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Fibrin glue and coats compromise the integrity of colonic anastomosis: an experimental trial on rats

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Abstract

Background Anastomotic leak remains a dreaded complication in colorectal surgery. Identifying optimal techniques that minimize its incidence is an active area of investigation. The aim of this experimental study was to evaluate the effect of commonly used hemostatic products on the integrity of colonic anastomoses.

Methods Male Wistar rats were randomized into 4 groups. In the control group (A), the anastomosis was performed using the standard hand-sewn technique in the ascending colon. In group B the hand-sewn technique was reinforced with a collagen–fibrinogen patch, in group C with fibrin glue, and in group D with a polyethylene glycol (PEG)-coated oxidized cellulose patch. On the 7th postoperative day, anastomotic bursting pressure measurements were obtained. A specimen surrounding the anastomosis was retrieved for histopathologic evaluation.

Results Of the 19 rats, 17 survived and 15 were included in the analysis (5 in each of groups A, B and C). Testing in group D was discontinued following adverse events in the preliminary experiments. The mean bursting pressure of the anastomosis was significantly higher in the control group (A: 221±19.41 mmHg, B: 151±14.42 mmHg, and C: 112±13.57 mmHg; P=0.001). Anastomotic healing parameters were not different between groups.

Conclusions Although experimental data support the use of sealants in defective anastomoses, in this study the reinforcement of colonic anastomosis with fibrin or oxidized cellulose-PEG sealants did not improve either bursting pressure values or anastomotic healing. More data from robust anastomoses of animals and humans are needed before sealing becomes common clinical practice in colorectal surgery.

Keywords Anastomotic bursting pressure, colonic anastomosis, anastomotic leak, fibrin glue, collagen patch

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Introduction

It is estimated that over one million intestinal resections and anastomoses are performed annually worldwide for various diseases, including colorectal cancer, diverticulitis, inflammatory bowel disease, morbid obesity, and trauma [1,2]. The most dreaded complication in those patients remains anastomotic dehiscence and eventual leak. Despite decades of dedicated effort in improving techniques, applying anastomotic devices and optimizing perioperative care, the risk of anastomotic leak (AL) in colorectal surgery remains at 3-14% [1-3]. Although the decrease in its incidence is noteworthy compared to older studies [4], there is still a need for active investigation to further reduce that percentage, since AL is linked to severe morbidity and has a consequent mortality rate of up to 18% [5-7].

Towards that goal, various biomaterials have been used to promote wound healing, anastomotic sealing and integrity.

The most frequent used materials are fleeces and glues with anticoagulant properties, fibrin, or cyanoacrylate [8-10]. Although numerous studies have been performed in both animal models and humans, the results thus far have been inconsistent in animals [9,11], and a significant difference was observed in humans only when results were pooled [12].

The above products have been more widely used in clinical practice over the last decade to reinforce an anastomosis or prevent fistula formation [12,13]. However, studies have mainly focused on upper gastrointestinal, pancreatic and hepatobiliary interventions [13-15]. It is of note that, in a recent systematic review, only 5 studies focused on colorectal interventions [12], and only 1 of them reported favorable outcomes [16].

The experimental data so far suggest that those sealants can be beneficial for closing defects, reinforcing anastomoses at risk, or in scenarios with comorbidities [17]. Nonetheless, in clinical practice, anastomotic leak occurs even when the construction of an anastomosis was deemed optimal and no risk factors were present. If the use of fibrin sealants in colon and colorectal operations aspires to become standard practice, more experimental and human studies are needed on their use in robust anastomoses.

The aim of this study was to incorporate in a common experimental protocol 2 of the main products for reinforcing anastomoses: a fibrin glue sealant (Tisseel®, Baxter Healthcare Corporation, Deerfield, IL, USA) and a collagen–fibrinogen sealant patch (Tachosil®, Takeda GMBH, Austria). Additionally, the effects of a PEG-coated oxidized cellulose patch (Veriset®, Hemostatic Patch, Covidien llc, Mansfield, USA), which has not been applied in enteric anastomosis experiments before, were evaluated.

The primary endpoint was the measurement of the anastomotic bursting pressure (ABP). Secondary endpoints were the formation and density of adhesions in the abdominal cavity, as well as histopathological features indicative of the anastomotic healing process, such as the presence and accumulation of polymorphonuclear neutrophils, lymphocytes, fibroblasts, and collagen.

