



Atraumatic laparoscopic intraperitoneal mesh fixation using a new laparoscopic device: an animal experimental study

R. N. Villalobos¹ · M. C. Mias² · C. Gas² · Y. Maestre² · M. Nogués³ · F. Vilardell⁴ · J. J. Olsina¹

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Abstract

Purpose Laparoscopic ventral and incisional hernia repair (LVIHR) has become a common procedure because of its feasibility and safety, but it is not free of complications. Acute and chronic post-operative pain and bleeding caused by traumatic fixation of the mesh are frequently prolonging the hospital stay. The aim of this study was to analyze the behavior of n-butyl-cyanoacrylate (GLUBRAN® 2) as only mesh fixation

Methods Ten female pigs were involved in the study and were divided into two groups of five (A and B). Animals in each group underwent a laparoscopic procedure in which two meshes were placed intraperitoneally and fixed with the same synthetic glue only. Animals in group A were sacrificed after 3 weeks, and those in group B were sacrificed after 12 weeks. We studied the morphological, biomechanical, and histological characteristics of the intraperitoneal mesh–tissue interface

Results No disruption, migration or folding was observed in any of the pigs. In group A, the mean tensile strength was 1.4 N/cm (± 0.2) while in group B, the mean tensile strength was 2.5 N/cm (± 0.8). Histological analyses, in areas where mesh was fixed using the glue, showed a chronic lymphocytic inflammatory reaction with a granulomatous component and a marked desmoplastic reaction made up of immature collagen and numerous fibroblasts acquiring myofibroblastic characteristics. In some areas corresponding to fixation, the desmoplastic reaction originated from mature lamellar bone tissue with osteocytes and osteoblasts.

Conclusion Laparoscopic mesh fixation with only the synthetic comonomer glue GLUBRAN® 2 is feasible, effective, and safe in intraperitoneal incisional/ventral hernia repair in this animal model.

Keywords Incisional hernia · Laparoscopic repair · n-Butyl-cyanoacrylate · Mesh fixation · Collagenogenesis · Tensile strength

Introduction

Abdominal wall surgery has expanded exponentially in the last decade. Many techniques have been developed, mainly in minimally invasive surgery. Laparoscopic ventral and incisional hernia repair (LVIHR) has become a

common procedure because of its feasibility and safety, but unfortunately, it is not free of complications. Acute and chronic post-operative pain [1] and bleeding are frequent complications, prolonging the hospital stay, and altering the quality of life [2] of patients. Absorbable or non-absorbable tacks are typically used for mesh fixation and are sometimes combined with transfascial sutures to secure the mesh. These two mechanical fixation methods pierce the abdominal wall with the possibility to cause nerve entrapment or vessel injury. Some studies [3, 4] have shown no differences between absorbable tacks, non-absorbable tacks, and transfascial sutures concerning remarkably high post-operative pain. Muysoms et al. [5] considered that non-penetrating mesh fixation by achieving an effective mesh–abdominal wall interface would significantly reduce post-operative pain after laparoscopic ventral hernia repair. Tissue glues are used in different

✉ R. N. Villalobos
rafovilla26@gmail.com

¹ Abdominal Wall Surgery Unit, General Surgery Department, Arnau de Vilanova University Hospital, 80 Rovira Roure Avenue, 25198 Lleida, Catalonia, Spain

² General Surgery Department, Arnau de Vilanova University Hospital, Lleida, Spain

³ Engineering Faculty, University of Lleida, Lleida, Spain

⁴ Pathology Department, Arnau de Vilanova University Hospital, Lleida, Spain

medical treatments and can be divided into two groups: biological and synthetic glues. Fibrin sealants, called as “biological glues,” originate from human or animal plasma and are classified as hemostatic drugs because the base mechanism of action is enhancing the coagulation cascade. These glues have been used successfully for extraperitoneal mesh fixation in laparoscopic inguinal hernia repair [6, 7] and open ventral hernia repair but not in laparoscopic ventral hernia repair, despite good results having been published in the literature [8].

Cyanoacrylate and its derivatives are “synthetic glues” and are classified as medical devices with stronger adherent properties than fibrin glues [9]. Experimental studies have reported good results compared with suture fixation [10], and the tissue toxicity of these glues does not lead to an increased foreign body reaction [11]. While some authors have studied the use of cyanoacrylate in laparoscopic inguinal hernia repair [12], unfortunately, there are no clinical trial reports in the literature of the use of cyanoacrylate glues in ventral and incisional hernia repair because of the lack of experimental studies demonstrating their safety in intra-abdominal mesh fixation and examining their interactions with intra-abdominal tissues.

Objectives

The present study was designed to investigate the morphological, biomechanical, and histological characteristics of the intraperitoneal mesh–tissue interface when formed using a comonomer glue derived from *n*-butyl-cyanoacrylate (GLUBRAN® 2). The aims of the study were (1) to evaluate the morphological changes in the mesh after positioning; (2) to describe the tensile strength of the mesh–tissue complex; (3) to evaluate the presence of collagenogenesis; and (4) to verify the functionality of a new device (GLUTACK®) built for the controlled application of the glue.

Materials and methods

Ten pigs were involved in the study and divided into two groups of five (A and B). Animals in each group underwent a laparoscopic procedure in which two meshes were placed intraperitoneally and fixed with the same synthetic glue only. In this study, two methods of mesh fixation were used: Glutack and drop by drop with a simple laparoscopic manually managed catheter. Animals in group A were sacrificed after 3 weeks, and those in group B were sacrificed after 12 weeks.

Experimental animals

The animals used for the repair model were female pigs. We chose female animals to avoid the interposition of the external male genitals at the midline below the umbilicus. The weight of the animals ranged from 30 to 35 kg. All procedures were performed at the CREBA (Applied Experimental Biomedical Research Center) in Lleida, Spain.

