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# Results of a New Patch to Externally Reinforce Colorectal Anastomosis: An Experimental Study

*Clara Gené Škrabec, MD,*[a,b] *Manel Cremades Pérez, MD, PhD,*[a,b,*] *Laia Gatell, MD,*[a] *Christine Weis, PhD,*[c] *Jesús M. Izco, PhD,*[d] *Anna Maria Rodriguez Rivero, PhD,*[c] *Teresa Zuñiga, MSc,*[d] *David Parés, MD, PhD, FACS,*[a,b] *and Joan Francesc Julián Ibáñez, MD, PhD*[a,b]

[a] *General Surgery Department, Hospital Universitari Germans Trias i Pujol, Badalona, Spain*
[b] *Department of Surgery and Morphological Sciences, Universitat Autònoma de Barcelona, School of Medicine, Bellaterra, Barcelona, Spain*
[c] *B. Braun Surgical S.A.U., Rubí, Barcelona, Spain*
[d] *Viscofan S.A., Tajonar, Navarra, Spain*

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ABSTRACT

*Introduction:* Anastomotic leak (AL) is a serious complication in colorectal surgery, associated with increased morbidity, mortality, and healthcare costs. Technological advances have yet to eliminate AL, which occurs from 5% to 14% of cases involving anastomosis. This study introduces and evaluates a new collagen-cyanoacrylate patch designed to externally reinforce colonic anastomoses with the aim to contain anastomotic leaks. The objective of the study is to evaluate the feasibility and safety of a novel collagen-cyanoacrylate patch to reinforce colonic anastomosis in a porcine model.

*Methods:* A preclinical study adhering to Good Laboratory Practices was conducted on 12 Landrace x Large White pigs. Following a previously validated model for a deficient anastomosis, a 21-mm defect was created at a colorectal anastomosis to simulate an AL. Pigs were randomized to receive either reinforcement with the collagen-cyanoacrylate patch or no reinforcement. Safety and feasibility were assessed, analyzing the integration of the patch in colorectal structures. In addition, as secondary outcomes, we assessed clinical monitoring, behavioral observations, blood tests for inflammatory markers, and histopathological analysis.

*Results:* The collagen-cyanoacrylate patch was easily applied, adhered effectively to the bowel surface, successfully sealed the defect, and was naturally degraded during the healing process. No significant differences in stenosis or adhesions were observed between the experimental and control groups, although minor variations in inflammatory and infectious markers were noted. All animals exhibited a 100% survival rate, and no clinical signs of AL were observed.

* *Corresponding author*. Department of Surgery and Morphological Sciences, Hospital Germans Trias i Pujol (HUGTIP) School of Medicine, Universitat Autònoma de Barcelona, Institut de Recerca Germans Trias i Pujol - IGTP, Carretera de Canyet s/n, Badalona, Barcelona 08916, Spain.
E-mail address: mcremades@outlook.com (M.C. Pérez).


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*Conclusions:* The use of collagen-cyanoacrylate patch is feasible, safe, and has good clinical outcomes, showing promise in preventing colonic AL. Further studies using an adequate anastomotic leak model or clinical studies are needed to confirm efficacy.


## Introduction

Anastomotic leak (AL) is one of the most serious and challenging complications in colorectal surgery, significantly increasing patient morbidity, mortality, and healthcare costs.[1,2] For patients undergoing surgery for colorectal cancer, AL can also compromise long-term oncological outcomes.[3-5] Despite advancements in surgical techniques and technology, the risk of AL persists, with an incidence rate of approximately 5% to 14% in colorectal surgeries.[6-8]

The reason why this risk persists is related to the fact that AL is a multifactorial complication. It results from the interplay between technical, mechanical, and biological factors. While mechanical integrity and tension-free, watertight closure are essential for proper healing, the vascular perfusion of the bowel ends, local tissue quality, microbiome composition, and systemic host conditions (e.g., nutritional status, comorbidities, immunosuppression) are all known to influence anastomotic outcomes. These factors may act synergistically or independently, and despite optimal surgical technique, leaks may still occur. Therefore, the clinical approach to AL should not only aim at preventing its onset but also at mitigating its consequences when prevention fails.

AL can also vary widely in severity, presenting as anything from asymptomatic or localized abscesses to life-threatening peritonitis that often requires urgent surgical intervention and the creation of a stoma.[9]

In recent years, numerous approaches have been developed to enhance anastomotic integrity in colorectal surgery. Current strategies have explored methods such as fluorescence imaging with indocyanine green to assess perfusion,[10,11] enhanced recovery programs,[12] and even studies examining the role of the microbiome[13] have shown potential. Additionally, tissue adhesives, like fibrin glue and cyanoacrylate or nanofibrous patches,[14-19] have been tested for their potential role to reinforce anastomoses. However, while these interventions are promising, the complete elimination of AL remains an elusive goal in clinical practice.