Materials and methods

This was an experimental study utilizing male Wistar rats. The animals were supplied by the "Demokritos" National Center for Scientific Research, Greece. The animals were brought to the facility 2 weeks prior to the experiment, to acclimatize to laboratory conditions. A total of 19 animals were enrolled. All animals had an age of 14 weeks and weighed 389±28 g at the time of surgery. They were housed in separate cages post operation. The animal house had a controlled environment of light alternating with dark (lights on at 7 am and off at 7 pm), temperature 21±2°C, relative humidity 50-70%, and ventilation with 15 air changes/h. The rats had *ad libitum* access to water and were fed a standard diet (code 4RF21, Analab®, Greece). Three days prior to surgery, Solid Drink-Diet Bio® (Triple A Trading, Netherlands) was added to the diet. On the last day before surgery and for the first 2 postoperative days the diet consisted of Solid Drink-Diet Bio® alone. A third-party lab technician randomized the experimental animals by drawing blinded ballots. Block randomization was applied, with block sizes of 4, and balanced to ensure allocation in a 1:1 ratio. Individual researchers were blinded concerning group allocation during outcome measurements and data analysis. All methods are reported in accordance with the ARRIVE guidelines for the reporting of animal experiments [18].

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Ethics approval

The experiments were held at the Experimental Educational Centre of ELPEN (European Ref Number EL 09 BIO 03), which conforms to the European Directive 2010/63/EU for the protection of animals used for scientific purposes. The center is authorized under the Greek National Legislation (Presidential Decree 56/2013). The study protocol was approved by the Research Committee of the Experimental Educational Centre of ELPEN and the Greek Ministry of Rural Development and Food: Directorate General of Veterinary Services (Protocol number: 1520/15.03.2017). All methods are reported in accordance with the ARRIVE guidelines for the reporting of animal experiments [18].

Materials used

Tisseel® – fibrin sealant

Tisseel® (Baxter Healthcare Corporation, Deerfield, IL, USA) is a biocompatible agent that includes 2 sterile, deep-frozen solutions: the sealer protein solution and a thrombin solution. The sealer protein solution consists of synthetic aprotinin, factor XIII and fibrinogen, while the thrombin solution contains human thrombin and calcium chloride as active ingredients.

Tachosil® – fibrin sealant patch

Tachosil (Takeda GMBH, Austria) is an absorbable fibrin sealant patch. Tachosil consists of a whitish equine collagen sponge coated with the active ingredient human fibrinogen and human thrombin, with riboflavin as yellow colorant.

Veriset® – PEG-coated oxidized cellulose patch

Veriset (Hemostatic Patch, Covidien llc, Mansfield, USA) is comprised of oxidized cellulose impregnated with buffer salts, trilysine and a reactive polyethylene glycol (PEG). It is

intended for use in solid-organ and soft-tissue procedures as an adjunct to hemostasis.

Experimental interventions

The experiment was divided into 2 phases. In the first phase the animals underwent laparotomy under anesthesia.

Anesthesia

Anesthesia was achieved with intramuscular injection of ketamine 50 mg/kg and medetomidine 0.5 mg/kg. An additional dose of ketamine (10 mg/kg) was injected if needed. For analgesia, ketoprofen 5 mg/kg s.c. was administered intraoperatively and on the first postoperative day. The animals had access to water with diluted paracetamol on the first 3 postoperative days. Each animal received perioperative antibiotic prophylaxis with long-acting oxytetracycline 20 mg/kg. During the operation, the animals received oxygen supplementation and were placed on a heating pad.

Surgical procedure

The procedures were performed by the same surgeon in all cases. The abdomen was shaved with a clipper and sterilized with povidone iodine. Access to the abdominal cavity was gained by a midline laparotomy approximately 3 cm in length. The abdominal cavity was inspected to ensure there was no gross intra-abdominal pathology. The ascending colon was transected approximately 3 cm distal to the cecum, and mesenteric vessel damage was avoided in all cases. In the control group (group A), a single-layer, end-to-end anastomosis with 8 interrupted sutures was performed using polypropylene 5-0 sutures (Ethicon™, M8325). In group B after the anastomosis was completed, a single layer (15×25 mm) of fibrin–thrombin coated sealant (TachoSil™) was wrapped around the anastomosis and gently pressed for at least 3 min to ensure adhesion. In group C, approximately 0.4 mL of fibrin sealant (Tisseel™) was applied evenly to the anastomotic site using the applicator provided. In group D, a single layer of PEG and oxidized cellulose patch (Veriset™) was wrapped around the anastomosis, after being properly hydrated, and pressure was applied for 30 sec (Fig. 1). Care was taken that all patches and sealants were dry before abdomen closure. The fascia and skin were closed with 3-0 vicryl and 4-0 silk sutures, respectively.