Preanesthesia: combined Zoletil 100 mg 2 ml and XILAGESIC 20 mg 0.5 ml IM.

Anesthesia: atropine 0.4 ml IM, O₂ 100% (5 l/min), and Fendivia 50 patch, followed by sevoflurane 4%, fentanest 3 ml, rocuronium 0.7 ml, and propofol 1% 5 ml sequentially for maintenance. All animals received one dose of cefazoline 1 g pre-operatively.

Mesh

Dyna Mesh®, an intraperitoneal onlay mesh (IPOM, FEG Textiltechnik mbH, Aachen, Germany), is a 100% synthetic two-component textile structure composed of 88% polyvinyl difluoride (PVDF) and 12% polypropylene (PP). The PVDF allows visceral contact, while the parietal PP side of the mesh faces the abdominal wall. The open pores of the mesh provide high intra-operative transparency and facilitate direct application of the glue.

Synthetic glue

GLUBRAN® 2 (GEM S.r.l., Viareggio, Italy) is a synthetic biodegradable comonomer adhesive consisting of NBKA + MS (*n*-butyl-cyanoacrylate + methacryloxy sulfolane). This comonomer polymerizes quickly (starts to set after 1–2 s and complete its setting reaction after about 60–90 s) in contact with live tissue and a wet environment, creating a thin, elastic film with a high tensile strength.

Device for mesh fixation

GLUTACK® (Fig. 1a) is a new device for laparoscopic mesh fixation that enables the precise and consistent delivery of calibrated drops of GLUBRAN® 2 (0.021 ml/shot) with each trigger pull, similar to traditional mesh fixation devices. It also has an articulating tip system for medial mesh fixation.

Surgical technique

After the administration of preanesthesia medications, a 12-mm optical trocar was introduced under CO₂-induced pneumoperitoneum into the upper left quadrant, followed by two 5-mm trocars in the left flank and lower quadrant.

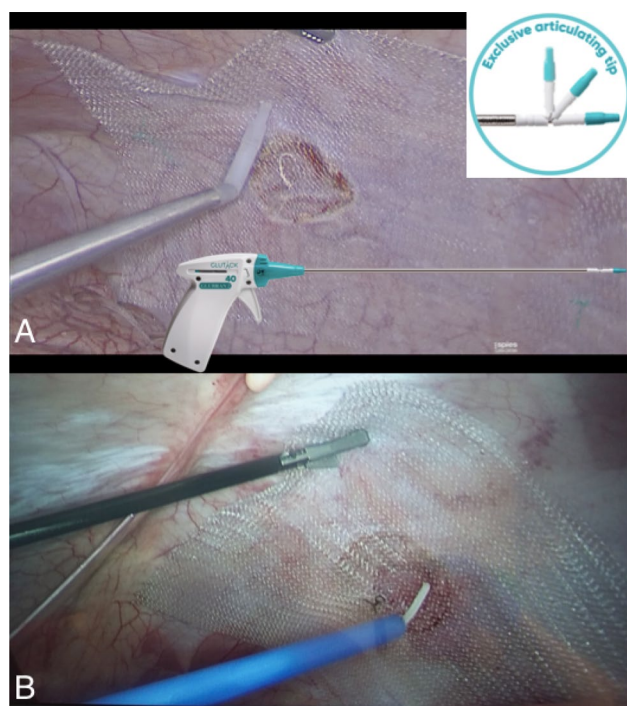


Fig. 1 Device mesh fixation. **a** Glutack. **b** Conventional applicator

Two intraperitoneal defects of 3 cm were created on the right side (upper and lower quadrants), including the transverse and internal oblique muscles. Dyna Mesh® (13 × 13 cm) was placed with an overlap of 5 cm and fixed with GLUBRAN® 2 using GLUTACK®.

The procedure for the lower defect was as follows:

1. Place a central stitch in the mesh for reference. Roll the mesh and introduce it through the optical trocar.
2. Unroll the mesh completely.
3. Attach the mesh to the lateral edge of the open defect with a grasper, verifying a good distal overlap. Initiate mesh fixation with GLUTACK®.
4. Begin mesh fixation in the middle of the distal edge and then continue laterally and vertically every 2–3 cm. Continue non-invasive fixation from the distal to medial edges.
5. Once the distal half of the mesh has been fixed, slide the grasper to the medial edge, and complete the procedure using the tip articulating for better ergonomic positioning.
6. Suture the skin with intradermal vicryl 3-0.

The same non-invasive procedure was performed for the upper defect using a conventional laparoscopic catheter applicator (Fig. 1b). All procedures were performed by a

Table 1 Canbaz scoring system

Score	No. adhesions
0	Without adhesion
1	1 thin adhesive band without vessels, and easily removable
2	2 thin adhesive bands without vessels, and easily removable
3	3 thin adhesive bands without vessels, and easily removable
4	More than three thin adhesive bands without vessels, easily removable or scattered adhesive band

single surgeon with one assistant and the defects created were not closed.

After surgery, the pigs were allowed to recover from anesthesia and were then extubated. Post-operative pain was controlled by Fentanyl patches and flunixin meglumine 2 mg/kg IM tid. Buprenorphine IM was reserved for cases of uncontrolled pain but was not required by any of the pigs. The animals were assessed daily and fed according to our protocols.

Follow-up

After 3 weeks, animals in group A underwent a new laparoscopic procedure to assess the morphological characteristics of the mesh and the presence of adhesions. The animals were euthanized with 200 mg/kg sodium pentobarbital; and then laparotomy was performed to remove the area where the meshes had been fixed.

Animals in group B underwent the same procedure after 12 weeks.

