Obsidian ASG® Autologous Platelet-Rich Fibrin Matrix and Colorectal Anastomotic Healing: A Preliminary Study

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ABSTRACT

Introduction: Anastomotic leakage (AL) following colorectal resection is a devastating complication affecting morbidity, mortality, and quality of life of patients in the long term. Different tissue sealants and biologic glues were tested showing conflicting results regarding their influence on anastomotic healing and leak prevention. Application of autologous platelet-rich fibrin (Vivostat A/S, Alleroed, Denmark), which acts as a source of angiogenic growth factors and cytokines, showed promising results in an in-vivo porcine model. Herein, we present the first human study of stapled colorectal anastomoses supplemented with an autologous-derived platelet-rich fibrin matrix (Obsidian ASG®, Rivolution GmbH, Rosenheim, Germany and Vivostat A/S, Alleroed, Denmark).

Materials and Methods: A retrospective analysis of prospectively accumulated data was performed in two colorectal centers (Linz, Vienna) on patients undergoing left-sided colorectal or coloanal stapled anastomosis between October 2018 and December 2019. The Obsidian ASG® Matrix was applied to the rectal stump, and after closure with the circular stapling device, at the circumference of anastomosis in every single case. Anastomoses were supplemented with intra- and extra-anastomotic application (IAA—intra-anastomotic application developed by Rivolution GmbH, Rosenheim, Germany) of Obsidian ASG® Matrix. The primary endpoints were incidence of perioperative complications and anastomotic leak rate.

Results: Two-hundred-sixty-one (138 female) patients underwent left-sided colonic (n=177) or rectal resection (n=84). In 253 (96.9%) cases, a laparoscopic or robotic-assisted approach was used. There were no complications attributable to the intraoperative application of the Obsidian ASG® Matrix. All intraoperative leak tests were

negative. Overall, anastomotic leak rate accounted for 2.3% (6/261). AL following colonic and rectal resection was seen in 2.3% (4/177) and 2.4% (2/84), respectively. Complication and leak rate was similar in the two participating centers. Postoperative fever and elevated CRP levels were significantly correlated to AL. There was no significant risk factor for AL on multivariate analysis.

Conclusion: Application of an autologous-derived platelet-rich fibrin matrix (Obsidian ASG®) at anastomotic site following colorectal resection is safe and associated with a low rate of anastomotic leakage.

INTRODUCTION

Anastomotic leakage (AL) remains a feared complication following colorectal resection with detrimental effects on health economics as well as perioperative morbidity and mortality effecting patient's quality of life in the mid and long term.[1-3] Construction of a safe (colorectal) anastomosis remains reliant upon a lack of tension and an adequate blood supply[4] with recent improvements afforded by technical advances such as the use of curved cutting staplers for lower coronal or sagittal rectal transection[5] and powered solutions designed to improve the precision and stability of the stapler.[6] However, leak rates remain high especially following (low) anterior rectal resection. There is extensive experimental literature on the impact of tissue sealants on anastomotic healing[7,8] where a range of biologic glues can be applied as an added layer of security after standard anastomotic construction.[9] Stapled anastomoses with a supplemental fibrin sealant have proven to be associated with less foreign body reaction and fibrotic scar tissue formation.[10] There is also a correlation between anastomotic integrity and thrombocyte activation, the extent of fibroblast infiltration, the level of angiogenesis, and the synthesis of fibrillar Type I and III collagen.[11,12] Although there are conflicting results, several animal models of colonic anastomosis supplemented by fibrin sealants have shown higher burst strength and fewer adhesions than the control group.[13-15] However, a human study in laparoscopically constructed rectal anastomoses supplemented by fibrin sealant failed to show any beneficial effect on AL rate.[16]