In this regard, our group recently presented a systematic review[20] on external reinforcements of colorectal anastomosis. Similar to what Cira *et al.*[21] described, among the tested techniques, fibrin sealants demonstrated the most significant reduction in AL rates in humans, while omentoplasty and collagen patches showed mixed results. All of these external reinforcements have in common that they act as a support to reduce anastomotic breakage; however, while they might reduce its appearance or severity, the leak, which bears the clinical consequences of an anastomotic tear, might still occur.

This study introduces a novel approach to address the challenge of AL by using a patch that combines a collagen matrix with a cyanoacrylate adhesive for external reinforcement of colonic anastomoses. Unlike current products in the market, this external patch is specifically designed not only to mechanically support the anastomosis but also to act as an impermeable and flexible barrier, allowing the healing to proceed beneath it even in the presence of a tear at the anastomotic site. This patch is intended to be routinely used in colorectal surgery, especially in high-risk settings for AL.

Using a previously validated porcine model—a gold standard for preclinical gastrointestinal research due to its anatomical and physiological similarities to humans—this study aims to assess the feasibility and safety of this novel collagen-cyanoacrylate patch (patent pending). Secondary objectives included assessing clinical, blood test, and histopathological differences between the control and reinforced groups.

## Methods

### *Study design*

An open-label, randomized study was designed according to experimental standards for animal model investigation. A porcine model based on a previously validated approach for simulating anastomotic leak, as described by Nordenfolt *et al.*,[22] was selected for investigational purposes.

### *Subjects of the study and anastomotic leak model*

Twelve (six males and six females) Landrace x Large White with a mean age 3-4 mo and mean weight 30-40 kg underwent a midline laparotomy. An end-to end stapled colorectal anastomosis was performed approximately at 15 cm from the anal verge. Next, a 21-mm defect at the anastomotic site to simulate anastomotic dehiscence and leak clinical condition was created following the described model.[22] The pigs were randomized using a computer randomized assignment into two groups: a control group (5), in which the defect was left uncovered, and a treatment group (7), in which the defect was covered by a 5-cm collagen-cyanoacrylate patch (Supplementary File [SF] 1) completely covering the defect previously performed. Animals were followed up for 7 d, except for two animals from the experimental group that were euthanized after 90 d, to guarantee the durability of the test product and assess its degradation.

### *Surgical procedure*

All procedures were performed by the same team of surgeons (C.G.S., J.F.J.I., M.C.P., and D.P.). Each pig underwent a midline laparotomy and an end-to-end stapled colorectal anastomosis

approximately 15 cm from the anal verge using a 29-mm circular stapler (CSC-KOL, Intraluminal Stapler CSC29, B. Braun). No resection and no devascularization were performed. A 21-mm defect was then created at the anastomotic site calibrated with a 21-mm Hegar stem. In the control group, the defect was left uncovered, while in the treatment group, it was covered with a 5-cm collagen-cyanoacrylate patch, covering completely the defect. Laparotomy was closed by an absorbable monofilament running suture of Monosyn 0, B. Braun for the fascia, and the skin was closed with a monofilament absorbable (Monosyn 2/0, B. Braun) running intradermal suture. A visual representation of the main steps is shown in Figure 1.

### *Animal care and monitoring*

This study was conducted in a referral center for animal studies. The Royal Decree 1369/2000 and applicable The Organization for Economic Co-operation and Development guidelines for implementing Good Laboratory Practice (GLP) principles concerning medicines and medical devices were followed. In accordance with GLP principles, this study was audited by a Quality Assurance Unit staff (Falcoquality).

The piglets were acclimated for 12 ± 2 d. During the acclimatization period, animals were group-housed in pens to allow social contact. Daily monitoring was conducted by technical staff to ensure the animals' well-being and maintain a stable environment. The base diet was specifically formulated for growing pigs, dosed according to their weight and age requirements. Food deprivation was 8 ± 1 h before the surgical procedure (coinciding with the animals' sleep and not the feeding period). Two days before and after the intervention, the animals were fed only with a normocaloric liquid diet (Nutricia, Nurison Multi Fibre), 2.5 L/animal per day. Drinking water from the public water supply, decalcified and filtered, was available ad libitum.