Adhesions

During the second laparoscopic examination, the abdominal cavity was explored. Intraperitoneal adhesions to the mesh were graded from 0 (absent) to 4 (severe) according to the Canbaz scoring system (CSS) (Table 1).

Morphological changes in the mesh

Four parameters were considered to evaluate these changes: disruption, folding, migration, and contraction. Cracks in the mesh were considered as disruptions, and the presence of ridges or wrinkles was considered as folding. Migration was noted if the reference central stitch was shifted. Contraction was calculated as a percentage by measuring the difference between the initial surface area (169 cm²) and the surface found on the excised abdominal wall (*S*):

$$\text{Percentage of contraction} = [(169 \text{ cm}^2 - S \text{ cm}^2) / 169 \text{ cm}^2] \times 100\%.$$

Biomechanical testing

Each mesh was divided transversely into two parts for biomechanical and histological studies. The tensile strength was determined by peel testing (PT) 110, also known as seal strength testing. In this test, the tissue is pulled in tension to one side while the mesh is pulled to the other side at a constant speed of 40 mm/min and an angle of 90°. Values are registered in N/mm (Fig. 2).

Histological study

Samples from representative areas were fixed in 10% buffered formaldehyde solution and embedded in paraffin. Histological sections were prepared at 5 µm and stained with hematoxylin-eosin (H&E) and Masson's trichrome (MT) to identify cellular types and the presence of collagen. An optical binocular microscope was used for the analysis.

For immunohistochemistry, tissue sections mounted on treated glass slides (IHC Microscope slides; Agilent-DAKO, United States) were used. Sections were dried for 1 h at 65° before pre-treatment procedure of deparaffinization, rehydration, and epitope retrieval in the Pre-Treatment Module, PT-LINK (Agilent Technologies-DAKO, United States) at 95 °C for 20 min in 50× Tris/EDTA buffer, pH 9. Before staining the sections, endogenous peroxidase was blocked.

A panel of primary antibodies was applied to serial sections and incubated for 30 min at room temperature using Autostainer Link 48 Immunohistochemistry staining system (Agilent Technologies-DAKO, United States).



Fig. 2 Peel testing

For myofibroblastic cells immunolabeling, an anti-alpha smooth muscle actin mouse monoclonal antibody (clone 1A4, Ready to use, Agilent Technologies-DAKO, United States) and an anti-β-catenin mouse monoclonal antibody were used (clone β-Catenin-1, Ready to use, Agilent-DAKO, United States). After incubation, the reaction was visualized with the EnVision™ FLEX Detection Kit (Agilent Technologies-DAKO, United States) using diaminobenzidine chromogen as a substrate, as indicated by manufacturers' instructions. Sections were counterstained with hematoxylin. Appropriate negative controls including no primary antibody were also tested.

Statistical analysis

Graphical and statistical analyses of the findings were subsequently conducted using SPSS 20.0 for Windows (SPSS, Chicago, IL, USA). The numerical data are presented as the mean ± standard deviation (SD), and a *p* value less than 0.05 was considered statistically significant.

Ethics and registration

This study was conducted in accordance with the authorization of the General Directorate of Natural Environment policy of Spain, Expedient No. FUE-2017-00602470, Project No. 9769 that was evaluated by the committee of animal experimentation of the IRB (Biomedical Research Institute of Lleida, Spain) according to article 34 of Royal decree 53/2013 of Spain, and ethics committee of animal experimentation No.: PI17-001.

Results

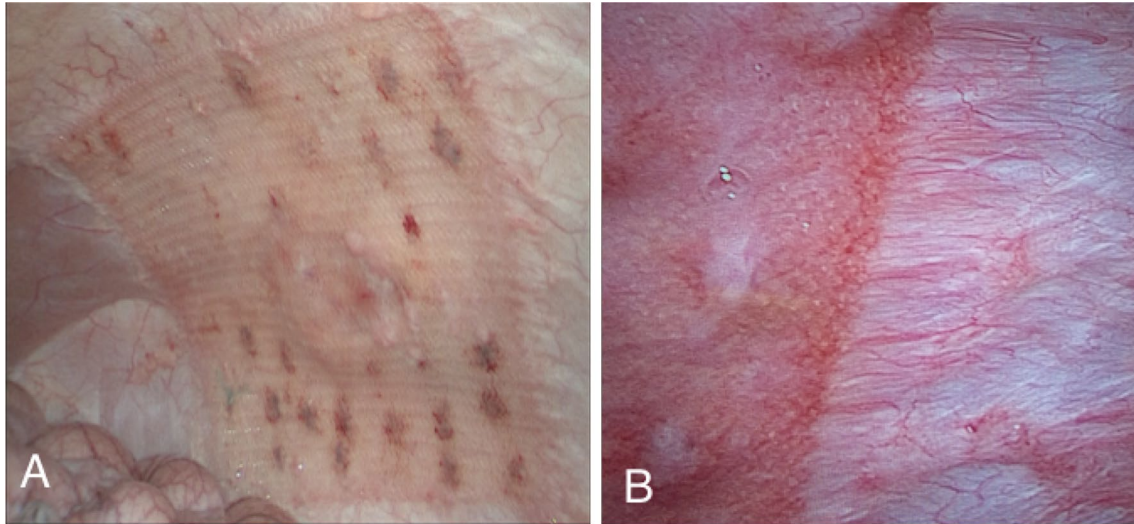
All ten pigs survived until sacrifice. None of the animals showed evidence of post-operative bleeding, bowel obstruction or infection. The mean weight in group A was 34.3 kg (± 2.3) initially and 46.2 kg (± 6.4) after 3 weeks. The mean operating time (OT) was 58 min (± 7.2) (Table 2). The mean weight in group B was 34.6 kg (± 3.1) and 85.5 kg (± 6.6) at the beginning and end (12 weeks) of the study, respectively. The mean OT in group B was 53.2 min (± 20.7).