Second experimental phase

On the 7th postoperative day, the animals underwent a second laparotomy under deep anesthesia with ketamine (100 mg/kg) and xylazine (20 mg/kg). After inspecting the abdomen for adhesions and other adverse events, ABP was measured. Subsequently, the animals were euthanized with a lethal dose of pentobarbital. A colonic segment 3 cm in length, containing the anastomosis, was acquired and stored for further histological examination.

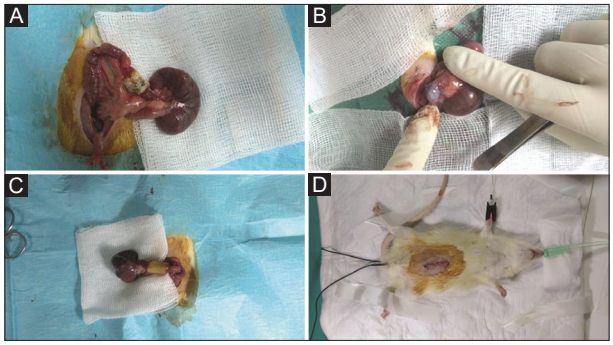

Figure 1 Reinforcing the anastomosis with (A) Tachosil, (B) Tisseel and (C) Veriset. In (D) experimental conditions: oxygen supplementation, oxygen saturation and rectal temperature measurement

Experimental endpoints

Anastomotic bursting pressure

The primary endpoint of this study was to compare the ABP between the 4 groups. To measure intraluminal pressure in mmHg, an Abbocath was inserted into each side of the anastomosis while the bowel was closed, using sutures to avoid leak. One Abbocath was connected to a volume control infusion pump (1 mL/min) and the other to a pressure monitor. All measurements were performed without lysis of the adhesions adjacent to the anastomosis and were recorded to preserve the results.

Adhesions and intra-abdominal findings

For classification of adhesions, the Zühlke score [19] was used, where: 0=No adhesions; 1=Filmy adhesions: easy to separate by blunt dissection; 2=Stronger adhesions: blunt dissection sufficient but partly sharp dissection possible; 3=Strong adhesions: lysis possible by sharp dissection only – clear vascularization; and 4=Very strong adhesions: lysis possible by sharp dissection only – organs strongly attached.

Other findings noted were mechanical ileus, anastomotic leak and presence of abscesses.

Histological evaluation

Standard hematoxylin–eosin staining and Masson's trichrome was performed. Four parameters were measured to assess the healing process: acute inflammation elements (presence of polymorphonuclear neutrophils), chronic inflammation elements (presence of lymphocytes, plasma cells, macrophages and mast cells), fibroblasts, and collagen content. These were scored according to the Ehrlich and Hunt numerical scale, as modified by Phillips *et al* [20], in which 0: no evidence, 1: occasional evidence, 2: light scattering, 3: abundant evidence, and 4: confluent cells or fibers. Scar tissue was also measured in thickness (mm) and maturity. For the maturity of the scar tissue, a scale of 1-3 was used where: 1: dominance of inflammatory elements only, 2: inflammatory elements and fibroblasts present, and 3: dominance of mature

collagen layer. A single-blinded pathologist evaluated the histopathological features of the specimens.

Statistical analysis

Normality was tested using the Shapiro-Wilk test. Normally distributed data were expressed as means and standard deviations, whilst skewed data were described as median values and interquartile range. Comparisons were performed with ANOVA or Kruskal-Wallis, with a Bonferroni *post hoc* correction test where appropriate. Fisher's exact test was used in the case of categorical variables. A P-value <0.05 was considered statistically significant. All tests were 2-tailed. The statistical package IBM SPSS Statistics for Windows Version 25 (IBM Corp., Armonk, N.Y., USA) was used for the analysis.

