Healing After Resection of Colonic Endometriosis and Growth Factor Enriched Agents: An Experimental Rat Model,

Anastasia Prodromidou MD , Kitty Pavlakis MD, PhD , Vasileios Pergialiotis MD, PhD , Maximos Frountzas MD , Nikolaos Machairas MD, PhD , Ioannis D. Kostakis MD, PhD , Laskarina M. Korou Vet PhD, MSc , Konstantinos Stergios MD , Dimitrios Dimitroulis MD, PhD , George Vaos MD, PhD , Despoina N. Perrea MD, PhD

PII: S1553-4650(18)30301-7
DOI: 10.1016/j.jmig.2018.04.025
Reference: JMIG 3531

To appear in: *The Journal of Minimally Invasive Gynecology*

Received date: 31 January 2018
Revised date: 28 March 2018
Accepted date: 2 April 2018

Please cite this article as: Anastasia Prodromidou MD , Kitty Pavlakis MD, PhD , Vasileios Pergialiotis MD, PhD , Maximos Frountzas MD , Nikolaos Machairas MD, PhD , Ioannis D. Kostakis MD, PhD , Laskarina M. Korou Vet PhD, MSc , Konstantinos Stergios MD , Dimitrios Dimitroulis MD, PhD , George Vaos MD, PhD , Despoina N. Perrea MD, PhD , Healing After Resection of Colonic Endometriosis and Growth Factor Enriched Agents: An Experimental Rat Model, *The Journal of Minimally Invasive Gynecology* (2018), doi: 10.1016/j.jmig.2018.04.025

Original Article

Healing After Resection of Colonic Endometriosis and Growth Factor Enriched Agents: An Experimental Rat Model

Anastasia Prodromidou, MD, Kitty Pavlakis, MD, PhD, Vasileios Pergialiotis, MD, PhD, Maximos Frountzas MD, Nikolaos Machairas, MD, PhD, Ioannis D. Kostakis, MD, PhD, Laskarina M Korou, Vet PhD, MSc, Konstantinos Stergios MD, Dimitrios Dimitroulis, MD, PhD, George Vaos, MD, PhD[e] and Despoina N. Perrea, MD, PhD

From the Laboratory of Experimental Surgery and Surgical Research N.S. Christeas, National and Kapodistrian University of Athens, Athens, Greece (Drs Prodromidou, Pergialiotis, Frountzas, Korou, Stergios, Vaos, and Perrea), Department of Pathology, National and Kapodistrian University of Athens, Athens, Greece (Dr Pavlakis), the Third Department of Surgery, Attiko Univesity Hospital, National and Kapodistrian University of Athens, Athens, Greece (Dr Machairas), the Second Department of Surgery, Laiko Univesity Hospital, National and Kapodistrian University of Athens, Athens, Greece (Drs Kostakis and Dimitroulis), and Department of Paediatric Surgery, "ATTIKON" University General Hospital, School of Medicine, National and Kapodistrian University of Athens, Athens, Greece (Dr Vaos).

Disclosure statement: The authors declare no conflicts of interest.

Corresponding author: Anastasia Prodromidou, MD, Laboratory of Experimental Surgery and Surgical Research N.S. Christeas, National and Kapodistrian University of Athens, Athens, Greece. Ag. Thoma 15B 11527, Goudi, Attiki, Greece +306972751000. E-mail: a.prodromidou@hotmail.com

Precis: PRP and TISSEEL can be safely applied in colon surface after shaving of experimentally induced endometriosis with a potential beneficial effect in collagen disposition, neo-vascularization and integrity of colon layers.

Word count: 2895

ABSTRACT

Study objective: To examine the potential beneficial effect of platelet rich plasma (PRP) and fibrin sealant (TISSEEL; Baxter Healthcare Corporation, Deerfield, IL) on bowel wound healing after shaving of an experimentally induced endometriotic lesion.

Design: Single-blind, randomized study (Canadian Task Force classification I).

Setting: Certified animal research facility.

Animals: Thirty female Sprague-Dawley rats.

Interventions: Experimental colonic endometriosis was induced by transplanting endometrial tissue to all animals (first surgery). Thirty rats were then randomized to 1 of 3 groups according to treatment: PRP (group I, n = 10), fibrin sealant (group II, n = 10), or no agent (group III, n = 10) was applied after shaving of the endometriotic nodule (second surgery).