### *Sedation and anesthesia*

Sedation and anesthesia were performed according to protocol on the day of surgery. Induction of sedation and analgesia was conducted with a premedication combination of dexmedetomidine (0.02 mg/kg), midazolam (0.3 mg/kg), ketamine (3 mg/kg), and buprenorphine (0.01 mg/kg). Deep anesthesia was induced with intravenous propofol (1-3 mg/kg), after which the animal was intubated and connected to a ventilatory system with isoflurane (1.3-1.65 MAC) for inhaled anesthesia. An enema with isotonic saline and transrectal iodine was administered, and female animals underwent urinary catheterization if possible. Throughout the procedure, anesthesia was closely monitored by capnography, pulse oximetry, electrocardiogram, and both invasive and noninvasive blood pressure monitoring. Additional analgesia was provided by a fentanyl patch (72 h) and intermittent intravenous fentanyl boluses, while enrofloxacin (5 mg/kg intramuscular) was administered as a single intraoperative antibiotic dose.

### *Test item preparation*

*Collagen mesh: Collagen Cell Carrier (Viscofan SA)*
The Collagen Cell Carrier is a thin (20 μm) and translucent membrane made of highly pure collagen type I fibers derived from bovine skin, without the treatment of chemical cross-linkers. The compact fiber network is nonporous but permeable for most soluble factors. It allows the combination with additional matrix molecules and/or growth factors. As a universal matrix, the Collagen Cell Carrier serves as a cell-supporting carrier for pinpointed cell implantation.

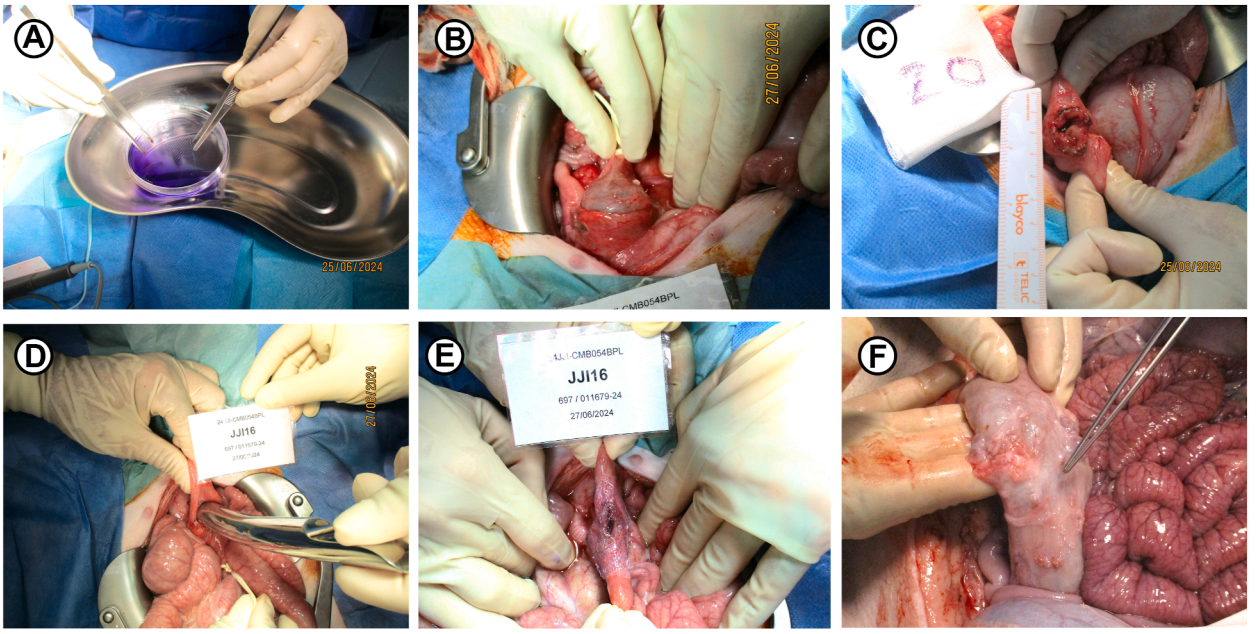


**Fig. 1 – Representative images of the anastomotic site. (A) Collagen-cyanoacrylate patch preparation, (B) anastomosis, (C) anastomotic defect, (D) defect calibration with Hegar stem, (E) collagen-cyanoacrylate patch placed covering the defect, and (F) anastomotic defect during necropsy.**

Suturable, flexible, and strong even in wet conditions, this membrane has proven biocompatibility and biodegradability *in vivo* and is stable under controlled room temperatures.

Histoacryl (B. Braun Surgical SAU) is a liquid tissue adhesive based on n-butyl-2-cyanoacrylate, a histocompatible and bactericidal adhesive compound that is fully absorbed through hydrolysis and decarboxylation. To ensure stability, Histoacryl was stored in refrigerated conditions (2°C-8°C) before use.

