutures. Polylactide degradation occurs via hydrolytic random scission of the ester bonds. Polylactide is degraded to lactic acid, a human metabolic byproduct. Products containing ϵ -caprolactone have been in clinical use since the early 1990s. The main degradation product following the hydro-

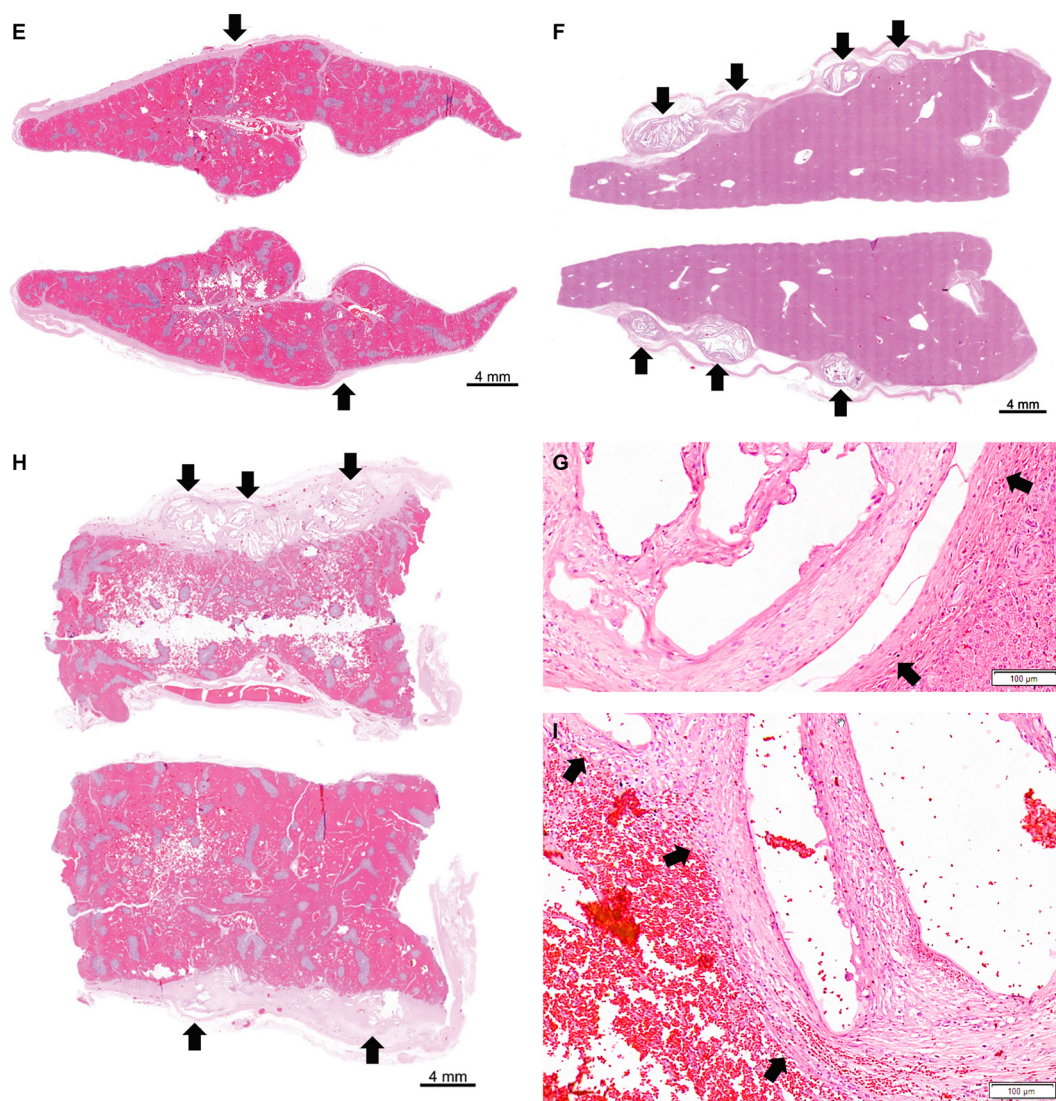


Fig. 3. Histopathology photographs of puncture sites.

E: Spleen puncture site from an animal treated with EVARREST. The puncture site is covered by a relatively thin, linear, mature connective tissue (arrows). H&E. **F:** Liver puncture site from an animal treated with sFilm-FS. The puncture site is covered by multinodular structures (arrows), composed of a relatively thick external mature connective tissue, surrounding cavities which previously contained sFilm-FS. H&E. **G:** Higher magnification of Figure 3F, demonstrating that within the nodular structures, the inner layer (i.e., interfacing the cavity) is characterized by a relatively thin, minimal foreign body reaction (composed of mixed mononuclear cells, such as polymorphonuclear cells, lymphocytes, macrophages, and giant cells) surrounding multiple cavities. Arrows indicate the interface between the normal tissue and the nodular structures. No traces of sFilm-FS are identified (scored as 0) within the cavities which previously contained the test compound. H&E. **H:** Spleen puncture site from an animal treated with sFilm-FS. The puncture site is covered by multinodular structures (arrows), composed of a relatively thick external mature connective tissue, surrounding cavities which previously contained sFilm-FS. H&E. **I:** Higher magnification of Figure H, demonstrating that within the nodular structures, the inner layer (i.e., interfacing the cavity) is characterized by a relatively thin, minimal foreign body reaction (composed of mixed mononuclear cells, such as polymorphonuclear cells, lymphocytes, macrophages, and giant cells) surrounding multiple cavities. Arrows indicate the interface between normal tissue and nodular structures. No traces of sFilm-FS were identified (scored as 0) within the cavities which previously contained the test compound, H&E.

lytic breakdown of ϵ -caprolactone is hydroxycaproic acid, which is mainly excreted in the urine. Reports on the long-term outcomes following the use of ϵ -caprolactone copolymers with lactide are known. The history of the clinical use of polymers based on lactide and ϵ -caprolactone suggests of

their safety^{41–43}. They are also approved for use by the Food and Drug Administration and have been in use for different clinical applications. The results of the current study further support its safety, compatibility, and degradability in the bleeding model of Göttingen minipigs⁴⁴.

Table 7. Mean Reaction Scores in the Liver and Spleen Puncture Sites

Group No.	Treatment	Group Mean Reaction Score for the Liver	Group Mean Reaction Score for the Spleen
1	Control	6.8	5.8
2	EVARREST® (Reference item)	3.4	3.4
3	sFilm-FS (Test item)	14	15.2

In conclusion, sFilm-FS was found to be safe and effective in bleeding control in a liver and spleen puncture model in Göttingen minipigs. Therefore, these results further promote the use of this device as a hemostatic treatment in humans.

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