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Sutureless closure of colonic defects with tissue adhesives: an in vivo study in the rat

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Abstract

BACKGROUND: Tissue adhesives (TAs) in gastrointestinal surgery are gradually gaining acceptance. Before implementation as colonic sealants, an evaluation of the sealing capability of a TA when in contact with fecal matter, as in a leaking anastomosis, is needed. In this study, we used clinically available TAs for the sutureless closure of colonic defects evaluating mechanical strength and tissue healing.

METHODS: A total of 160 rats were divided into 8 groups. Two .5-cm incisions were created, one in the proximal and another in the distal colon. Incisions were sealed with a TA: Histoacryl Flex, Bioglue, Dermabond, Tissucol, Duraseal Xact, gelatin-resorcinol-formaldehyde or Glubran 2. A control group was included in which the colonic defects were not sealed. Follow-up time was 3 or 10 days. Clinical complication rate, bursting pressure, and histopathologic analysis was included.

RESULTS: Leakage rates in the TA groups were highest for Duraseal Xact, Bioglue, and gelatin-resorcinol-formaldehyde at 3 and 10 days. The cyanoacrylates Glubran 2, Histoacryl Flex, and Omnex, and the fibrin glue Tissucol showed the lowest overall clinical complication rates while maintaining the highest bursting pressure at day 10. Histoacryl Flex exhibited significantly higher collagen formation at day 10 than the other TAs.

CONCLUSIONS: This experimental model evaluates the protective effect of a TA seal on a leaking colonic defect. We found large differences in leakage rates and inertness of the tested TAs. The cyanoacrylates Histoacryl Flex, Omnex, and Glubran 2 as well as the fibrin glue Tissucol demonstrated the lowest leakage rates and the most inert histopathologic profile while maintaining high mechanical strength.

Anastomotic leakage (AL) rates in gastrointestinal (GI) surgery remain unacceptably high, ranging from 5% to 15%, with subsequent mortality rates of up to 32%.[1–3] The sealing of a GI anastomosis with a tissue adhesive (TA) has been a major focus of surgical research during the past years.[4–7] Present-day TAs can be grossly divided into 4 categories based on their chemical composition:

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cyanoacrylates (CAs), fibrin glues (FGs), polyethylene glycol (PEG) adhesives and, at last, biological adhesives, which contain albumin and/or gelatine.[8] In upper GI surgery, the use of TAs has become standard clinical practice, for example, in staple line sealing with FG after gastric bypass in bariatric surgery.[9] Furthermore, research indicates that the sealing of the esophageal and the pancreaticoduodenal anastomosis with PEG adhesives and FGs may decrease AL and leakage-related complications.[10–16] In colorectal surgery, despite a broad range of experimental studies, anastomotic sealing with TAs has not yet been implemented into regular clinical practice.[6,8]

To investigate the potential of TA use in colorectal surgery, we have proposed a stepwise validation of TAs for the sealing of the colorectal anastomosis, minimizing confounding factors and enabling a sound comparison between various TAs by using the same experimental model for all TAs. In this bottom-up approach, we started with an experimental model in which 11 TAs were applied on ex vivo rat colon to evaluate mechanical strength. Rheologic characteristics of the TAs were also studied to provide information on their degree of cohesiveness, and in turn, flexibility. We found that CAs were the most promising TAs, maintaining high mechanical strength and flexibility of the glue bond with a high amount of cohesiveness, enabling the absorption of external forces.[8]

In a follow-up in vivo study, the best performing 7 of the 11 TAs were used to glue the serosal surface of 2 intact (eg, without any defect) colonic segments to each other in a sutureless manner, providing information on the inertness of each TA when used on the colon. Clinical, mechanical and (immuno)histopathologic analysis pointed toward large differences between TAs, with the biological TAs (gelatin-resorcinol-formaldehyde [GRF] and Bioglue) showing high mechanical strength but also toxic effects on the colonic wall, leading to ulceration and necrosis. FGs and PEG adhesives exhibited an inert (immuno)histopathologic profile, combined with low mechanical strength. CAs demonstrated high mechanical strength while remaining inert, not causing any toxic effects on colonic tissue.[17]

In the present study, we continue this stepwise validation with a novel in vivo model in which iatrogenic colonic defects are sealed using the same set of 7 TAs, as included in our previous in vivo study. The present model evaluates the protective effect of a TA barrier in terms of intraperitoneal leakage of bowel contents and healing capability, when used to seal a colonic defect in a sutureless manner.