The collagen-cyanoacrylate patch was prepared immediately before surgery under sterile conditions. The preparation involved the following steps: in the operating room, 12 vials of Histoacryl (each containing 0.5 mL of n-butyl-cyanoacrylate) were poured into a Petri dish. The collagen mesh was immersed in the Histoacryl for 60 s on each side, using sterile tweezers to turn it over. Excess adhesive was allowed to drip off onto sterile gauze, after which the collagen-cyanoacrylate mesh was applied directly over the anastomotic defect, ensuring a complete coverage.

### *Outcome measures*

Study endpoints included: safety and feasibility, clinical, analytical, macroscopic, and histologic indicators of anastomotic leak and the possibility to develop stenosis and adhesions.

### *Follow-up*

After the intervention, the animals were awakened and kept under observation, applying the established supervision protocol. Postoperative care included behavioral observations and blood tests to monitor inflammatory markers and assessment of signs of infection, peritonitis, or other complications. Blood tests were performed during the acclimatization period (baseline), at day 3 ± 1 postprocedure and at the end of the study (7 or 90 d). For the two animals that were followed for 90 d, a blood sample was obtained at days 30 and 60.

### *Necropsy*

At study completion, all animals were euthanized under general anesthesia using 50-200 mg/kg pentobarbital sodium administered intravenously. A midline laparotomy was performed, and the "Peritoneal Adhesion Index" was recorded.[23] All macroscopic findings such as abscesses, visible leakage, and stenosis were registered. During necropsy, the anastomotic site was carefully dissected, freed from adhesions, and resected. Additionally, tissue samples were collected from the liver, kidney, and lung for further toxicity analysis. The resected anastomosis was longitudinally cut opposite to the reinforcement to unfold the colon into a flat sheet for detailed examination.

### *Histological analysis*

Histological analysis was conducted (Patconsult SL) to assess tissue response and healing at the anastomotic site. All specimens were processed through trimming, dehydration, and embedding in paraffin. Histological tissue processing was completed by cutting sections at a nominal thickness of 2-4 μm using a microtome. Slices were stained with hematoxylin-eosin and then coverslipped. The Intestinal Wall Integrity score,[15] adapted for this study, was employed to assess these outcomes comprehensively (SF 2).

### *Sample size, randomization, and statistical analysis*

Sample size was calculated using the arcsine approximation. Accepting an alpha risk of 0.05 and a beta risk of less than 0.2 in a bilateral contrast, seven subjects are needed in the first group and five in the second to detect the difference between two peritonitis proportions as statistically significant, which for group 1 is expected to be of 0.95 and group 2 of 0.25. A 2% rate of loss to follow-up was estimated. As such, the number of animals needed was 12.

The expected proportion of peritonitis in each group was determined as follows:

- Group 1: Nordentoft *et al.* demonstrated a 100% incidence of peritonitis in an experimental porcine model when 21 mm disruptions were made in colorectal anastomoses.[22]
- Group 2: Prior to this study, the research group tested the new patch under *in vitro* conditions. The results were excellent, with the patch successfully containing intestinal contents for several days. Therefore, assuming good adhesion to the intestinal wall could be achieved, a very low rate of peritonitis was anticipated.

Animals were randomly assigned into two groups with a computer randomization allocation and based on their arrival at the facility, as detailed in the protocol (SF 1).

Statistical analysis was performed with SPSS.24 version. For categorical variables, a Chi-square test with Fisher's exact test was used. For quantitative variables, a nonparametric Mann-Whitney *U* test or a Wilcoxon rank sum test was used depending on the analyzed groups.

### *Ethical approval and compliance*

This preclinical study adhered to Animal Research: Reporting of In Vivo Experiments guidelines[24] for animal research reporting. Measures to minimize animal suffering were implemented throughout the study. The project was reviewed and approved by the Animal Experimentation Ethics Committee and authorized by the competent authorities, with compliance to GLP principles (The Organization for Economic Co-operation and Development C(97)186/Final).

## Results

### *Baseline characteristics*

Between June 13, 2024 and September 23, 2024, twelve piglets were included in the study. There were no significant statistical differences between the control and treatment groups in sex, weight, and age.