Adhesions

In group A, no adhesions were observed in the lower meshes (Fig. 3a), while adhesion with a severity score of 1 was observed in the upper meshes. These adhesions were related to the liver, and in some cases, the spleen and omentum. The findings in group B were similar to those in group A.

Table 2 Post-operative findings

Mesh variables	Group A		Group B		<i>p</i>
	Upper	Lower	Upper	Lower	
Initial weight (kg)	34.3 ($\pm$ 2.3)		34.6 ($\pm$ 3.1)		NS
Final weight (kg)	46.2 ($\pm$ 6.4)		85.5 ($\pm$ 6.6)		NS
Operative time (min)	58 ($\pm$ 7.2)		53.2 ($\pm$ 20.7)		NS
Adhesions (Canbaz score)	1	0	1	0	–
Wrinkles/folding	No	No	No	No	–
Contraction	10 ($\pm$ 3.1)	9 ($\pm$ 2.2)	13.6 ($\pm$ 2.2)	13.8 ($\pm$ 2.6)	NS
Peeling test (N/cm)	1.4 ($\pm$ 0.2)	1.3 ($\pm$ 0.4)	3.3 ($\pm$ 1.5)	2.5 ($\pm$ 0.8)	

**Fig. 3** Adhesions and morphological changes. **a** No adhesions. **b** No disruption, migration or folding

Morphological changes in the mesh

No disruption, migration or folding was observed in any of the pigs. During the second laparoscopy, all meshes exhibited a preserved integrity, with a flat and central position (Fig. 3b). In group A, the mean contraction of the lower meshes was 9.02% ($\pm$ 2.2), while that of the upper meshes was 10.01% ($\pm$ 3.1). In group B, mean contraction values of 13.86% ($\pm$ 2.6) and 13.58% ($\pm$ 2.2) were observed for the lower and upper meshes, respectively (Table 2).

Peeling strength

After collection, the specimens were maintained in a cooler for up to 6 h until testing. In group A, the mean tensile strength was 1.4 N/cm ($\pm$ 0.2) and 1.3 N/cm ($\pm$ 0.4) for the lower and upper meshes, respectively. Group B showed a mean tensile strength of 2.5 N/cm ($\pm$ 0.8) and 3.3 N/cm ($\pm$ 1.5) for the lower and upper meshes, respectively. No significant differences were found in either group (Table 2 A: 0.211, B: 0.805).

Histology

In group A, examined 3 weeks post-surgery, the areas where the prosthetic material was fixed using the comonomer glue showed a chronic inflammatory reaction characterized by macrophages and small lymphocytes; and a reduced number of neutrophils was present around the mesh and glue deposits (Fig. 4a) delimited by a marked desmoplastic reaction (red spots, see Fig. 3a) that was constituted by immature collagen and numerous fibroblasts acquiring myofibroblastic characteristics (Fig. 4b). Such myofibroblastic differentiation was demonstrated by means of smooth muscle actin immunostaining showing diffuse cytoplasmic immunoreactivity (Fig. 4b insert). These samples were also immunolabeled for β -catenin, which revealed non-nuclear but cytoplasmic expression, ruling out the benign neoplastic nature of this desmoplastic reaction secondary to an insult and typical on scar tissue (adhesions) within the abdomen after abdominal surgery. A Masson's trichrome stain showed the presence of immature collagen (Fig. 4b).