Methods

This study was approved by the ethical committee on animal experimentation, under supervision of the Erasmus University Rotterdam (permit number 105-12-03). This manuscript was written according to the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines.[18] One hundred and sixty inbred specified-pathogen-free male Wistar rats of 2-month-old weighing 250 to 300 gm were obtained from a licensed breeder (Charles River Laboratories, MA). Rats were housed according to standard laboratory conditions, including individually ventilated cages with unrestricted access to standard rat chow and water. An acclimatization period of 1 week was observed before the start of the experiment. Rats were scored daily using an adapted wellness score to assess the onset of peritonitis.[19]

We evaluated 7 TAs, as listed in Table 1. In total, 20 rats were included per TA: 10 rats for short-term (3 days) and 10 rats for long-term (10 days) follow-up. A power analysis was calculated based on an increase of 25 mm Hg (δ) in bursting pressure (BP) between the different experimental groups at day 3. With a standard deviation of 20 mm Hg and an alpha of .05, for a power of 80%, 10 rats were needed per group. All TAs except GRF and Glubran 2 were approved by the US food and drug administration at the time of the study and were used in an off-label manner for the purposes of the present study. Glubran 2 and GRF TAs were CE approved at the time of the study. A control group was also included, in which no TA was applied to the defect, simulating the natural course of an untreated colonic perforation. Rat allocation to each group was performed in a randomized manner by an independent researcher not involved in the experiment. In this study, we opted for a novel model in which the colonic defect location and technique was highly standardizable and comparable to our previous in vivo study.[17] It was decided not to use a colonic anastomosis model, as to minimize confounding factors associated with variations in surgical technique and TA application. Furthermore, AL especially when due to

Table 1 Included study groups and information on included tissue adhesives

Group	Tissue adhesive	TA category	Composition	Manufacturer
0	None	—	—	—
1	Bioglue	AB	Glutaraldehyde-albumin	Cryolife (Kennesaw, GA)
2	GRF glue	AB	Gelatin-resorcinol-formaldehyde	Microval (St. Just Malmont, France)
3	Histoacryl Flex	CA	n-butyl-2-cyanoacrylate	B. Braun (Tuttingen, Germany)
4	Omnex	CA	2-octyl-cyanoacrylate/butyl lactoyl cyanoacrylate	Ethicon (J&J, Sommerville, NJ)
5	Glubran 2	CA	n-butyl-2-cyanoacrylate and methacryloxy sulfolane	GEM S.r.l. (Viarregio, Italy)
6	Duraseal Xact	PEG	Polyethylene glycol with N-hydroxy succinimide	Covidien (Mansfield, MA)
7	Tissucol	FG	Fibrin glue with aprotinin	Baxter (Deerfield, IL)

AB = albumin-based glue; CA = cyanoacrylates; FG = fibrin glues; GRF = gelatin-resorcinol-formaldehyde; PEG = polyethylene glycol; TA = tissue adhesive.

technical factors and to differences in colonic perfusion would have played a confounding role in the evaluation of TAs.[6]

Surgical technique

Fig. 1 depicts the surgical model. Rats received analgesia (Temgesic; .05 mg/kg subcutaneously) preoperatively and were anaesthetized by isoflurane/O_2 inhalation. The abdomen was shaved, and the skin was disinfected with ethanol 70%, after which the abdominal cavity was opened through a 3-cm midline incision. After identification of the cecum, two .5-cm longitudinal incisions were created: one in the ascending (proximal) colon and another in the descending (distal) colon. The proximal colonic segment was used for histopathologic testing and the distal segment for BP testing. Afterward, the wound edges of each incision were approximated, enabling TA application over the full length of the defect. For each rat, .05 mL of TA was used per incision. Sufficient glue curing time was allowed, ranging between 60 and 240 seconds, based on the manufacturers' guidelines of each TA. The abdominal wall was closed in 2 layers using a continuous suture technique (Safil, 5-0. B. Braun, Germany). An equal second dose of Temgesic was administered 24 hours postoperatively.

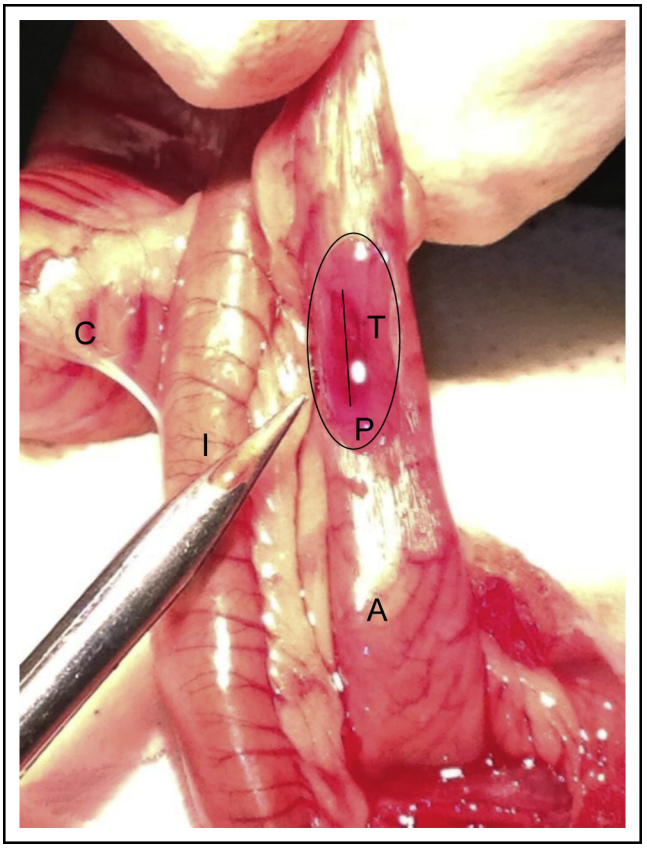

Figure 1 Proximal incision directly after application of Histoacryl Flex. A = ascending colon; C = cecum; I = ileum; P = proximal incision; T = tissue adhesive.