Measurements and Main Results: Colonic endometriosis was successfully induced in all subjects. Four days following the second surgery, the animals were euthanized, and microscopic evaluation was performed. The pathologist was blinded to the treatment method. Histopathologic analysis revealed that compared with the control group, collagen disposition was found in significantly higher expression in both the PRP and fibrin sealant groups ($p = .011$ and $p = .011$, respectively). Distortion of the integrity of the colon layers was statistically more pronounced in the control group compared with the fibrin sealant group ($p = .033$) while greater new blood vessel formation was observed in the fibrin sealant group compared with control ($p = .023$). No histologic evidence of residual or recurrent disease was detected.

Conclusion: Both PRP and fibrin sealant appear to be safe and associated with improved tissue healing during shaving for the excision of colonic endometriosis, attributed to the enhanced collagen disposition, neovascularization, and protection of the integrity of colon layers. Clinical trials are warranted to confirm the feasibility of PRP and fibrin sealant in the clinical setting.

Keywords: Collagen; Colorectal endometriosis; Growth factors; Neoangiogenesis; Shaving

Introduction

Deep infiltrating endometriosis (DIE) constitutes the most severe form of endometriosis affecting 1% to 2% of women [1]. Colorectal involvement is diagnosed in 3% to 37% of cases [2]. Two surgical approaches have been proposed for the management of colorectal endometriosis; en bloc resection of endometriotic lesions with the colon or minimally invasive techniques including shaving or disc excision [3]. Shaving has been proposed in select patients who suffer from colorectal endometriosis with lesions that are <3 cm, occupying less than 33% to 50% of the colon circumference [4]. Even for large DIE lesions, functional outcomes were comparable after minimally invasive techniques or radical excision [5].

Platelet-rich plasma (PRP) has been efficiently applied in medical fields such as orthopedics and cosmetic surgery [6,7] as it is associated with faster wound healing, tissue restoration, and recovery [8]. Fibrin sealant is widely used as adjunct for hemostasis, as a sealant, and wound healing enhancer in many surgical procedures including gastrointestinal anastomoses, plastic surgery, urologic procedures and gynecologic procedures [9,10]. Specifically, favorable outcomes after fibrin sealant application have been reported in patients who underwent laparoscopic ovarian cystectomy, while efficient hemostasis and accelerated wound healing have additionally been noted after laparoscopic myomectomy [11,12]. In contrast to colonic surgery for other benign or malignant pathology, shaving of endometriosis does not necessarily preclude the existence of remnant cells. Therefore, to date, it remains unclear whether agents such as PRP and fibrin sealant may result in disease recurrence or if it benefits tissue healing.

The objective of the present study was to compare the effect of PRP and fibrin sealant on wound healing of the bowel after shaving of an experimentally induced colonic endometriosis.

Materials and Methods

Animals

Thirty female, 4-month-old, non-pregnant Sprague Dawley rats with a median weight of 255 grams (range, 220–324) were initially used in this study. The number of subjects included was determined based on previous studies in this field [13,14]. The animals were separately caged in the animal section of the laboratory in a controlled environment at 22°C with 12-hour cycles of light per day. Standard rat food and water were provided ad libitum. The surgical procedures were performed under sterile conditions between 9:00 AM and 1:00 PM. The experiments were conducted in the laboratory of Experimental Surgery 'NS Christeas', National and Kapodistrian University of Athens. All animal experiments comply with the Animal Research Reporting of In Vivo Experiments (ARRIVE) guidelines, and all procedures were in accordance with the European Union Directive 2010/63 for the protection of animals used for scientific purposes and approved by the competent Veterinary Service of Attica Region.

Experimental Protocol and Surgical Procedures

Induction of experimental endometriosis of the colon in rats by transplanting endometrial tissue to the colorectal site, has meticulously been described in a previous study from our group [15]. Under anesthesia with intraperitoneal administration of 60 mg/kg ketamine and 7 mg/kg xylazine hydrochloride, a 4- to 5-cm ventral vertical incision was performed, and a single uterine horn from the uterus of each rat was removed. The endometrium was exteriorized by a longitudinal excision, and the specimen was then sutured around the colon with the endometrial surface in direct contact with the bowel serosa. The specimen was attached in the area of the proximal colon in continuity with the ampulla ceci of the colon, as it can ensure standardized reproduction of the procedure and be easily identified. The "uterine loop" was thoroughly inspected to ensure that it did not suffocate the bowel