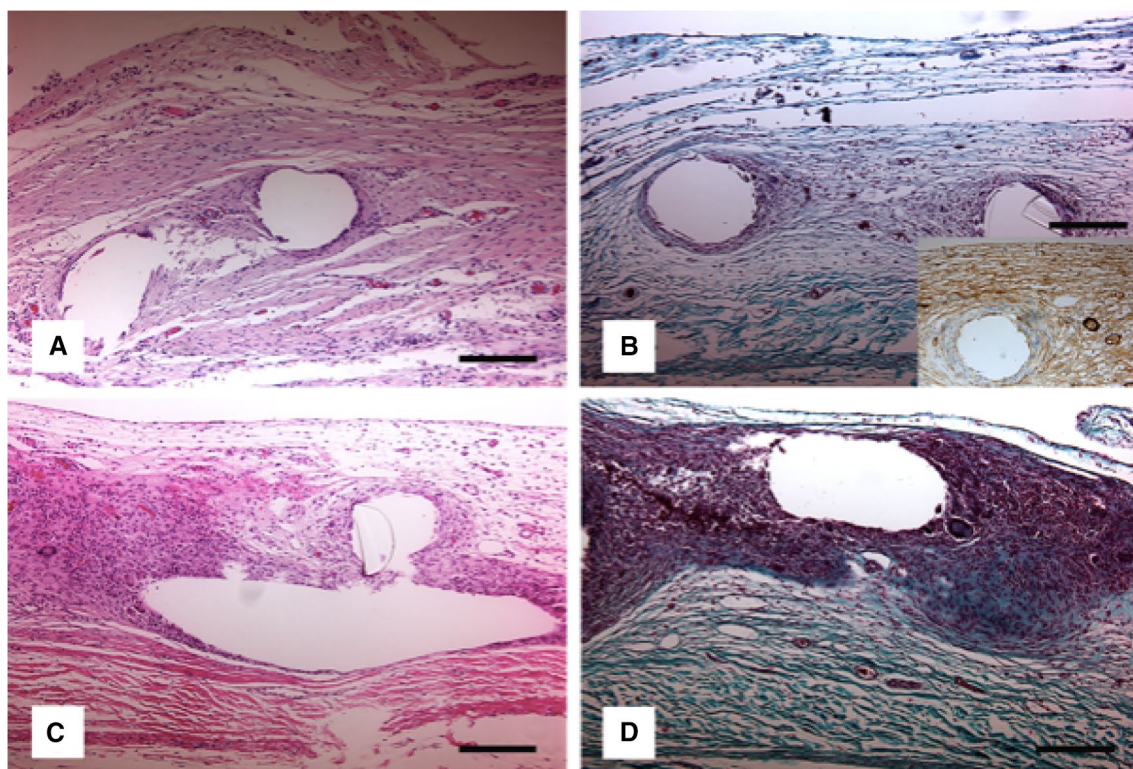


Fig. 4 Histology

In group B, examined 12 weeks post-surgery, histological findings revealed the presence of a chronic lymphocytic inflammatory reaction with a granulomatous component characterized by numerous macrophages and giant cells (Fig. 4c) delimited by a well differentiated fibrous connective tissues (Fig. 4d). In some areas corresponding to fixation points of the prosthetic, the desmoplastic reaction accompanied by bone metaplasia was observed in the muscular tissue that was characterized by mature lamellar bone tissue with osteocytes and osteoblasts (red spots turned to nodes, see Fig. 5a).

Discussion

LVIHR has increased abroad because of some advantages over the open approach. However, it is important to know its limitations because they depend on the complexity of the hernia. Laparoscopic IPOM placement is one of the most common procedures in incisional or ventral hernia repair, and it involves placing a mesh intraperitoneally overlapping the defect by at least 5 cm around its edges. Tacks and sutures are frequently used to fix mesh, but their use is traumatic and can, thus, cause acute and chronic pain and bleeding. In 2006, Schwab et al. [13] compared the use of fibrin glue vs. staples for mesh fixation and found that 20.7%

of patients in the staple group reported issues with chronic pain compared with 4.7% of patients in whom the mesh was fixed with glue. Reynvoet et al. [4] concluded that the development of atraumatic mesh fixation techniques will be of major significance in the future of laparoscopic ventral hernia repair. Burza et al. [14] reported the advantages of synthetic-modified cyanoacrylate glue for mesh fixation in laparoscopic transabdominal preperitoneal inguinal hernia repair compared to titanium tacks, demonstrating that the use of NBCA + MS glue was associated with a significantly reduced risk of developing chronic groin pain and shortened the hospital stay. Despite these encouraging results, further studies with longer follow-up periods are needed to confirm the findings. Studies did not demonstrate statistical differences between traumatic vs. atraumatic fixation related to the post-operative pain because there could be many other causes (suture of the defect, preperitoneal fat, patient idiosyncrasy, number of tacks, etc.).

Our experimental study is the first to use only a modified cyanoacrylate (NBCA + MS) glue for intraperitoneal mesh fixation applied using a newly developed drop control device (GLUTACK®) in a porcine abdominal wall defect model. Eriksen et al. [15] developed an experimental study in pigs comparing fibrin sealant vs. tacks for mesh (cPTFE) fixation with only fibrin but after using corner sutures and applying a spray. Although they concluded that the use of