Platelet-rich plasma (PRP) permits the release of multiple growth factors involved in cellular proliferation and angiogenesis. Subsequently, PRP enables platelet cascade activation providing a richer source of stable fibrinogen compared to fibrinogen production derived from polymerization of autologous blood after the addition of exogenous thrombin.[17,18] In particular, platelet-rich fibrin (PRF) offers an alternative where the framework of the concentrate allows a slower release of proteolytic growth factors.[19,20] Technique of optimal automated PRF production capable of generating predictable levels of fibrin regardless of the initial fibrinogen concentration was already published in 1997 and 2002.[21,22] Dauser et al. published the results of a porcine model with the application of a novel autologous platelet-rich fibrin Obsidian ASG® Matrix (hereafter "matrix", Rivolution GmbH, Rosenheim, Germany) as a supplement to colonic anastomotic healing.[23] In this study, matrix-treated animals showed a greater deposition of mature collagen and a stronger angiogenic response than that of untreated animals, although there was no matrix effect on anastomotic line thickness, granulation tissue formation, or foreign-body reactivity. These findings were accompanied by a higher in vitro bursting strength of the matrix-treated anastomoses. Herein, we present the first human study using an autologous platelet-rich fibrin matrix as a supplement during formation of a double-stapled colorectal or coloanal anastomosis investigating anastomotic leak rate.

PATIENTS AND METHODS

With ethics committee approval by the State Ethics Commission for Upper Austria (Kepler University Hospital, Linz, Austria) and the local Ethics Committee of the St. John of God Hospital, Vienna, Austria, patients undergoing elective left-sided colonic or rectal resection between October 2018 and December 2019 were enrolled. Written informed consent was obtained from every patient. Patients with underlying hematologic malignancy and those undergoing emergency surgery were excluded. Demographic, peri- and postoperative data as well as any adverse events associated with Obsidian ASG® Matrix application were recorded. All procedures were performed by consultant colorectal surgeons especially trained to enable a standardized sequence for retrieval and application of the Obsidian ASG® Matrix. Level of anastomosis was recorded measuring the distance from the anal verge.

Besides platelet-rich fibrin matrix application to anastomosis, there were no changes regarding the standard protocol of pre-, peri-, and postoperative patient care. In every case, oral mechanical bowel preparation was given followed by oral antibiotics (paromomycin sulfate 8g) one day before surgery. In addition, an intravenous single shot with amoxicillin/clavulanic acid or cefuroxime and metronidazole was administered on the day of operation. Indocyanine green (ICG) fluorescence imaging was done intraoperatively.[24]

All operations in center 1 were performed by five experienced laparoscopic and colorectal surgeons. In center 2, operations were performed by two experienced laparoscopic and colorectal surgeons.

Surgical technique and preparation of matrix

To reduce any bias, construction of anastomoses was standardized and performed using a double-stapled technique with linear and circular stapling devices (easyEndo™ Flex, easyCS, EziSurg Medical, Shanghai, China). In the upper rectal third (anastomosis located within 12–16cm from the anal verge), a 60mm blue cartridge was used for transection of the rectum; whereas, for the middle (7–11cm) and lower rectal third (≤6cm), two (or more) 45mm green cartridges were applied. Preparation of the Obsidian ASG® Matrix has been previously described.[23] Briefly, all patients had 120mL of whole blood withdrawn preoperatively with a minimum time of

45 minutes between the perioperative antibiotic infusion and blood removal since transient alteration of the pH with the antibiotics (e.g., cephalosporines) can interfere with fibrin polymerization.[25] The matrix is prepared with 300mg tranexamic acid and transferred to a processing unit[26] comprised of a fully automated Vivostat microprocessor-controlled system (Vivostat A/S, Alleroed, Denmark). Blood is heated to 36°C and separated by centrifugation in the upper reservoir chamber of the processing unit with transfer of the plasma to a mixing chamber where it is merged with 30 units of Batroxobin. The mixture contains acid-soluble fibrin 1, which polymerizes and allows excess fibrinogen- and thrombocyte-depleted serum to return to the waste chamber leaving concentrated fibrin 1 and thrombocytes. The fibrin concentrate is then mixed with 3.5mL of sodium acetate buffer (pH 4) to dissolve the available fibrin and enable transfer of the acidified fibrin to an empty syringe. The result is a stable clot matrix with the characteristics of high elasticity and tensile strength as well as crack resistance, each of which permits better tissue adherence during intestinal peristalsis.[27] Finally, the matrix contains up to 10-fold thrombocytes (compared to the normal concentration) which are embedded in a fibrin scaffold (Fig. 1). The waste package is a single-use application system which is applicable in open or minimally invasive surgery.