**Table 1 – Blood test results at PO day 2-3.**

| Parameter | Control | | | | | Experimental (7 d) | | | | | P value |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | ♀ | | ♂ | | | ♀ | | | ♂ | | |
| | S1 | S2 | S3 | S4 | S5 | S1 | S2 | S3 | Se | S5 | |
| Hemoglobin (g/dL) | 8.5 | 10.1 | 12.0 | 9.5 | 10.6 | 9.9 | 10.2 | 8.8 | 10.3 | 0 | 0.82 |
| White blood cell count ($10^6$ cel/μL) | 27.00† | 29.87† | 22.07† | 18.56 | 13.28 | 21.22 | 19.42 | 10.72* | 25.17† | 10.54* | 0.36 |
| Lymphocytes ($10^3$ cel/μL) | 11.92† | 12.97† | 11.51† | 11.40† | 10.62 | 9.14 | 11.25† | 7.68 | 16.53† | 7.51 | 0.12 |
| Neutrophils ($10^3$ cel/μL) | 14.76† | 16.70† | 10.36† | 7.07 | 2.59* | 11.90† | 8.04 | 2.91* | 8.52 | 2.87* | 0.73 |
| AST (U/L) | 25* | 26* | 35 | 24* | 39 | 28* | 23* | 29* | 14* | 38 | 0.73 |
| ALT (U/L) | 61† | 31* | 37 | 35 | 40 | 30* | 43 | 37 | 33 | 44 | 0.84 |
| Potassium (mmol/L) | 4.3* | 4.1* | 4.1* | 4.7 | 4.8 | 4* | 3.6* | 4.5 | 4.5 | 4.8 | 0.98 |

ALT = alanine aminotransferase; AST = aspartate aminotransferase; PO = post operative; U/L = units/liter.
* Below reference values.
† Above reference values.

All animals completed follow-up and were euthanized as per protocol: five animals in the control group, five animals in the experimental group (7-d follow-up), and two experimental animals with a 90-d follow-up.

### *Safety and feasibility*

All anastomoses were successfully performed without intraoperative complications. The collagen-cyanoacrylate patch application was feasible and got correctly stuck to the bowel wall in an open surgery setting. No meaningful trends between the two groups were observed in regards to the overall surgical time (*P* value: 0.20), although interpretation is limited by sample size.

### *Postoperative clinical outcomes*

Despite the intentional creation of significant colorectal defects, none of the animals in either the control or experimental group exhibited signs of peritonitis or abdominal distress during postoperative follow-up. All animals maintained normal appetite, adequate fecal output, activity levels, and social interactions throughout the study. No weight changes were observed at postoperative day 7. Pigs at 90 d gained the expected amount of weight. No adverse effects related to the collagen-cyanoacrylate patch were noted.

### *Laboratory results*

No relevant differences were observed between the two groups in baseline blood test parameters. Similarly, during the early postoperative period (days 2-3), results remained comparable (Table 1). A consistent trend toward higher values of total white blood cell count, lymphocytes, and neutrophils was noted in the control group. By the end of the study, blood test results continued to show similar patterns across groups.

### *Necropsy results*

Regarding laparotomy wounds, after the 7-d follow-up period, abdominal wall abscesses or seromas were observed in 3/5 (60%) control animals and 2/7 (28%) experimental animals. *P* value for wound complication between control and experimental groups was 0.49, but this result may be limited by the sample size.

Free abdominal fluid was present in 3/5 control animals *versus* 1/7 experimental animals. The fluid observed in the majority of cases was serous, clear, and nonmalodorous and lacked characteristics typically associated with peritonitis. In one of the control animals (S4c), the fluid was cloudy, so it was analyzed. It revealed no parenchymal cellularity, some leukocytes (insufficient for a leukocyte count), normal protein levels, and presence of *Staphylococcus aureus* (sample contamination cannot be ruled out). No fibrin deposits, abscesses, or additional macroscopic signs of infection or inflammation were noted.

Regarding the outcome of the Peritoneal Adhesion Index, adhesion grades ranged between 0 and 3 for most animals. One experimental animal (S1e) had a total score of 6, while one control animal, S1c, had no adhesions (score 0) (Table 2). In all cases, adhesions of different tissues (urinary bladder, fallopian tubes, small intestine, etc.) to the area of the intervention were observed. The results were comparable between the experimental and control groups ($P = 0.47$), although the study was not powered to detect differences in this outcome.

Macroscopically, no peritonitis, intra-abdominal abscess, or macroscopic signs of stenosis were observed. Almost all macroscopic observations of the liver, kidneys, and lungs were normal. Pulmonary congestion was observed macroscopically in three control animals (S3c, S4c, and S5c). These observations were not correlated by the histopathological study. A purulent anterior neck abscess was observed in animal S6e (Experimental group 90d), unrelated to the study.