and that passage of stools was not interrupted. Two weeks later, a second procedure was performed to confirm the establishment of endometriosis to allow for the second step of the protocol. A specimen of approximately 6 cm of the area recognized as part of the proximal colon in continuity with the ampulla ceci of the colon was excised and sent for histologic examination. As described by Roman et al, shaving was performed with the use of scissors as deeply as possible to completely remove the endometriotic lump along with the infiltrated colon layers [16]. Because shaving involves an intact colon lumen, suturing is not considered mandatory in this technique. In the current study, the depth of the shaving technique was not evaluated (in terms of pathology analysis) because this would render necessary a segmental resection that would prohibit the introduction of PRP and fibrin sealant and further analysis of the tissue. However, macroscopically, all subjects had serosal involvement and the potential involvement of the muscularis layer (if any) remained superficial; hence rendering suturing of the lesion was not necessary. Following the shaving, a meticulous evaluation of the integrity of the remaining colon layers was performed to ensure that no perforation during shaving had occurred. Following shave excision, the rats were randomly divided into three groups. In the PRP (group I: n = 10) and fibrin sealant (group II: n = 10) groups, the agents were applied, respectively in the shaved area while no agent was applied in group III (n = 10). Fig. 1 depicts the application of fibrin sealant in the affected colon area from which the endometriotic lesion had been shaved and excised.

Platelet-Rich Plasma Preparation

Platelet-rich plasma was prepared according to standard protocol for rats proposed by Messora et al [17]. In particular, 2 weeks before the first surgery, blood samples of approximately 3 mL were extracted from each subject after intraperitoneal anesthesia via jugular vein cannulation [17]. A 5-mL vacuum silicone tube was used to preserve blood samples at –40^{o}C until final preparation of PRP in the second

phase of the experiment. After that, the amount of blood extracted from each subject was then replaced through the jugular cannula with sterile saline. During the second phase of the experiment to prepare PRP, a centrifuge of two steps was adopted. Initially, the blood that was collected from each subject was mixed with 10% sodium citrate (0.11 mL of sodium citrate for every 1 mL of blood) and was centrifuged at 160 G, for 20 minutes at 22^{o}C. For the second spin, blood plasma was collected in the upper phase to 1.4 mm below the formatted line to isolate the platelets and blood plasma and then was further centrifuged at 400 G for 15 minutes at 22^{o}C. Platelet rich plasma was collected and activated with 10% calcium chloride solution (0.05 mL to each 1 mL of PRP). As a result, a gel was formed and about 0.4 mL of PRP was then applied to the affected bowel surface of each subject.

Fibrin Sealant

The fibrin sealant (TISSEEL^{TM}; Baxter Healthcare Corporation, Deerfield, IL) is a biocompatible agent that contains a mixture of coagulation factors, the active ingredients of which include two sterile, deep-frozen solutions: the sealer protein solution and thrombin solution, each in a separate preloaded double-chamber syringe in a mixture of coagulation factors. 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, fractionated from pooled human plasma. After thawing and warming to 37ºC, the two solutions were mixed and approximately 0.4 mL was applied to the shaved area (Fig. 1).

Macroscopic Evaluation

Two weeks after the first surgery, the peritoneal cavity was opened and explored for the macroscopic confirmation of cystic endometriotic nodules in the implanted area as well as the detection of additional endometriotic lesions elsewhere in the peritoneal cavity owing to cell migration from the uterine horn. The peritoneal

cavity was further explored for the presence of any other adverse events such as obstruction or adhesions. Four days following the second surgical procedure, the subjects were euthanatized after deep anesthesia followed by cervical dislocation and bowel examination for bowel perforation, peritonitis, abscesses, and adhesions.

Histological Examination

A pathologist was recruited to the study (KP) and performed the histopathologic analysis of all specimens that were excised following the sacrifice of the animals. The procedure was blinded to the examiner. The tissue was fixed and preserved in 10% solution of formaldehyde and then embedded in paraffin blocks and cut in 4 μm sections with the use of microtome. Standard hematoxylin-eosin staining was applied. Moreover, in select cases, immunohistochemical staining with CK7 and vimentin was performed for the verification of the endometrial nature of the epithelium lining the cystic nodules and histochemistry with Masson trichrome for the identification of the fibroblastic proliferation. Specimens were evaluated and photographed. The shaved area was examined for fibroblasts, inflammatory cells, granulation tissue, blood vessels, collagen disposition, and fecal material in the colon lumen of the resected specimen, as well as for integrity of the colon layers, defined as thinning or complete replacement by fibrous tissue or collagen of one or both circular and longitudinal muscle layers or as extension of the inflammatory process up to the submucosa. The assessed parameters were scored using a score system proposed by Zhou et al [13]. Accordingly, each parameter was scored 0 to 4 as follows (0, no evidence; 1, minimal; 2, slight; 3, moderate; 4, abundant). A score of ≤2 was considered low expression, and scores ≥3 were interpreted as high expression of the parameters under examination. Furthermore, the presence of residual or recurrent columnar to cuboidal epithelium of endometrioid type in the specimens was evaluated.