As the strength of an anastomosis in the early phase of healing is mainly reliant on submucosal healing, intra-anastomotic application (IAA) is done spraying 2mL of the matrix directly on to the rectal stump prior to closure of the circular stapling device as shown in Figure 2. Following approximation of bowel ends, additional 2.5–3mL of matrix are sprayed on the outside of the anastomosis circumferentially whenever possible. Integrity of the anastomosis was confirmed in several ways. These included assessment of the completeness of the staple rings, performance of an air-leak test after filling the pelvis with saline, and by intraoperative endoscopy. IAA was first described and developed by Rivolution GmbH, Rosenheim, Germany.

At the site of application, thrombocytes are slowly released and activated over time by fibrin degradation. It is postulated that the matrix acts like other regenerative products by stimulating anastomotic healing through a dense concentration of thrombocytes that can be activated for up to seven days.[28]

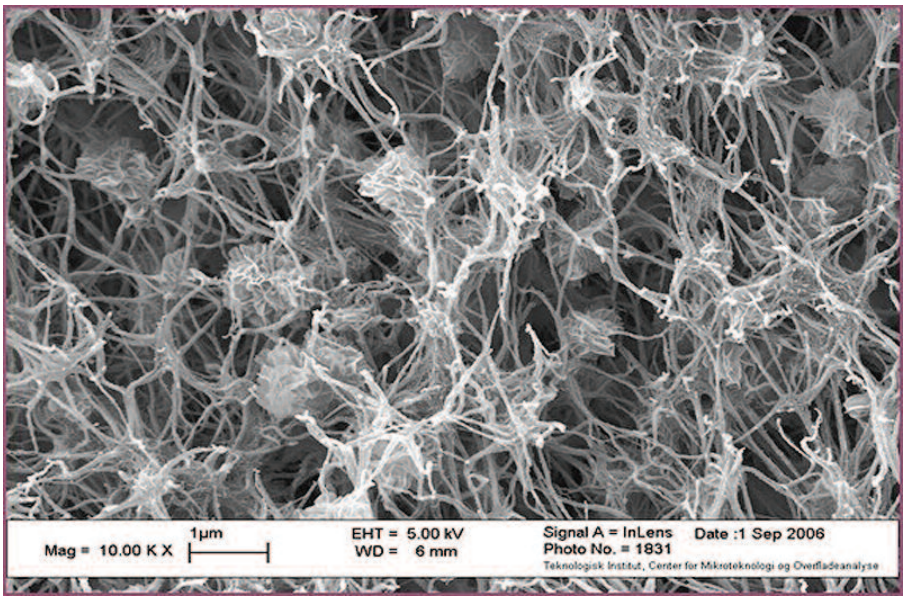

Figure 1. Scanning electron microscope image of the Obsidian ASG© scaffold, magnification.

Anastomotic leakage

In case of postoperative fever >38°C at any point up to 30 postoperative days, signs of AL—leukocytosis, elevated C-reactive protein (CRP) levels >150mg/L, and/or conspicuous fluids via drainage—were sought by imaging (computed tomography [CT] scan with rectal contrast enema), endoscopy, or repeat surgery. All patients with diverting stoma had endoscopy and CT scan with gastrografin enema prior to the closure of the stoma. The definition of an AL was in accordance with the published guidelines of the International Study Group of Rectal Cancer,[29] where severity of a leak is based upon the impact on clinical management. This may range from no change in postoperative care (Grade A), through administration of antibiotics, percutaneous and/or trans anal drainage/revision (Grade B), to repeat abdominal surgery (Grade C). Complications were recorded in accordance with the Clavien-Dindo classification.[30]

Statistics

All data of continuous variables were checked for normal distribution (test of normality: Kolmogorov-Smirnov with Lilliefors significance correction, type I error=10%), and in the case of normal