### *Histopathological findings*

Histological analysis revealed a complete ulceration of the intestinal wall in the control group samples at necropsy on postoperative day 7, characterized by granulation tissue with severe acute and granulomatous inflammation. This inflammation was predominantly composed of neutrophilic

**Table 2 – Peritoneal Adhesion Index (PAI) grades outside the surgically intervened area.**

| Group | Region | A | B | C | D | E | F | G | H | I | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Right upper | Epigas-trium | Left upper | Left flank | Left lower | Pelvis | Right lower | Right flank | Central | |
| Control | S1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | **0** |
| | S2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 2 | **3** |
| | S3 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | **2** |
| | S4 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | **3** |
| | S5 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | **1** |
| Experimental | S1 | 0 | 0 | 0 | 2 | 0 | 2 | 0 | 0 | 2 | **6** |
| | S2 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | **1** |
| | S3 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | **3** |
| | S4 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | **1** |
| | S5 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | **2** |

Grade ranging from 0 to 3. 0: no adhesions; 1: filmy adhesions, blunt dissection; 2: strong adhesions, sharp dissection; 3: very strong vascularized adhesions, sharp dissection, damage hardly preventable.

polymorphonuclear leukocytes, macrophages, and multinucleated giant cells. Increased angiogenesis and extensive adhesions to the urinary bladder and oviducts were also observed.

In the experimental group, at necropsy on postoperative day 7, similar ulcerative lesions with inflammatory granulation tissue were noted, mirroring the findings in the control group. Additionally, traces of collagen and cyanoacrylate mesh were detected, indicating the presence of the applied reinforcement material. Adhesions to the urinary bladder and oviducts were also observed in this group.

By day 90, the two animals in the experimental group showed significant healing at the anastomotic site. Microscopic examination of samples from female S6e demonstrated complete re-epithelialization of the mucosa, with mature granulation tissue replacing the submucosa and minimal fibrosis. Disruption of the muscle layer was identified, with a gap measuring approximately 4.1 mm. In male S7e, an almost fully re-epithelialized mucosa was observed, accompanied by chronic inflammatory infiltrates in the submucosa, mature granulation tissue, and a slightly larger muscle layer disruption of 5.6 mm (Table 3 and Fig. 2).

A foreign body granuloma containing mesh debris was identified in both experimental groups, in accordance with the normal degradation process of the reinforcement material.

## Discussion

In this study, we assessed the feasibility, safety, and clinical outcomes of a novel collagen-cyanoacrylate patch as an external reinforcement to contain AL in colorectal anastomoses using a porcine model.

Experimental models are essential to test new methods and technologies. While smaller animal models, such as rodents, are more susceptible to infection and more closely mimic human immune responses, the porcine model remains the most anatomically similar for large-scale testing.[25,26] Many authors have described their anastomotic healing studies using a porcine model.[27,28] In 2007, Nordentoft[22] described an experimental model of anastomotic leak in pigs, creating a progressively bigger defect at the anastomosis.

In our study, all animals in both groups survived the observation period in good clinical condition, without any relevant complications. The findings suggest that the application of this patch is well tolerated, with no significant adverse events *in vivo*. Furthermore, no animals developed ileus or

**Table 3 – Intestinal wall integrity scores (WIS).**

| Intestinal wall layer | Control | | | | | Experimental (7 d) | | | | | P value |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | ♀ | | ♂ | | | ♀ | | | ♂ | | |
| | S1 | S2 | S3 | S4 | S5 | S1 | S2 | S3 | S4 | S5 | |
| Mucosa | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | **0.49** |
| Submucosa | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | **0.49** |
| Muscularis (mm) | 0 (NV) | 0 (NV) | 0 (16.2) | 0 (27.2) | 0 (20.0) | 0 (16.7) | 0 (11.3) | 0 (NV) | 0 (18.5) | 0 (25.1) | **1.0** |
| Serosa | 3/12 | 3/12 | 3/12 | 2/12 | 3/12 | 2/12 | 1/12 | 1/12 | 1/12 | 2/12 | **0.07** |

NV = not valuable.