Statistical Analysis

Statistical analysis, was performed with SPSS v.23.0 statistical software (IBM Corp., Version 23.0. Armonk, NY). Continuous variables (weight of the animals) were interpreted as mean ± standard deviation, whereas categoric variables (histologic parameters as scored by the pathologist) for comparisons between groups were analyzed with two-tailed chi-square test and Fisher's exact test. The level of statistical significance was set at $p < .05$.

Results

Outcomes of First and Second Surgery

Two weeks after the first operation, no serious adverse events were reported, and no intraoperative or postoperative deaths occurred. During the second surgery, macroscopic exploration of the affected area revealed the presence of one or more cystic lumps filled with fluid at the site of surgical endometriotic intervention in 9/10 (90%) rats in group I, 9/10 rats in group II (90%), and in 10/10 (100%) rats in group III. Concerning the 2 subjects from groups I and II with no grossly detectable endometriosis, only the implanted "uterine loop" was found. Nevertheless, specimens from the 2 subjects were also shaved and histologically examined. No obstruction of the bowel owing to the surrounding cystic lump was identified in any case. During second surgery and before shaving, macroscopic evaluation of disease extent indicated that the ampulla ceci was infiltrated to the serosa or subserosa in all animals of the 3 groups. Hence, the shaved specimen included the lesion en bloc with the affected underlying colon tissue (serosa or subserosa) in all 30 subjects. In all cases, the muscularis propria was exposed and no further damage to the muscular layers, submucosa, or mucosa was macroscopically recognized after meticulous evaluation. Consequently, no sutures were necessary to remedy the potential defect in the colon after shaving. Histopathology of the shaved lesions revealed cystic endometriosis in all 30 subjects. The epithelium lining the cystic

spaces was columnar to cuboidal of endometrioid type, as recognized in endometrial tissue of female rats; it is immunohistochemically positive for staining of both antibody CK7 and vimentin as described in our previous study [15]. No endometrial-like stroma was detected. This was expected as sections from the rat uterus comprising both endometrial and myometrial tissue demonstrated that the endometrial stroma in rats is composed of loose fibrous tissue bearing no resemblance to the hypercellular human endometrial stroma.

Main Outcomes

Four days following shaving of endometriosis, no serious adverse events were noted, and all rats were alive. No signs of perforation, peritonitis, abdominal abscess, or adhesions were detected in any group. A significant mean weight loss was observed in the experimental models of the PRP and fibrin sealant groups between the first and second surgery ($p = .01$ and $p = .005$, respectively), while no weight difference was observed in the control group ($p = .498$). No significant difference was observed in any group when weights of the second surgery were compared with those at the time of sacrifice (Table 1).

Endometriotic epithelium was not identified in any of the 30 subjects, indicating no residual or recurrent disease. Histopathology of the specimens from the PRP group revealed the presence of fibroblasts, blood vessels, granulation tissue, and collagen disposition with moderate presence of inflammatory cells and fecal material and limited impaired colon layers (Fig. 2A). In the fibrin sealant group, intensive fibroblasts concentration, collagen, granulation tissue disposition, and blood vessel formation were observed with the presence of inflammatory cells, fecal material, and a slight loss of colon layer integrity (Fig. 2B). In the control group, less fibroblasts, granulation tissue, blood vessels, and poor collagen disposition were noted while intensive infiltration of inflammatory cells, loss of colon layer integrity, and moderate fecal material were described (Fig. 2C).

Table 2 depicts the scores for each animal and Table 3 lists each parameter according to low and high expression along with significance for each comparison. Despite the high scores in fibroblastic concentration and granulation tissue formation in the treated area in the PRP and fibrin sealant groups compared with the control group, no statistical significance was detected. Collagen disposition was found in significantly higher expression in both the PRP and fibrin sealant groups (6/10 subjects, p = .011 and 6/10 subjects, p = .011, respectively) compared with the control group (0/10). In the PRP group, no significant difference in new blood vessel formation or presence of feces in the colon lumen was observed compared with the control group (p = .35 and p = .628, respectively). On the other hand, when the control group was compared with the fibrin sealant group regarding high blood vessel concentration, a significant difference was found (3/10 rats vs 8/10 rats with, p = .023), while a difference of the presence of feces in the colon lumen was not found in the two groups (p = 1.0). Infiltration with inflammatory cells was noted in the PRP and fibrin sealant groups but there was no statistical difference compared with the control group (p = 1.0 and p = 1.0, respectively). Finally, distortion of the integrity of the colon layers was statistically more pronounced in the control group compared with the fibrin sealant group (5/10 vs 0/10, p = .033), while no statistical difference was observed when compared with PRP group (p = .650). For all parameters under evaluation, no statistical difference was detected when the PRP and fibrin sealant groups were compared.