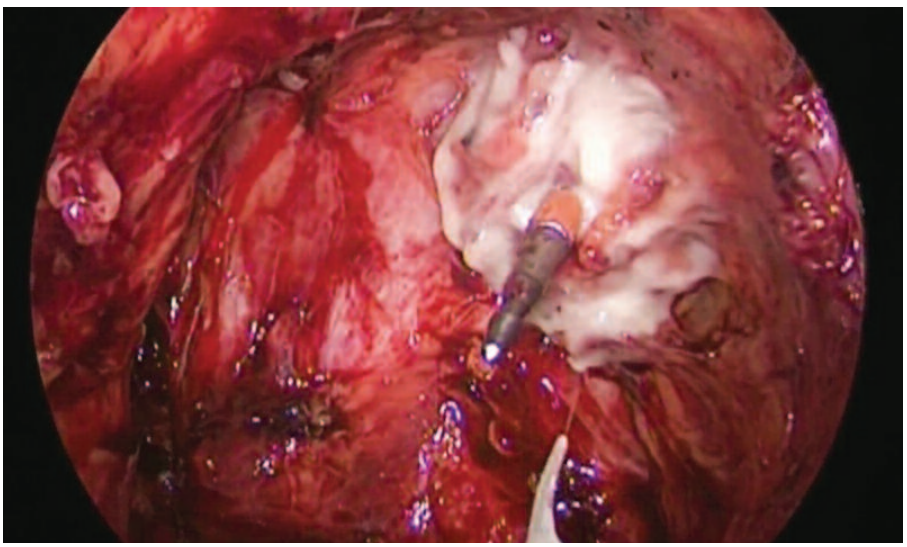

Figure 2. Intra-anastomotic application (IAA) technique—the laparoscopic spray application with the spray applicator on the rectal stump prior to stapled anastomosis.

Table I
Patient demographics of Obsidian-ASG® Matrix-treated colonic left-sided and rectal resections (n=261)

Number	177	84
Male Female	77 100	46 38
Age (years)	64 (23–87)	63 (35–91)
BMI (kg/m²)	26.35 (18.13–39.92)	25.5 (16.0–38.5)
Comorbidities		
Smokers	24 (13.5%)	5 (6.0%)
Diabetes	11 (6.2%)	10 (11.9%)
Hypertension	49 (27.7%)	15 (17.9%)
Heart failure	12 (6.8%)	5 (6.0%)
Asthma/COPD	9 (5.1%)	2 (2.4%)
Hyperlipidaemia	8 (4.5%)	5 (6.0%)
Hypothyroidism	17 (9.6%)	1 (1.2%)
Cirrhosis	1 (0.6%)	1 (1.2%)
Chronic renal failure	10 (5.6%)	2 (2.4%)
Other	1 (0.6%)	2 (2.4%)
Neoadjuvant Therapy		
Chemotherapy alone	2 (1.1%)	30 (35.7%)
Radiotherapy alone	2 (1.1%)	6 (7.1%)
Chemoradiation		5 (6.0%)
		19 (22.6%)

distribution, also for heteroscedasticity (Levene test, type I error=5%). In the case of normality and variance homogeneity, the independent two-sample t-test was used for group comparisons. In the case of normality, but no variance homogeneity, Welch's t-test was applied. For variables without normally distributed data, the exact Mann-Whitney U test was used. Dichotomous variables were compared by Fisher's exact test, the other categorical variables by the exact chi-square test.

For categorical variables, two-sided 95% confidence intervals (CI), according to Clopper-Pearson, were calculated. Continuous variables are reported as medians and quartiles (minimum and maximum in brackets).The type I error was not adjusted for multiple testing. Therefore, the results of inferential statistics are descriptive only.