Sample size

To calculate the sample size, preliminary experiments were conducted with 3 animals of each group. To achieve a power of 80% and significance level of 0.05, 5 successful experiments per group were needed. A successful experiment was defined as an animal surviving up to the euthanasia day and providing measurement of ABP.

Results

Animal inclusion and complications

Seventeen of the 19 animals survived the experiment and 15 were included in the final analysis.

In the Tisseel group, 1 animal died 5 min after the laparotomy was concluded and was replaced. The operation was prolonged by 30 min because of product denaturation (either fault at thawing or defective product).

In the Veriset group, all 3 animals showed poor performance in terms of oral intake, feces production, postoperative pain, and cachexia. One underwent early euthanasia because of detrimental clinical signs. On autopsy, mechanical ileus was observed, while adhesions at the anastomotic site involving multiple organs were noted. The other 2 animals survived up to day 7 but with obvious abdominal bulging. On autopsy, intestinal obstruction was observed (Fig. 2). Because of extensive adhesions, the anastomosis was accessible in only 1 animal and ABP measurements were obtained (99 mmHg). Testing with Veriset was discontinued after mutual agreement by the experimental team and the laboratory's protocol evaluation committee. Blocks for randomization of the following experiments were adjusted to 3.

ABP

The highest ABP measurements were observed in the control group, followed by the Tachosil group and Tisseel group (Table 1). The mean bursting pressure of all groups was 161.7±57.4 mmHg. There were statistically significant differences between group A and the other groups (group A vs. B, P=0.026; group A vs. C, P=0.001). There was no statistical difference between groups B and C (P=0.342).

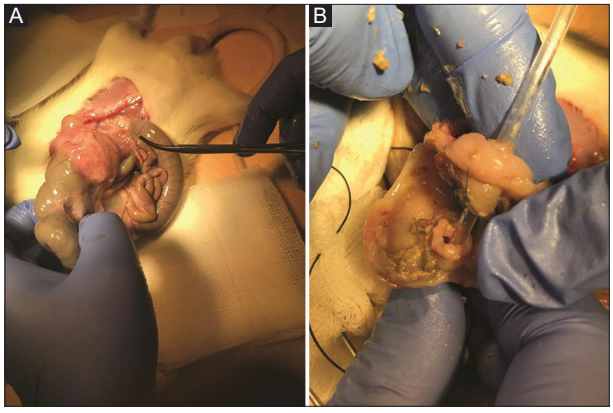

Figure 2 Intestinal obstruction in the Veriset group (A). Opening the anastomosis, visible wall edema and a constricted lumen were observed (B)

Clinical outcomes

Weight loss between groups had no statistical difference when calculated in absolute numbers (P=0.908), or percentages of initial weight (P=0.874) (Table 1). The difference between the initial weights in the 3 groups ranged between 5-6%. Adhesions as scored by the Zühlke score were less intense in the control group, while the Tisseel group had the densest adhesions (Table 1).

Histological evaluation

The detailed scoring on the Elrich and Hunt numerical scale of the anastomotic healing in the 3 groups is depicted in Fig. 3. From the analysis, no statistical difference was found between groups (acute inflammation, P=0.134; chronic inflammation, P=0.6; fibroblasts, P=0.126; and collagen, P=0.097), therefore no *post hoc* analysis was warranted. A slight trend was observed towards fewer elements of acute inflammation and higher concentrations of fibroblasts and collagen in the control group. Concerning scar tissue maturity, it should be noted that all specimens of the control group had dominance of the collagen layer. However, there was no significant difference in this parameter between groups (Table 1, Fig. 4,5).

Discussion

This study demonstrated that fibrin glue and coats did not improve the integrity or healing process of colonic anastomoses

Table 1 Characteristics, outcomes, and comparative analysis between the 3 experimental groups