Discussion

Shaving of colonic endometriotic nodules is considered low risk for adverse outcomes regarding bowel integrity [18]. Nevertheless, the colon layers can be injured and mechanisms of the healing process such as hemostasis, concentration of inflammatory cells, proliferation of fibroblasts, and remodeling of the affected tissue by new blood vessels and granulation tissue are activated [19,20].

The model used in the current study aimed to mimic human colonic endometriosis in terms of tissue contact and infiltration; in our previous study histopathologic analysis revealed the endometriotic focus adherent to the bowel wall with replacement of part of the outer longitudinal muscle layer or by a thin layer of fibrous tissue and fat [15]. Respectively, studies concerning histologic confirmation of human colorectal endometriosis described a penetration of endometriosis to the serosa and intense fibrous reaction in the affected area [21]. Concerning shaving of endometriosis, stitching was avoided unless the colonic wall integrity was compromised. This is in accordance with studies on human DIE in which suturing was performed in cases of perforation or severe damage in the muscular layers [18,22]. No such defect was detected in the present study. Adding stiches might impact the evaluation of the efficacy of the applied factors on the healing of the shaved area, although placement of stiches after shaving when the bowel wall integrity is not compromised has not been determined. While Donnez et al placed stiches only in cases of detection of defect in the bowel lumen, other investigators described adding stiches to the muscularis layers in all cases after shaving [18,23]. Future studies could add an additional group in which only stiches are placed in the shaved area or groups treated with a combination of stiches and the examined agents, however, this was beyond the scope of the present study.

By using a previously proposed scoring system [13], no differences were observed between the study and control group regarding fibroblastic proliferation, inflammation, granulation tissue and fecal material in the resected colon lumen. However, application of growth agents led to significantly elevated scores of collagen disposition compared with the control group. The high scores observed in both our study groups for neoangiogenesis, are consistent with the role of both PRP and fibrin sealant in wound healing. High concentrations of platelets in PRP are responsible for secretion of growth factors and are related to formation of new blood vessels and tissue regeneration [24], which are responsible for providing injured tissues with

oxygen and nutrients [25–28]. Respectively, fibrin sealant has been noted to augment re-epithelialization, neoangiogenesis, and fibroblastic concentration in the treated area thus promoting wound healing [29]. The absence of remnant endometriotic cells in the examined specimen is important owing to the infiltrating nature of the disease and indicates the efficacy of shaving to achieve complete lesion excision. Moreover, application of growth factors was not shown to promote disease recurrence.

To the best to our knowledge, this is the first study to evaluate the above factors in shaved colonic surface. Various experimental protocols have reported comparable outcomes on the efficacy of growth factors on colonic anastomosis [14,30]. Yol et al and Yamaguchi et al detected high collagen disposition, pronounced fibroblasts in colonic anastomosis of rats in the PRP group [14,30]. On the other hand, reduced collagen disposition was demonstrated by Van der Vijver et al 5 days after application of fibrin glue in intestinal anastomosis in rats compared with controls [31]. Nevertheless, studies considering also several morphologic findings, demonstrated the role of specific tissue adhesives in inducing fibroplasia and collagen disposition [13,32].

The randomization of the animals for the second phase of the experiment (agent application) suggests a major strength of the current study. Additionally, the pathologist who performed the histopathologic analysis was blinded to the agent applied to each animal and the experiments were performed by the same investigator. Furthermore, adoption of score for evaluating the healing parameters enhanced the validity of the outcomes. One major limitation in the current study is that the findings cannot necessarily indicate the impact of the examined agents on humans. The small size of each group may also interfere in the validity of the outcomes. Nonetheless, as this is the first study examining the healing and regenerative effect of PRP and fibrin sealant on shaved surfaces of experimentally induced endometriosis, it could be the basis for further investigation in this field.

Conclusion

The findings of the current study indicate that both PRP and fibrin sealant are safe and associated with improved tissue healing during shaving for the excision of colonic endometriosis. This is attributed to the enhanced collagen disposition, neovascularization, and protection of the integrity of colon layers. However, these findings are based on an experimental model, and clinical trials are warranted to confirm the feasibility of PRP and fibrin sealant in the clinical setting.