RESULTS

Two-hundred-sixty-one (138 female) patients were included in this analysis derived from both participating centers. Median age was 64 years (23–91 years) and median body mass index (BMI) accounted for 26kg/m^2 (18–40kg/m^2). Comorbidities and details concerning neoadjuvant therapy are listed in Table I. In 253 out of 261 cases (96.9%), a laparoscopic (n=210) or robotic-assisted (n=43) approach was used. An open access was done only in four colonic and four rectal resections as primary access. There were no conversions to open surgery. Anterior rectal resection with anastomosis located within 7 to 12cm from the anal verge was done in 32 cases. Low anterior rectal resection with anastomosis within 6cm from the anal verge was done in 52 cases. Demographic and perioperative data are also presented in Table II and III. Application of the matrix was possible in all cases with proper polymerization.

Intraoperative air leak tests to check anastomotic integrity were negative in every single case. Overall, leak rate accounted for 2.3% (6/261; CI=0.9–4.9%)). Four times leaks occurred following colonic resection (4/177; 2.3%; CI=0.6–5.7%). In two patients, AL was observed following low anterior rectal resection (2/52; 3.8%; CI=0.5–13.2%). Following anterior rectal resection (n=32) with anastomosis located between 7 and 12cm from the anal verge, no leaks were observed.

Leaks following colonic resection (grading according to the ISREC classification)[29]

One leak occurred in a 78-year-old female patient and was diagnosed on the third postoperative day following laparoscopic colonic resection for sigmoid cancer. A laparotomy with dismantling of the anastomosis and colostomy was done (Grade C). Intestinal reconstruction was performed five month later without further complications.

Another leak was seen following laparoscopic left hemicolectomy in a 72-year-old man for chronic diverticular disease. In this case, the anastomosis was refashioned on the sixth postoperative day with the addition of a protective ileostomy which has subsequently been closed (Grade C).

A further 79-year-old male patient underwent a laparoscopic extended left hemicolectomy for cancer which became ischemic on the third postoperative day, and which was converted to a Hartmann's procedure (Grade C).

Finally, a leak occurred in a 51-year-old woman after laparoscopic sigmoidectomy for diverticular disease. The patient presented with fever, pain, and bowel obstruction on the third postoperative day. The leak was seen at the intersection of the linear and circular staple line. A redo anastomosis without a protective stoma was done and the patient was discharged from hospital on postoperative day 11 (Grade C).

Leaks following rectal resection (grading according to the ISREC classification)[29]

In a 53-year-old female patient, following neoadjuvant chemotherapy due to advanced cancer stage, a laparoscopic low anterior rectal resection was performed. Initially, no stoma was raised. The patient left the hospital on postoperative day six but returned with pain and fever one week later. On CT scan, air bubbles around the anastomosis were visible without abscess formation. Intravenous antibiotics were administered, and no further intervention was necessary due to resolution of symptoms (Grade B).

Another 65-year-old obese (BMI= 35kg/m²) male patient received neoadjuvant chemoradiation and consecutive laparoscopic low anterior rectal resection with a protective ileostomy. CT scan was performed due to renal colic and, again, free air surrounding the site of

Table II
Patient demographics and perioperative details for the participating centers (Linz, Vienna)

	COLONIC RESECTION		RECTAL RESECTION	
	LINZ	**VIENNA**	**LINZ**	**VIENNA**
Protective Stoma			28	15
Neoadjuvant Therapy				
None	136	38	24	28
Chemotherapy alone	1	1	0	6
Radiotherapy alone			2	3
Chemoradiation			14	5
Total	137	39	40	42
Access				
Laparoscopic	134	20	38	18
Robotic	0	19	0	24
Open	3	1	2	2
Type of procedure				
Left sided resection	137	40		
Anterior resection			10	22
Low anterior resection			30	22
Complications (Clavien-Dindo Classification)				
None	124	34	35	43
Grade III/IV	8	3	2	2
Length of postoperative hospital stay (range)	7 (4–31)	5 (4–19)	13 (6–22)	6.5 (3–17)
Anastomotic leakage				
Grade A	0	0	1	0
Grade B	0	0	0	1
Grade C	4	0	0	0

Grading of leakage according to the ISREC[29] classification.

anastomosis was found. Due to the presence of ileostomy and lacking (systemic) symptoms, no intervention was necessary (Grade A).