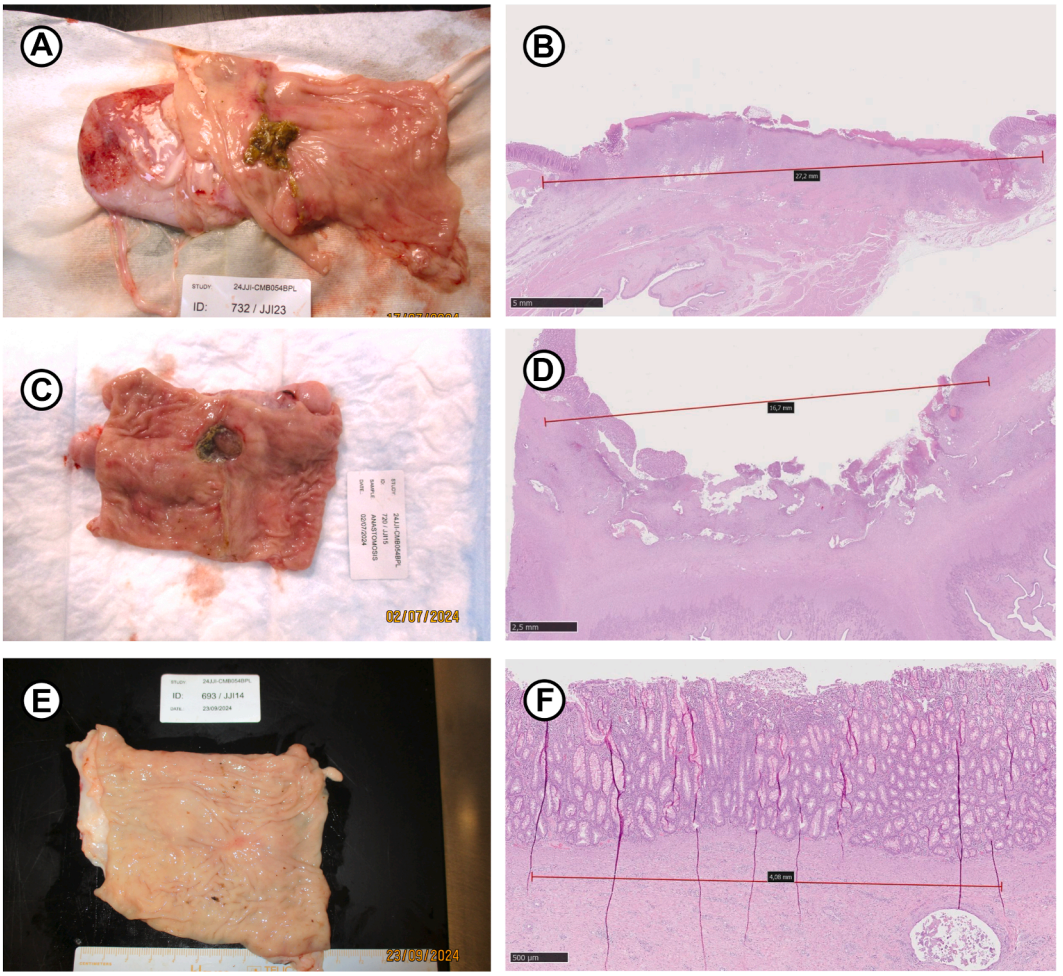


**Fig. 2 – Histological comparison of intestinal healing between control and experimental groups (7-d and 90-d). (A) Control subject necropsy at 7 d; (B) microscopy same animal, H&E, ulceration muscle layer gap (line), scale bar: 5 mm; (C) experimental subject necropsy at 7 d; (D) microscopy same animal, H&E, ulceration muscle layer gap (line), scale bar: 2.5 mm; (E) experimental subject necropsy at 90 d; (F) microscopy same animal, H&E, normal mucosa, muscle layer gap (line). Scale bar: 500 μm. H&E = hematoxylin and eosin.**

sepsis. Accordingly, no strictures, local abscesses, other serious pathological reactions, or target organ toxicity secondary to the foreign material were observed in the necropsy. The presence of free abdominal fluid lacking the typical characteristics of peritonitis was interpreted as a mild postoperative response rather than a pathological condition. Abdominal wall abscesses were localized and not associated with widespread peritoneal signs such as diffuse inflammation, fibrin deposits, or purulent ascites, suggesting they were secondary to a localized tissue reaction at the surgical access site or to minor contamination during the procedure rather than originating from a deeper intraperitoneal source. Altogether, supporting the assumption that the collagen-cyanoacrylate patch is safe to apply. At 90 d, the patch showed appropriate degradation, with significant mucosal healing and granulation tissue replacement at the anastomotic site. The integrity score,[15] adapted for this study, showed no differences between the experimental and control groups.

Adhesions are a frequent consequence of abdominal surgery, forming in different degrees after surgical procedures, infections, or other injuries to the peritoneal cavity. While their formation is a natural protective response to peritoneal damage, adhesions often contribute to long-term postoperative complications, including gastrointestinal obstruction, infertility, and persistent abdominal discomfort.[15,29] The underlying causes of the adhesions are unclear, but they may be related to inflammatory responses to the surgical procedure, infection, or the presence of foreign material. In our study, no significant differences in adhesion intensity were observed between the two groups.

Although the results of our reinforcement patch were satisfactory, demonstrating feasibility and safety, further studies are needed to assess its effectiveness to contain leaks. The absence of anticipated clinical consequences of leakage in the control group complicates the assessment of the patch's efficacy, as the model failed to consistently replicate the

conditions required to fully evaluate its potential. Consistent with our group's findings, Hoeppner *et al.*[30] concluded that large anastomotic dehiscence and localized ischemia of the bowel wall in pigs do not reliably result in intra-abdominal abscesses, peritonitis, or sepsis. The lack of reproducibility of Nordentoft's[22] results, with 0% signs of peritonitis in our control group, suggests that the porcine innate resistance to infection may limit the translatability of these results to human applications, where immune responses to AL differ significantly.