Author contribution: Vasilios Pergialiotis and Despina Perrea had the principal idea, designed the experiments and provided consultation during MS writing. Anastasia Prodromidou performed the operations, performed the statistical analysis and wrote the manuscript. Machairas Nikolaos wrote the manuscript. Kostakis D Ioannis performed the statistical analysis and wrote the manuscript. Maximos Frountzas performed the operations. Laskarina M Korou was the principal veterinary doctor, provided anaesthesia, and analgesia and handled postoperative animal care. Kitty Pavlakis performed the pathology analysis and provided consultation to the manuscript. Dimitrios Dimitroulis and Georgios Vaos wrote the MS. All authors reviewed the final draft of the MS.

References

1. Koninckx PR, Ussia A, Adamyan L, Wattiez A, Donnez J. Deep endometriosis: definition, diagnosis, and treatment. *Fertil Steril*. 2012;98:564–571.

2. Malzoni M, Di Giovanni A, Exacoustos C, et al. Feasibility and safety of laparoscopic-assisted bowel segmental resection for deep infiltrating endometriosis: a retrospective cohort study with description of technique. *J Minim Invasive Gynecol*. 2016;23:512–525.

3. Ercoli A, D'Asta M, Fagotti A, et al. Robotic treatment of colorectal endometriosis: technique, feasibility and short-term results. *Hum Reprod*. 2012;27:722–726.

4. Landi S, Pontrelli G, Surico D, et al. Laparoscopic disk resection for bowel endometriosis using a circular stapler and a new endoscopic method to control postoperative bleeding from the stapler line. *J Am Coll Surg*. 2008;207:205–209.

5. Roman H, Bubenheim M, Huet E, et al. Conservative surgery versus colorectal resection in deep endometriosis infiltrating the rectum: a randomized trial. *Hum Reprod*. 2018;33:47–57.

6. Leo MS, Kumar AS, Kirit R, Konathan R, Sivamani RK. Systematic review of the use of platelet-rich plasma in aesthetic dermatology. *J Cosmet Dermatol*. 2015;14:315–323.

7. Filardo G, Kon E, Roffi A, Di Matteo B, Merli ML, Marcacci M. Platelet-rich plasma: why intra-articular? A systematic review of preclinical studies and clinical evidence on PRP for joint degeneration. *Knee Surg Sports Traumatol Arthrosc*. 2015;23:2459–2474.

8. Tambella AM, Attili AR, Dupre G, et al. Platelet-rich plasma to treat experimentally-induced skin wounds in animals: a systematic review and meta-analysis. *PloS One*. 2018;13:e0191093.

9. Stergios K, Kontzoglou K, Pergialiotis V, et al. The potential effect of biological sealants on colorectal anastomosis healing in experimental research involving severe diabetes. *Ann R Coll Surg Engl*. 2017;99:189–192.

10. Jung MS, Choi CH, Yu MS. Comparison of the effect of aerosolized fibrin sealant and biodegradable synthetic polyurethane foam on hemostasis and wound healing after endoscopic sinus surgery: a prospective randomized study. *Int Forum Allergy Rhinol.* 2017;7:1089–1094.

11. Angioli R, Plotti F, Ricciardi R, et al. The use of novel hemostatic sealant (Tisseel) in laparoscopic myomectomy: a case-control study. *Surg Endosc.* 2012;26:2046–2053.

12. Liu YC, Li YC, Kuo HH, Wang CJ, Wu KY. The use of fibrin sealant (Tisseel) in laparoscopic excision of ovarian endometrioma. *Taiwan J Obstet Gynecol*. 2017;56:342–345.

13. Zhou B, Ren J, Ding C, et al. Protection of colonic anastomosis with platelet-rich plasma gel in the open abdomen. *Injury*. 2014;45:864–868.

14. Yol S, Tekin A, Yilmaz H, et al. Effects of platelet rich plasma on colonic anastomosis. *J Surg Res*. 2008;146:190–194.

15. Prodromidou A, Pergialiotis V, Pavlakis K, et al. A novel experimental model of colorectal endometriosis. *J Invest Surg*. 2017;May 25:1–7.

16. Roman H, Moatassim-Drissa S, Marty N, et al. Rectal shaving for deep endometriosis infiltrating the rectum: a 5-year continuous retrospective series. *Fertil Steril*. 2016;106:1438–1445.

17. Messora MR, Nagata MJH, Furlaneto FAC, et al. A standardized research protocol for platelet-rich plasma (PRP) preparation in rats. *RSBO*. 2011;8:299–304.

18. Donnez O, Roman H. Choosing the right surgical technique for deep endometriosis: shaving, disc excision, or bowel resection? *Fertil Steril*. 2017;108:931–942.

19. Zubaidi AM, Hussain T, Alzoghaibi MA. The time course of cytokine expressions plays a determining role in faster healing of intestinal and colonic anastomatic wounds. *Saudi J Gastroenterol*. 2015;21:412–417.