Characteristics	Control (n=5)	Tachosil (n=5)	Tisseel (n=5)	P-value
Preoperative weight (g) (mean±SD)	390±25.5	392±8.4	398±16.4	0.773
Operative time (min) (mean±SD)	52.8±2.6	60±9.3	58±8.4	0.318
Adhesions (Count of specimens where adhesions were present)	4	5	5	>0.99
Adhesions (density[a])				
0	1	0	0	
1	2	2	1	0.241
2	2	0	1	
3	0	2	0	
4	0	1	3	
Intestinal obstruction (presence)	0	1	0	>0.99
Weight difference[b] (%) (median, IQR)	4.1 (0.65-10.8)	6.3 (-3.85-8.75)	9.5 (-3.6-11.3)	0.377
Bursting pressure (mmHg) (mean±SD)	221.8±43.4	151±32.3	112.4±30.3	0.001 (Control vs. Tisseel, P=0.001; Control vs. Tachosil, P=0.026; Tisseel vs. Tachosil, P=0.342)
Scar tissue maturity[c] (Dominance of)				
Inflammation	0	0	0	
Inflammation and fibroblasts	0	3	3	0.126
Collagen layer	5	2	2	
Scar tissue thickness (mm) (median and IQR)	2.3 (2-2.5)	2.3 (2.15-3.15)	3 (2.55-3.75)	0.051

[a]Adhesion density according to the Zühlke score [20], where: 0=No adhesions, 1=Filmy adhesions: easy to separate by blunt dissection, 2=Stronger adhesions: blunt dissection sufficient but partly sharp dissection possible, 3=Strong adhesions: lysis possible by sharp dissection only – clear vascularization, 4=Very strong adhesions: lysis possible by sharp dissection only – organs strongly attached.
[b]Weight difference percentage between day of sacrifice and day of operation.
[c]Maturity of the scar tissue: This value represents what was the predominant feature in each specimen's scar tissue per group
SD, standard deviation; IQR, interquartile range

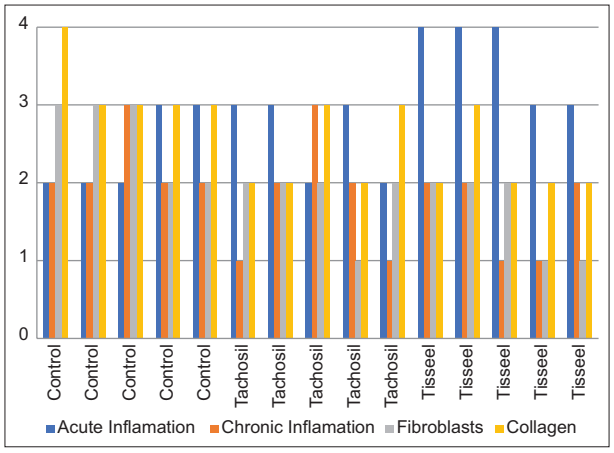

Figure 3 Histological evaluation of anastomoses according to the Elrich and Hunt numerical scale, as modified by Phillips *et al*, in which 0: no evidence, 1: occasional evidence, 2: light scattering, 3: abundant evidence, and 4: confluent cells or fibers