Focusing on the clinical impact, numerous methods have been proposed to reinforce colorectal anastomoses. Current products primarily emphasize hemostatic or adhesive properties, with anastomotic reinforcement serving as a secondary function. Fibrin glue, for instance, is effective in certain scenarios but lacks the robust mechanical properties required for comprehensive anastomotic reinforcement.[17,31,32] Cyanoacrylate has been used on colonic anastomosis, both in experimental settings[17] and in human studies, within the ReAL Trial.[18] However, the rigidity of cyanoacrylate after polymerization may result in the formation of small cracks, potentially compromising its impermeability.[33,34] Moreover, cyanoacrylate has another drawback, as its quick polymerization makes applicability and repositioning difficult.[29]

In fact, cyanoacrylate and collagen each have limitations when used independently. Cyanoacrylate, as noted before, is brittle and prone to cracking after polymerization, compromising its impermeability.[34] Collagen, on the other hand, is permeable and lacks sufficient structural support for anastomotic reinforcement. However, when combined, these materials exhibit a synergistic effect, producing a patch with novel properties of malleability and impermeability.

This external patch is specifically designed to reinforce the anastomosis and act as a flexible barrier, not to reduce the rate of anastomotic dehiscence, but to contain potential leaks and allow healing to proceed underneath. By preventing intestinal contents from entering the abdominal cavity in the event of an anastomotic dehiscence, the clinical consequences of AL do not appear, thus avoiding the life-threatening complications surgeons dread. However, the current preliminary nature of the patch design highlights the need for further refinement in both its preparation and application methods to improve clinical usability. While future studies may indeed demonstrate clear clinical benefits, the preparation process must be simplified—shifting toward a ready-to-use, integrated product rather than one requiring prior assembly. This refinement is particularly important for minimally invasive surgical settings—the current standard of care—where the applicability of the patch in its present form would be especially challenging.

Despite its strengths, this study has several limitations. The sample size, although statistically justified and aligned with the 3 Rs principle, may be insufficient to capture the full variability of some outcomes. Moreover, while the porcine model offers anatomical and physiological similarities to the human gastrointestinal tract and is well suited for assessing feasibility and safety, it does not adequately replicate the clinical consequences of anastomotic leakage in humans.

Importantly, our model does not fully reflect the multifactorial nature of AL. Although a mechanical defect was induced, no bowel resection or devascularization was performed, which limits the simulation of ischemia-related risk. In addition, despite the use of bowel preparation to approximate clinical conditions, the porcine gut microbiota differs significantly from that of humans and may reduce susceptibility to peritonitis. These two factors—vascular perfusion and microbial environment—are key contributors to AL pathogenesis and were not fully represented in our model, which may partially explain the unexpectedly benign clinical course observed in the control group. Future studies should consider incorporating these variables to better replicate human AL conditions.

Now that the patch's applicability and safety have been demonstrated, further research is needed to confirm its efficacy. Potentially, human trials may be necessary, as alternative animal models are unavailable and both components of the patch are already approved for human use.

If validated, the collagen-cyanoacrylate patch could represent a significant advancement in reducing or preventing the clinical consequences of AL in colorectal surgery.

### *Conclusions*

This study confirms the feasibility and safety of a novel collagen-cyanoacrylate patch specifically designed to reinforce colonic anastomoses and mitigate leakage.

While the results are encouraging, testing of this new patch in AL models would be essential to ensure accurate evaluation of new devices and facilitate their translation into clinical practice.

Despite these challenges, the collagen-cyanoacrylate patch represents a significant innovation with the potential to enhance outcomes in colorectal surgery, offering a promising step forward in addressing the complications of anastomotic leakage.

## Supplementary Data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jss.2025.07.047.

## CRediT authorship contribution statement

**Clara Gené Škrabec:** Writing – original draft, Methodology, Funding acquisition, Conceptualization. **Manel Cremades Pérez:** Writing – original draft, Methodology, Conceptualization. **Laia Gatell:** Writing – original draft, Methodology. **Christine Weis:** Resources, Methodology, Funding acquisition. **Jesús M. Izco:** Resources, Methodology, Funding acquisition. **Anna Maria Rodriguez Rivero:** Resources, Methodology. **Teresa Zuñiga:** Resources, Methodology. **David Parés:** Writing – review & editing, Writing – original draft, Methodology. **Joan Francesc Julián Ibáñez:** Writing – review & editing, Methodology, Conceptualization.

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None declared.

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