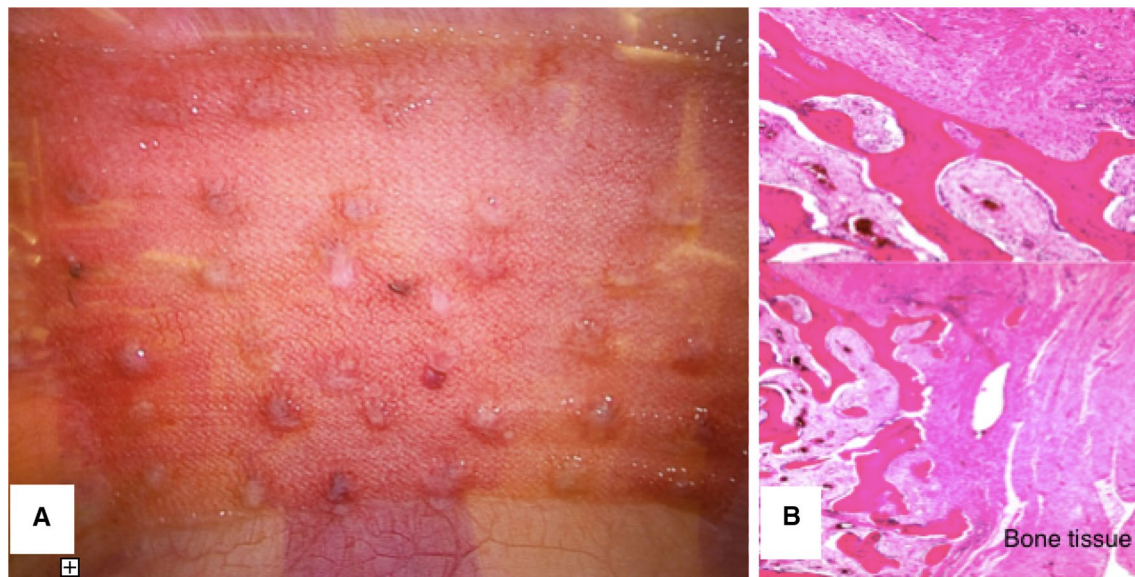


Fig. 5 **a** Nodes. **b** Mature bone tissue

fibrin was safe and feasible, there was no evidence that fibrin glue alone was appropriate for the IPOM technique. Later, the same group [16] performed a randomized clinical trial and concluded that mesh fixation with fibrin sealant in the repair of small umbilical hernias improves the general well-being and reduces the acute post-operative pain of patients. Meanwhile, Olmi et al. [17] performed a non-randomized trial and concluded that the use of dual-sided composite mesh with fibrin glue fixation can achieve very good results in patients. Fortenly et al.⁸ reported that mesh fixation using GLUBRAN® 2 impairs tissue integration, elicits inflammation, and unfavorably alters the biomechanics of both the macroporous mesh and the abdominal wall. Nevertheless, some aspects must be considered as the main bias of the experimental design: the animal model that was chosen to reproduce the human abdominal wall was small (rat), and the amount of product placed extraperitoneally was 30 times greater than the quantity indicated by the manufacturer, as experimentally verified by Losi et al. [10] Pascual et al. [18] described that seroma was evident, especially in the GLUBRAN® 2 group, after extraperitoneal mesh fixation in rats. In our study, the mean animal weight in both groups was 34 kg (± 0.8); thus, in these animal models, one drop of glue is not comparable to one drop of glue in very small animal models, such as rats. An excess of glue could lead to the formation of non-absorbed, rigid residues in the tissue, so we applied controlled drops of the fixing glue directly onto the peritoneum without compromising the muscle structures of the abdominal wall, thereby avoiding biomechanical alterations. It is possible that a variety of biomechanical behaviors of the mesh should be studied when placed at different levels of the abdominal wall. In several cases, seroma is directly

related to the extension of dissected tissue, and when glue is used, it is necessary to avoid dead spaces before it polymerizes. In our study, we did not observe seroma in the defects. We divided the animal population into two groups to differentiate the effects of the mesh–glue complex over time. In each group, two meshes were placed to differentiate fixation with a conventional, manually controlled laparoscopic catheter applicator, and an automatic, controlled device named GLUTACK®.

In this study, we performed a second laparoscopy in all animals just before they were sacrificed. The aim was to directly determine the “in vivo” characteristics and the extent of any adhesions, as well as morphological changes in the mesh–tissue complex. The close contact between the mesh and viscera will stimulate the formation of adhesions, causing major complications, such as small bowel obstructions and fistulae. Therefore, one of our concerns was to investigate whether intraperitoneal mesh fixation using GLUBRAN® 2 involves visceral complications. Zinther et al. [19] described the formation of dense adhesions with the use of absorbable or non-absorbable tacks and showed that fewer adhesions formed with the use of coated meshes than with non-coated meshes. Some authors [20] have reported a decrease in the number and extent of adhesions with the use of fibrin glue. We found adhesions on the upper meshes related to the omentum and liver, similar to the findings described by Clarke et al. [21] In the lower meshes, no adhesions were observed in either group, probably because of the movement of the bowel, in contrast with the liver, which is fixed, as well as the controlled drop method used for mesh fixation. It is known that any inflammatory events in the peritoneal cavity result in local

peritoneal irritation with the loss of regional mesothelial cells. Defects in the mesothelial lining are repaired by the “metastasis” of nearby mesothelial cells. Peritoneal defects heal everywhere simultaneously. A large peritoneal defect heals in the same amount of time as a small defect, usually within 3–5 days. This process is rapid and usually reconstitutes the peritoneal continuity without adhesion formation. Usually, the majority of fibrinous attachments formed in this way are lysed within a few days of development. If they persist for 3 days or longer, fibroblastic proliferation may occur within them, causing adhesion formation. In case of a drop of glue inside the peritoneum, we did not see this problem with GLUTACK® and only in one case with the laparoscopic catheter. In this case, we identified the drop and waited for 60–90 s after polymerization of the glue.

Disruption or wrinkling represents a failure at the mesh–abdominal wall interface (MAI), which weakens the procedure and contributes to recurrence. Clarke et al. [21] described the lowest mean score of buckling with the use of only fibrin glue for mesh fixation, while the use of tacks and sutures creates “hollows” in the mesh that make it difficult for the mesh to remain flat against the abdominal wall. In our study, we fixed the mesh along its surface approximately every 3 cm, creating a very good MAI and achieving fixation stability. These characteristics prevent mesh migration and can decrease mesh contraction. On the other hand, with the use of only fibrin glue, the same group described significant mesh contraction of up to 28% after 4 weeks; they implemented a sprayer method to fix the mesh, and the resulting excessive inflammation could explain these findings. In our study, the GLUBRAN® 2 applied dropwise resulted in a mesh contraction of approximately 10% after 3 weeks and 14% after 12 weeks. One limitation is that it remains to be determined whether the mesh would have continued to shrink after 12 weeks. In any case, mesh contraction was very low with the use of either the laparoscopic applicator or GLUTACK®.

Non-coated meshes are more appropriate than coated meshes for fixation using glue because glue can be released directly to the MAI with non-coated meshes, while with coated meshes, glue must be released on the parietal side followed by pulling the mesh up against the abdominal wall. This maneuver can decrease the efficacy of the glue and lead to mesh stability failure. Eriksen et al. [22] found a high recurrence rate, especially in cases of larger hernia defects, with the use of fibrin glue to fix a composite mesh coated with collagen. Jenkins et al. [23] also observed a lower acute fixation strength with these types of meshes than with non-coated meshes. Schug-pass et al. [24] observed the best mesh fixation results with PP meshes. In our study, Dyna Mesh IPOM fixation achieved very good results.