20. Gurtner GC, Werner S, Barrandon Y, Longaker MT. Wound repair and regeneration. *Nature*. 2008;453:314–321.

21. Kaufman LC, Smyrk TC, Levy MJ, Enders FT, Oxentenko AS. Symptomatic intestinal endometriosis requiring surgical resection: clinical presentation and preoperative diagnosis. *Am J Gastroenterol*. 2011;106:1325–1332.

22. Lagana AS, Vitale SG, Trovato MA, et al. Full-thickness excision versus shaving by laparoscopy for intestinal deep infiltrating endometriosis: rationale and potential treatment options. *Biomed Res Int*. 2016;2016:3617179.

23. Abrao MS, Borrelli GM, Clarizia R, Kho RM, Ceccaroni M. Strategies for management of colorectal endometriosis. *Semin Reprod Med*. 2017;35:65–71.

24. Carmeliet P, Jain RK. Molecular mechanisms and clinical applications of angiogenesis. *Nature*. 2011;473:298–307.

25. Mammoto T, Jiang A, Jiang E, Mammoto A. Platelet rich plasma extract promotes angiogenesis through the angiopoietin1-Tie2 pathway. *Microvasc Res*. 2013;89:15–24.

26. Schar MO, Diaz-Romero J, Kohl S, Zumstein MA, Nesic D. Platelet-rich concentrates differentially release growth factors and induce cell migration in vitro. *Clin Orthop Relat Res*. 2015;473:1635–1643.

27. Kakudo N, Morimoto N, Ogawa T, et al. Angiogenic effect of platelet-rich plasma combined with gelatin hydrogel granules injected into murine subcutis. *J Tissue Eng Regen Med*. 2017;11:1941–1948

28. Matsui M, Tabata Y. Enhanced angiogenesis by multiple release of platelet-rich plasma contents and basic fibroblast growth factor from gelatin hydrogels. *Acta Biomater*. 2012;8:1792–1801.

29. Jeschke MG, Finnerty CC, Shahrokhi S, Branski LK, Dibildox M. Wound coverage technologies in burn care: novel techniques. *J Burn Care Res*. 2013;34:612–620.

30. Yamaguchi R, Terashima H, Yoneyama S, Tadano S, Ohkohchi N. Effects of platelet-rich plasma on intestinal anastomotic healing in rats: PRP concentration is a key factor. *J Surg Res*. 2012;173:258–266.

31. van der Vijver RJ, van Laarhoven CJ, de Man BM, Lomme RM, Hendriks T. The effect of fibrin glue on the early healing phase of intestinal anastomoses in the rat. *Int J Colorectal Dis*. 2012;27:1101–1107.

32. Vakalopoulos KA, Wu Z, Kroese LF, et al. Sutureless closure of colonic defects with tissue adhesives: an in vivo study in the rat. *Am J Surg*. 2017;213:151–158.

Fig. 1 The application of the fibrin sealant (TISSEEL; Baxter Healthcare Corporation, Deerfield, IL) in the treated colon area (serosa) from which the endometriotic lesion was shaved and excised.