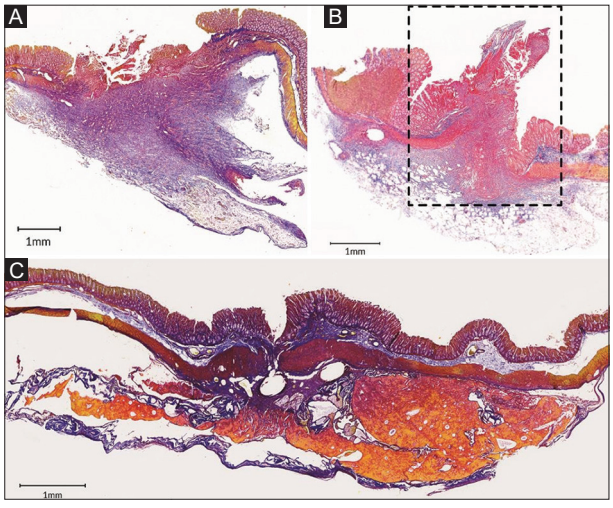

Figure 4 Histopathology images of (A) Tisseel, (B) control, and (C) Tachosil

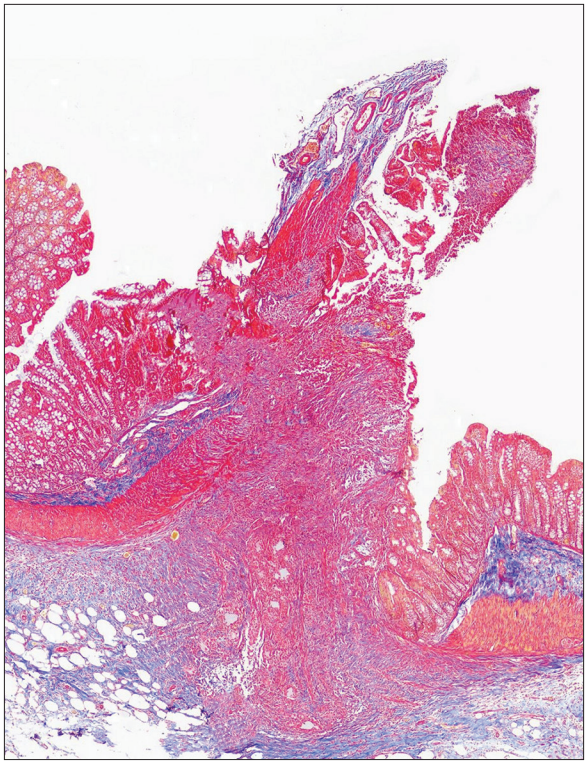

Figure 5 Control anastomosis (Fig. 4B) magnified (3×)

in a rat model. There has been a great improvement in the safety of colorectal surgery over the last 50 years, due to the optimization of surgical techniques and perioperative management [2]. However, the integrity of colorectal anastomoses remains a critical concern among surgeons. AL is considered to be an outcome of disrupted healing, and while technical aspects cannot be neglected, even perfect anastomoses made by skilled surgeons can still develop leakage. Although there is a multitude of publications in experimental settings, the pathophysiology of AL remains relatively obscure, and the healing process is not fully understood [21,22]. Furthermore, the reporting parameters and protocols applied have been considered problematic, according to researchers in the field [23].

To reduce leak incidence or improve healing, surgeons have experimented with various materials to reinforce the anastomosis, the most prominent being fibrin glues and fibrinogen–thrombin coats or patches. Concerning fibrinogen–thrombin patches in experimental settings with animal models, Pantelis *et al* [24] reported that, in rats with compromised anastomoses, the use of Tachosil reduced AL, increased bursting pressure, and improved the histological findings of wound healing. Similar experiments have been carried out by other groups, with various results in rats, mice and pigs. Pommergaard [25] *et al* reported lower AL rates, no difference in ABP and more intestinal obstructions, while Suarez-Gran [26] observed no difference in adhesion formation, healing process or survival rates. Two studies in porcine models reported a decrease in AL and no effect on anastomotic healing, while in 1 of them a greater formation of microscopic abscesses was noted [27,28]. In another study in rats, the Tachosil patch had a negative effect on anastomotic healing compared to controls (compromised anastomosis with TachoSil vs. hand-sewn complete anastomosis) [29]. Another experimental use of Tachosil was as a sealant patch in colonic defects of compromised rats (defect and ischemia), where placing Tachosil was viable, but no difference was found in ABP and healing [30]. In all the above publications, compromised anastomoses were constructed either by using fewer sutures or through ischemia.

Regarding human patients who underwent colorectal anastomosis reinforced with fibrinogen–thrombin patches, 2 studies have been published by the same scientific group (de Stefano *et al*) claiming that Tachosil was a highly protective anastomotic sealant, reducing hospital stay and AL incidence [31,32]. Moreover, in a feasibility study by Parker *et al* [33], the use of Tachosil was well tolerated and applicable.

In the present study, conducted in non-compromised anastomoses of rats, the Tachosil group exhibited lower ABP measurements (P=0.026), while no benefit was observed in anastomotic healing parameters. One animal of the group had a mechanical obstruction on autopsy due to anastomosis stricture. Taking into consideration the smaller diameter of the rat's lumen, that might not have occurred in humans or larger experimental animals.

The other main representative for reinforcing anastomosis, fibrin glue, has been more thoroughly investigated. In a systematic review published in 2015 by Nordentoft *et al* [9], it was reported that, out of 28 experimental studies included in the qualitative synthesis, 7 reported a positive effect on healing, 8 a negative effect, 11 no effect, while 2 had unclear results. The differences in the studies' outcomes were independent of the study design and the type of fibrin glue used. This study included publications involving all fibrin glue materials. Twenty-three studies evaluated liquid fibrin glue (Tisseel/Tissucol, Bioseal, Beriplast), while fibrin patches (Tachosil, Tachocomb) were used in 5 studies. The histological methodology used to assess healing also varied.