In terms of the fixation strength, Ladurner et al. [9] observed that sutures and tacks were superior to

cyanoacrylate in terms of the tensile strength (14.5 N vs. 9.6 N). An interesting study by Reynvoet et al. [25] tested different absorbable tacks with a tensile strength up to 26 N/cm² after 2 weeks in a pig model. Ladurner et al. [26] observed a similar tensile strength with PP/PVDF mesh glue fixation and ePTFE mesh spiral tack fixation. Rieder et al. [27] proposed the combination of glue and sutures for less invasive mesh fixation. In our study, we found a greater tensile strength in group B than in group A, but the difference was not statistically significant (3.3 N/cm vs. 1.4 N/cm). These tensile strengths are similar to those previously described by Eriksen et al. [15] However, it is very important not to consider that increasing the tension increases the safety of mesh fixation. We do not know the suitable tensile strength for mesh fixation, so we must consider multiple factors that are involved in achieving optimal results, for example to close the defect. Most of the surgeons performing LVIHR are closing the defect and changing the biomechanical behavior of the tissue–mesh complex. Only the glue mesh fixation could have more support when it is implanted. Considering the morphological findings in this study, the tensile strength obtained seems to be effective, and a higher tension may not be necessary.

The histological study demonstrated the formation of collagen and the presence of myofibroblastic cells but only in areas where GLUBRAN® 2 was applied, and after 12 weeks, the presence of mature bone tissue was also observed in these areas (Fig. 5b). Heterotopic mesenteric ossification (HMO) refers to the formation of bone-like lesions in abnormal locations inside the abdominal cavity, and it has been described in the literature. HMO is a disorder with an innocuous manifestation that develops after abdominal surgery or trauma related to inflammatory stimuli due to abdominal injury. Peritoneal ossification is a pathological condition that is often associated with patients undergoing peritoneal dialysis (PD) or occurs as a result of traumatic splenic rupture or peritonitis [28]. Although inflammatory responses are believed to contribute to peritoneal ossification, the processes regulating peritonitis-mediated ossification are not clear. Several recent works have indicated the presence of progenitor cells in the peritoneum, similar to stem cells in the bone marrow. Multipotent cells have the ability to differentiate into osteogenic, adipogenic, neurogenic, and myogenic phenotypes. Simply, the mechanism seems to be related to the recall of undifferentiated mesenchymal cells to the site of inflammation and their stimulation to develop into osteoblasts or chondroblasts [29]. Estrogen activity [30] has also been shown to promote the activity of osteogenic differentiation of mesenchymal stem cells.

In conclusion, laparoscopic mesh fixation with only the synthetic comonomer glue GLUBRAN® 2 is feasible, effective, and safe in intraperitoneal incisional/ventral hernia repair in this animal model, but the glue must be applied

using a device for drop control to avoid excess glue release. GLUBRAN[®] 2 interacts with the tissue and promotes the formation of myofibroblasts and collagen. The clinical studies described in the literature are insufficient, and randomized controlled trials must be developed to support these results.

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Compliance with ethical standards

Conflict of interest Author RV, author CM, author CG, author YM, author MN, author FV, and author JO declare that they have no conflict of interest.

Ethical approval All applicable international, national, and institutional guidelines for the care and use of animals were followed: Resolution No .9769 of directorate general for environmental policy, Generalitat de Catalunya, Spain.

Human and animal rights This study does not contain any studies directly involving human participants.

Informed consent For this type of study formal consent was not necessary.