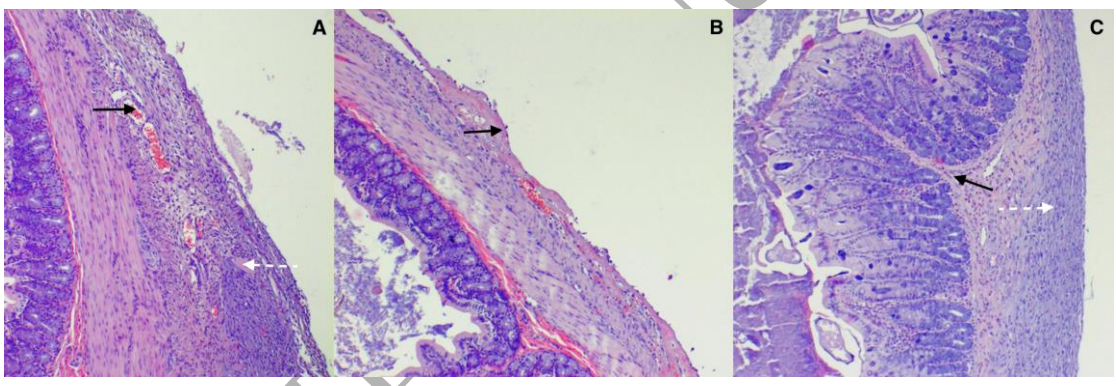

Fig. 2 Histopathological appearance of the colon layers 4 days following shaving of the endometriotic lesion. (**A**) After application of platelet rich plasma (PRP group), increased fibroblasts, blood vessels (black arrow), granulation tissue, and collagen disposition (white arrow) with moderate presence of inflammatory cells and limited impaired colon layers (hematoxylin and eosin [H&E] x40). (**B**) After application of the fibrin sealant (TISSEEL; Baxter Healthcare Corporation, Deerfield, IL), intensive fibroblasts concentration, collagen (black arrow), granulation tissue disposition, and blood vessel formation were observed with moderate presence of inflammatory cells and slight loss of colon layer integrity (H&E x40). (**C**) The control group with no agent applied showing less fibroblasts, granulation tissue, blood vessels, and poor collagen

disposition with intensive infiltration of inflammatory cells (white arrow) and loss of colon layer integrity (black arrow) (H&E x40).

Table 1. Weight outcomes among studied groups

	Control (n = 10)	Platelet rich plasma (n = 10)	Fibrin sealant (n = 10)
First surgery (mean ± SD)	274.8 ± 27	252.2 ± 6.8	253 ± 15.8
Second surgery (mean ± SD)	269.8 ± 25.7 p = .498	246.6 ± 10.2 **p = .01**	239.7 ± 16.8 **p = .005**
Sacrifice (mean ± SD)	270.5 ± 24.5 p = .734	248.9 ± 12.9 p = .148	237.3 ± 17.6 p = .528

The p value represents statistical significance among groups comparing first and second surgery and second surgery and sacrifice of the subjects.
SD = standard deviation. Animal weights were measured in grams.

Table 2. Parameter scores

Subject number	Fibroblasts/fibrosis 0–3	Inflammatory cells 0–4	Granulation tissue 0–4	Blood vessels 0–4	Collagen disposition 0–4	Fecal material 0–4	Loss of integrity of colon layers 0–4
Control group							
1	1	4	0	1	0	2	4
2	2	2	1	2	0	1	2
3	0	1	0	0	0	4	1
4	0	2	0	0	0	1	1
5	3	3	3	3	1	4	4
6	3	4	3	3	0	2	3
7	1	2	2	2	1	3	4
8	2	3	2	2	2	1	3
9	3	2	2	2	2	4	2
10	2	2	0	0	0	2	1
Platelet rich plasma group							
11	2	2	0	2	1	3	1
12	3	3	3	4	3	2	4
13	2	3	4	4	3	2	2
14	2	3	3	3	2	3	3
15	3	2	2	3	2	2	0
16	3	3	3	3	3	2	3
17	1	2	2	2	1	1	0
18	1	3	2	2	3	2	2

19	2	1	2	1	3	1	1
20	1	1	1	1	3	2	1
Fibrin sealant group							
21	2	2	1	2	4	2	0
22	2	1	2	3	2	2	1
23	1	3	2	3	1	3	2
24	3	2	3	3	4	2	0
25	3	2	3	2	3	2	2
26	3	2	2	3	2	2	2
27	3	3	3	4	2	2	0
28	3	3	3	4	4	3	2
29	3	3	3	3	3	3	1
30	3	2	3	3	3	3	1

Score 0: no evidence; score 1: minimal parameter expression; score 2: slight parameter expression; score 3: moderate parameter expression; score 4: abundant parameter expression.

Table 3. Analysis of each parameter according to low and high expression (low expression for scores ≤2 and high expression for scores ≥3).

	Fibroblasts-Fibrosis		Inflammatory cells		Granulation tissue		Blood vessels		Collagen disposition		Fecal material		Loss of integrity of colon layers	
	Low	High	Low	High	Low	High	Low	High	Low	High	Low	High	Low	High
Control, n/N	7/10	3/10	6/10	4/10	5/10	5/10	8/10	2/10	10/10	0/10	6/10	4/10	5/10	5/10
Control vs PRP	p = 1.00		p = 1.00		p = .628		p = .35		p = .011		p = .628		p = .650	
Fibrin sealant, n/N	3/10	7/10	6/10	4/10	4/10	6/10	2/10	8/10	4/10	6/10	6/10	4/10	10/10	0/10
Control vs fibrin sealant	p = .179		p = 1.00		p = .170		p = .023		p = .011		p = 1.00		p = .033	
PRP, n/N	7/10	3/10	5/10	5/10	5/10	5/10	5/10	5/10	4/10	6/10	8/10	2/10	7/1	3/10

																		0	
PRP vs fibrin sealant	p = .179		p = 1.00		p = .656		p = .350		p = 1.00		p = .628		p = .211						

Statistical significance was $p < .05$.
PRP = platelet rich plasma.