In general, the number of postoperative days with fever ($p=0.004$) and elevated CRP levels ($p>0.001$) were clearly associated with AL. In patients receiving neoadjuvant therapy ($n=32$), AL was associated with the level of anastomosis ($p<0.001$), postoperative fever ($p=0.017$), prolonged time for operation ($p<0.001$), absence of fecal diversion ($p<0.001$), and history of diabetes mellitus ($p=0.007$).

Other complications

There was no difference regarding the incidence of Grade III/IV Clavien-Dindo complication following colonic (5.8% vs. 7.5%) or rectal resection (5% vs. 4.5%) between the two centers (for each comparison: $p>0.05$). One patient presented with trans anal hemorrhage on the first postoperative day following low anterior resection. In this case, anastomotic staple line bleeding was evident during endoscopy and successfully clipped consequently. In one further patient, fever on day 10 following surgery was observed which resolved spontaneously. Postoperative endoscopy and CT scan showed no evidence of anastomotic leakage in this patient. The other complications according to Clavien Dindo III/IV are listed in Table IV.

The length of postoperative hospital stay in the two participating centers was seven days (six to eight) versus five days (five to seven) ($p=0.003$) following colonic and 13 days (10–21) versus 6.5 days (five to eight) ($p<0.001$) following rectal resection (Linz, Vienna).

The mortality rate was zero within the study cohort.

DISCUSSION

This single-arm study from two participating colorectal units is the first report on the clinical use of the autologous platelet-rich fibrin, Obsidian ASG® Matrix as a supplement to colorectal anastomosis. The matrix was specifically applied over the intra- and extra-anastomotic areas with performance of a standardized double-stapling technique. Application of the matrix was safe and was not associated with any particular complication recognized so far. The overall AL rate was low (2.3%). On univariate analysis, postoperative fever, elevated CRP levels, prolonged operating time, no protective stoma, and a history of diabetes was correlated with AL.

Since the introduction of the double stapling technique, rates of AL remained inadmissibly high, ranging between 3–19%.[31–35] Lack of any tension and

Table III
Patient demographics and perioperative details between the two participating centers

	COLECTOMY			LAR /TME		
	CENTER 1	**CENTER 2**		**CENTER 1**	**CENTER 2**	
Age (years)	63 (23–87)	69 (38–86)	*	64 (35–86)	62 (37–91)	
Males **Females**	65 72	12 28		23 17	23 21	
BMI (kg/m²)	26 (18.13–39.92)	27.1 (19.4–39.0)		25.49 (18.21–34.72)	25.54 (18.0–38.5)	
Operative Duration (min)	134 (63–271)	N/A		181 (62–376)	N/A	
Anaesthetic Duration (min)	191 (107–403)	N/A		242.5 (88–421)	N/A	
Smokers	24	N/A		5	N/A	
Protective Stoma				28	15	**
Neoadjuvant Therapy None Chemotherapy alone Radiotherapy alone Chemoradiation	 136 1	 38 1		 24 0 2 14	 28 6 3 5	 **
Site 1 Upper-third Middle-third Lower-third				 8 19 11	 9 11 16	
Benign Pathology				2	6	
Operative Method Laparoscopic Robotic Open	 134 0 3	 20 19 1	 **	 38 0 2	 18 24 2	 **
Operation Sigmoidectomy High Anterior Resection Low Anterior Resection TME	 70 67	 35 5	 **	 10 30	 22 22	 *
<u>Clavien-Dindo Complications</u> None Grade III/IV	 124 8	 34 3		 35 2	 42 2	
LOHS (days)	7 (4–>20)	5 (4–19)	**	13 (6–>20)	6.5 (3–17)	**