If the studies that only concerned colonic anastomosis and the use of Tisseel/Tissucol were isolated from the aforementioned systematic review, 16 publications would remain. Among those, 2 studies showed a positive effect on anastomotic healing [34,35], 7 a negative effect [36-41] and 7 no effect or inconclusive results [42-48]. As far as ABP is concerned, 7 studies reported an increase, 4 no effect, 2 a decrease, and 3 a decrease in certain subgroups.

More recently, in a series of publications by Vakalopoulos *et al* [49,50], Tisseel proved to be a promising adhesive for sealing colonic defects. However, in the first experimental series Tisseel was inert histopathologically, while in the second an increase of inflammation elements in the specimens with Tisseel was noted on day 10. Thus, the conflicting results regarding Tisseel's interaction with colonic tissue are repeated, not only in different research groups, but also in publications from the same team.

In the present study, Tisseel anastomoses exhibited significantly lower ABP measurements, while no benefit in anastomotic healing was observed. There was also a strong trend

for thicker scar tissue in the Tisseel group (P=0.051), without the anastomotic site containing more fibroblasts (P=0.126) or collagen (P=0.097). It is of note that 3 of the 5 specimens in the Tisseel group exhibited very strong adhesions (4 on the Zühlke scale), while the Tachosil group had 1 such case and the control group none.

Fibrin glue sealants have also been tested in humans in various circumstances requiring anastomosis. In a recent meta-analysis by Cira *et al* [12], 4 studies investigating colorectal anastomosis reinforced with fibrin glue were included, of which only 1 showed a significant benefit regarding anastomotic leak [16].

Veriset is a relatively newer hemostatic product and its effect on colonic anastomosis has not yet been studied. Its individual contents, oxidized cellulose and PEG, have been tested individually, with mixed results. PEG glues have been applied in the rat colon without causing major inflammatory reactions [49]. In our limited experience from this study, Veriset caused stenosis of the anastomosis and dense adhesions involving multiple organs. However, the sample size was too small to produce safe results, and we had to discontinue its use because of the high morbidity and mortality rates in that group of rats.

In our study, although measurements of ABP were low, none of the interventional groups showed any signs of macroscopic or microscopic AL. Thus, coating with either fibrin glue or patch may have a negative effect on ABP, but could have a mechanical sealing role in less robust, "leaky" anastomoses.

In the clinical setting, the rate of anastomotic leak, and not the bursting pressure, is the optimal endpoint to examine the success of different interventions applied in the construction of an anastomosis. However, anastomotic leak and bursting pressure measurements have been strongly correlated in previous studies [8,50], thus providing a viable alternative that requires a smaller sample size to yield results. Since our intention was to test those products in robust anastomoses, where rates of AL would be low, we opted to test the anastomotic integrity with ABP to reduce the number of animals needed and comply with the "3Rs" principles [23]. This decision resulted in one of the main limitations of this study, which is the small sample size. Setting ABP as the main endpoint, and having only 3 comparison groups, since Veriset was discontinued, led to a sample size needed of 5 animals per group.

Another limitation is the external validity of this study in humans. One probable cause of the reduced bursting pressure measurements observed in this study might be linked to impaired healing of the anastomotic site. However, this argument cannot be safely supported, since no healing parameters exhibited a significant difference between groups, although the results of the control group were slightly favorable. This is purely hypothetical, but both products contain human fibrinogen and thrombin, and the potential immunologic reaction might not have occurred in humans.

In conclusion, there are several risk factors for AL in colorectal surgery, such as diabetes, smoking, excessive blood loss and immunosuppressive therapy. However, AL might occur in patients with none of the above. Although fibrin materials may be of benefit in compromised anastomoses (patching a defect or in special conditions such as diabetes), the results of our study do not support their use as common practice.

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Summary Box

What is already known:

- Anastomotic leak remains a dreaded complication in colorectal surgery
- Fibrin glues and coats are the most actively investigated materials to prevent it
- Outcomes so far vary, prohibiting clinicians from applying them routinely
- Results are more consistent for impaired patients/ anastomosis

What the new findings are:

- Reinforcing robust colonic anastomosis of rats led to significantly lower anastomotic bursting pressures
- None of the materials used led to improved outcomes in anastomotic healing
- Experimentation with Veriset, a material not previously tested in similar protocols, was discontinued because of adverse events

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