References

- Rosenberg J (2009) Pain, quality of life and recovery after laparoscopic ventral hernia repair. *Hernia* 13:13–21. <https://doi.org/10.1007/s10029-008-0414-9>
- Langbach O (2015) Long term recurrence, pain and patient satisfaction after ventral hernia mesh repair. *World J Gastrointest Surg* 7(12):384. <https://doi.org/10.4240/wjgs.v7.i12.384>
- Wassenaar E, Schoenmaeckers E (2010) Mesh-fixation method and pain and quality of life after laparoscopic ventral or incisional hernia repair: a randomized trial of three fixation techniques. *Surg Endosc* 24:1296–1302. <https://doi.org/10.1007/s00464-009-0763-1>
- Reynvoet E, Deschepper E, Rogiers X, Troisi R, Berrevoet F (2014) Laparoscopic ventral hernia repair: is there an optimal mesh fixation technique? A systematic review. *Langenbeck's Arch Surg* 399:55–63. <https://doi.org/10.1007/s00423-013-1126-x>
- Muysoms F, Vander Mijnsbrugge G, Pletinckx P et al (2013) Randomized clinical trial of mesh fixation with “double crown” versus “sutures and tackers” in laparoscopic ventral hernia repair. *Hernia* 17(5):603–612. <https://doi.org/10.1007/s10029-013-1084-9>
- Antoniou SA, Ph D, Ko G et al (2015) Meta-analysis of randomized trials comparing nonpenetrating vs mechanical mesh fixation in laparoscopic inguinal hernia repair. *Am J Surg*. <https://doi.org/10.1016/j.amjsurg.2015.06.008>
- Shah NS, Fullwood C (2014) Mesh fixation at laparoscopic inguinal hernia repair: a meta-analysis comparing tissue glue and tack fixation. *World J Surg* 38:2558–2570. <https://doi.org/10.1007/s00268-014-2547-6>
- Fortelny RH, Petter-Puchner AH, Glaser KS, Redl H (2012) Use of fibrin sealant (Tisseel/Tissucol) in hernia repair: a systematic review. *Surg Endosc Other Interv Tech* 26(7):1803–1812. <https://doi.org/10.1007/s00464-012-2156-0>
- Ladurner R, Drosse I, Bu D et al (2011) Cyanoacrylate glue for intra-abdominal mesh fixation of polypropylene-polyvinylidene fluoride meshes in a rabbit model. *J Surg Res* 162:157–162. <https://doi.org/10.1016/j.jss.2009.11.710>
- Losi P, Burchielli S, Vet MD et al (2010) Cyanoacrylate surgical glue as an alternative to suture threads for mesh fixation in hernia repair. *J Surg Res* 6:6–11. <https://doi.org/10.1016/j.jss.2010.05.003>
- Reynvoet E, Van Cleven S, Van Overbeke I et al (2015) The use of cyanoacrylate sealant as simple mesh fixation in laparoscopic ventral hernia repair: a large animal evaluation. *Hernia* 19:661–670. <https://doi.org/10.1007/s10029-015-1347-8>
- Shen Y-M, Liu Y-T, Chen J, Sun L (2017) Efficacy and safety of NBCA (*n*-butyl-2-cyanoacrylate) medical adhesive for patch fixation in totally extraperitoneal prosthesis (TEP): a prospective, randomized, controlled trial. *Eur Rev Med Pharmacol Sci* 21(4):680–686. <https://doi.org/10.1007/s10029-016-1482-x>
- Schwab BR (2006) Less chronic pain following mesh fixation using a fibrin sealant in TEP inguinal hernia repair. *Hernia* 10:272–277. <https://doi.org/10.1007/s10029-006-0080-8>
- Burza A, Avantifiori R, Curinga R, Santini E, Delle Site PSF (2014) Comparison between two different mesh fixation methods in laparoscopic inguinal hernia repair: tackers vs synthetic cyanoacrylate glue. *Minerva Chir* 69(6):321–329
- Rosenberg J (2008) Laparoscopic intraperitoneal mesh fixation with fibrin sealant (Tisseel®) vs. titanium tacks: a randomised controlled experimental study in pigs. *Hernia* 12:483–491. <https://doi.org/10.1007/s10029-008-0375-z>
- Eriksen JR, Bisgaard T, Assaadzadeh S, Jorgensen LN, Rosenberg J (2009) Randomized clinical trial of fibrin sealant versus titanium tacks for mesh fixation in laparoscopic umbilical hernia repair. *Br J Surg* 98:1537–1545. <https://doi.org/10.1002/bjs.7646>
- Stefano O, Giovanni C, Luca S, Claudio P, Giuseppe V, Enrico C (2010) Laparoscopic incisional hernia repair with fibrin glue in select patients. *JSLs* 14:240–245. <https://doi.org/10.4293/108680810X12785289144359>
- Pascual G, Rodríguez M, Pérez-köhler B et al (2017) Host tissue response by the expression of collagen to cyanoacrylate adhesives used in implant fixation for abdominal hernia repair. *J Mater Sci Mater Med*. <https://doi.org/10.1007/s10856-017-5869-8>
- Zinther NB, Wara P, Friis-Andersen H (2010) Intraperitoneal onlay mesh: an experimental study of adhesion formation in a sheep model. *Hernia* 14(3):283–289. <https://doi.org/10.1007/s10029-009-0622-y>
- Gungor B, Malazgirt Z, Topgül K, Gök A, Bilgin M, Yürüker S (2010) Comparative evaluation of adhesions to intraperitoneally placed fixation materials: a laparoscopic study in rats. *Indian J Surg* 72(6):475–480. <https://doi.org/10.1007/s12262-010-0168-3>
- Clarke T, Katkhouda N, Mason RJ (2011) Fibrin glue for intraperitoneal laparoscopic mesh fixation: a comparative study in a swine model. *Surg Endosc* 25:737–748. <https://doi.org/10.1007/s00464-010-1244-2>
- Eriksen JR, Bisgaard T, Assaadzadeh S, Jorgensen LN, Rosenberg J (2013) Fibrin sealant for mesh fixation in laparoscopic umbilical hernia repair: 1-year results of a randomized controlled double-blinded study. *Hernia* 17:511–514. <https://doi.org/10.1007/s10029-013-1101-z>
- Jenkins ED, Melman L, Deeken CR, Greco SC, Frisella MM, Matthews BD (2011) Biomechanical and histologic evaluation of

- fenestrated and nonfenestrated biologic mesh in a porcine model of ventral hernia repair. *J Am Coll Surg* 212(3):327–339. <https://doi.org/10.1016/J.JAMCOLLSURG.2010.12.006>
24. Schug-pass C, Lippert H (2010) Mesh fixation with fibrin glue (Tissucol/Tisseel®) in hernia repair dependent on the mesh structure—is there an optimum fibrin—mesh combination?—investigations on a biomechanical model. *Langenbeck's Arch Surg* 395:569–574. <https://doi.org/10.1007/s00423-009-0466-z>
 25. Reynvoet E, Berrevoet F, De Somer F, Troisi R (2012) Tensile strength testing for resorbable mesh fixation systems in laparoscopic ventral hernia repair. *Surg Endosc* 26:2513–2520. <https://doi.org/10.1007/s00464-012-2224-5>
 26. Roland L (2008) Tissue attachment strength and adhesion formation of intraabdominal fixed meshes with cyanoacrylat glues. *Eur J Med Res* 13:185
 27. Rieder E, Stoiber M, Scheikl V et al (2010) Mesh fixation in laparoscopic incisional hernia repair: glue fixation provides attachment strength similar to absorbable tacks but differs substantially in different meshes. *ACS* 212(1):80–86. <https://doi.org/10.1016/j.jamcollsurg.2010.08.015>
 28. Agarwal A, Breiman RS, Coakley FV (2004) Peritoneal calcification : causes and distinguishing features on CT. *Am J Roentgenol* 182:441–445
 29. Shen J, Nair A, Saxena R, Zhang CC, Borrelli J Jr, Tang L (2014) Tissue engineering bone using autologous progenitor cells in the peritoneum. *PLoS ONE* 9(3):1–7. <https://doi.org/10.1371/journal.pone.0093514>
 30. Paper O (2012) Estrogen and its receptor enhance mechanobiological effects in compressed bone mesenchymal stem cells. *Cells Tissues Organs* 195:400–413. <https://doi.org/10.1159/000328003>

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