1 Includes an additional two cases where a sigmoidectomy was also performed
N/A Data not available
LOHS Length of hospital stay
TME Total mesorectal excision
* $p<0.05$
** $p<0.01$

well-vascularized bowel ends are basic requirements for appropriate anastomotic healing. However, rate of leakage has been stable for decades and did not improve even after introduction of novel instruments and techniques.[36] However, standardization may improve surgical quality. For example, Kuroyanagi et al. used a standardized protocol for rectal transection and construction of anastomosis resulting in a favorable low leak rate.[37] In addition, indocyanine-green (ICG) fluorescence has been shown to be able to reduce the rate of AL.[38] A standardized protocol and ICG fluorescence were used in this series as well, contributing to the low rate of leakage. The leakage rate in our center was in the period from 2010 to 2015—4.9% for rectal cancer with low anastomosis and 4.6% for colonic anastomosis. These data are from the database from our clinic for quality control. From 2016 to 2018, the leak rate for low anastomosis was 6.5%. These data are from the Upper Austrian quality control program which is comparable to the US NSQIP system. All patients who underwent rectal resection in one of the 10 public clinics were analyzed by the health authority.

Comparing our results to other series is difficult for various reasons. First, there is no clear definition for anastomotic leak and its diagnosis and treatment. Second, the use of proximal fecal diversion affects the appearance and diagnosis of an AL as well as the likelihood of surgical reintervention. Subclinical radiological leak rate is estimated to be about 2.7 times higher than the clinical one.[31] Therefore, the clinical benefit of an adjunctive tissue sealant may be less evident in diverted patients.

The technique of the matrix application as used in our study was designed to take advantage of the importance of the submucosal healing to gain sufficient anastomotic tensile strength. The submucosal layer contains the bulk of collagen and elastin and has traditionally been the suture-holding layer in hand-sutured anastomoses.[39] Staples crushing through the entire bowel wall make no distinction of these individual layers. Strength of anastomosis in the very early phase after construction is dependent upon the staple-holding capacity of the prevalent collagen. Future work is needed to assess the molecular dynamics of collagen synthesis and degradation with matrix application during the acute inflammatory (lag), proliferative and remodeling/maturation phase of normal anastomotic healing. Dauser et al. examined some of these effects in a pre-clinical study in a porcine model.[23]

Table IV
Other complications Clavien-Dindo III/IV (without leakage)

Patient Nr.	Clavien-Dindo	Complication
8	3b	Day 4: Incarcerated trocarhernia—revision pop
50	4	Day 9: Stroke
76	3b	Day 18: Readmission: small bowel obstruction, anastomosis was fine
99	3b	Day 10: Stenosis of loop ileostomy—revisional surgery—new ileostomy
108	3b	Day 8: revisional surgery due to serös peritonitis—anastomosis without leak intraoperatively: lavage
137	3b	Day 1: Revisional surgery due to postoperative bleeding in a hemophilia patient
161	3b	Day 3: Ischämia of the transverse colon, descending colon was fine—no leak. Revisional surgery

Clinical data using fibrin sealant application in stapled colorectal anastomosis is limited. Huh et al.[16] used fibrin glue applied to stapled anastomosis externally following low anterior rectal resection reducing the rate of leakage compared with untreated cases (5.8% vs. 10.9%, respectively; p=0.169). However, this study was not randomized, and the majority of the sealant-treated cases were included later on with the growing experience of surgeons using laparoscopy for rectal cancer surgery. Experimental animal data focusing on the use of fibrin sealants or PRP is conflicting with elevation of leak rate in a rat model.[40] Others reported paradoxical increase in adhesion formation in combination with lower local tissue collagen concentration despite higher in vitro anastomotic bursting pressure of anastomosis.[41] However, available data is difficult to interpret as studies have used unique experimental designs attempting to simulate high-risk colorectal anastomoses.[42] Recently, the focus has set on alteration of the gut microbiome having an impact on AL.[43] Future research is necessary to study the effect of fibrin and PRF sealants on the local microbiome in stapled anastomoses.

Interpretation of data collected for this series is limited as there was no control group for comparison. Nevertheless, our group has obtained institutional review board approval to perform a prospective controlled randomized, double-arm, multi-institutional study. The effect of the matrix on AL following rectal resection and double-stapling anastomosis will be examined.

In summary, application of intra- and extra-anastomotic Obsidian ASG® Matrix is safe and associated with a low risk of AL following colorectal resection. STI

AUTHORS' DISCLOSURES

The authors have no conflicts of interest to disclose.

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Surg Technol Int. 2021, November 23;39. pii: sti39/1508