Clinical outcomes

At the end of the follow-up time of 3 or 10 days, or on the onset of clinical signs of peritonitis based on the above mentioned wellness score, rats were anaesthetized, and the abdomen was opened using a U-shaped incision. The abdomen was macroscopically inspected for signs of leakage or TA-related complications, that is, the presence of intraperitoneal abscess or fecal matter and ileus formation. The Zühlke score, which depicts the tenacity of intra-abdominal adhesions, was also determined.[20] Each animal was euthanized by cardiac puncture on completion of the experimental protocol.

BP testing

An air-infusing probe was introduced into the distal colonic segment transanally, and the colon was ligated proximally and distally to the incision site, to ensure an airtight compartment. Air was infused through the probe at a rate of 99 mL/h via an automatic syringe pump (Perfusor Secura, B. Braun, Melsungen, Germany). The setup was connected to a digital pressure indicator (DPI 101, Druck, Leicester, UK). The maximum BP was recorded for each rat.

Histopathologic analysis

Before sacrifice, the proximal incision site was resected and used for histopathologic analysis. All samples were processed with standard histopathologic techniques resulting in 5-μm hematoxylin and eosin (HE)-stained sections for evaluation. On staining, HE slides were scored on inflammatory cell infiltration, fibroblast activity, neoangiogenesis, and collagen deposition using the Modified Phillips Scale.[21] In this scale, each of the histologic parameters is scored from 0 to 4 (0 = no evidence; 1 = occasional evidence; 2 = light scattering; 3 = abundant evidence, and 4 = confluent cells or fibers). H&E scoring was performed during a single session using a multiple-head microscope, in which 3 of the authors (K.V., Z.W., and K.L.), including an experienced pathologist (K.L.), evaluated each slide and provided their scores independently while blinded to the type of TA used. In the (rare) event of interobserver discrepancies in scoring, slides were reexamined and discussed until consensus was reached.

Statistical analysis

For the clinical and histologic data, a Kruskal–Wallis one-way analysis of variance was used, followed by post hoc Dunn's multiple-comparison test. For BP, one-way ANOVA was used, followed by post hoc Bonferonni multiple-comparisons test. A P value of .05 or less was chosen to define statistical significance. All data analyses were performed using MATLAB (Version R2015a; The MathWorks, Inc., Natick, MA).

Results

Clinical outcomes

A synopsis of the clinical outcomes is presented in Table 2. At day 3, mortality in the control group was significantly higher than in the TA groups [$P < .001$, χ^2 (7,71) = 45.45; post hoc analysis: all P values between the control group and the TA groups less than .001] and did not differ significantly between the TA groups. Also, fecal peritonitis rate at day 3 was higher for the control group [$P < .001$, χ^2 (7,70) = 35.74; post hoc analysis: all P values between the control and the TA groups ranging between .0001 and .031, expect for GRF: $P = .101$]. No significant differences in terms of fecal peritonitis were observed between TA groups. Rate of abscess formation at day 3 did not differ significantly between groups, except for Bioglue, which showed a higher rate than the control group ($P = .002$).

At day 10, no significant differences were found in mortality rates between groups [$P = .07$, χ^2 (7.71) = 12.92]. The total leakage rate, including abscess formation and fecal peritonitis was higher for the control group than for Histoacryl Flex ($P = .018$) and Tissucol ($P = .025$). Post hoc analysis showed no further differences between groups for either fecal peritonitis or rate of abscess formation.

Fig. 2 shows the number and Zühlke score of adhesions for each group at days 3 and 10. At day 3, significant differences were found between groups for the number of proximal adhesions [$P = .010$, $\chi^2(7,63) = 18.39$] and the Zühlke score of proximal [$P = .001$, $\chi^2(7,63) = 25.90$] and distal adhesions [$P = .010$, $\chi^2(7,63) = 18.41$] but not for the number of distal adhesions [$P = .524$, $\chi^2(7,64) = 6.13$]. The most prominent results were the lower Zühlke score of proximal adhesions for Glubran 2 as compared to Duraseal Xact ($P = .004$), GRF ($P = .009$), Bioglue ($P = .013$), and Histoacryl Flex ($P = .046$), and the higher Zühlke score of distal adhesions for Bioglue as compared to Glubran 2 ($P = .031$).

At 10 days, significant differences were found between groups for the number of proximal [$P < .001$, $\chi^2(7,60) = 32.53$] and distal adhesions [$P = .007$, $\chi^2(7,60) = 19.28$] but not for the corresponding Zühlke scores [proximal: $P = .100$, $\chi^2(7,60) = 12.02$; distal site: $P = .037$, $\chi^2(7,60) = 14.91$]. The most prominent result was that Duraseal Xact exhibited a higher number of proximal adhesions as compared to Glubran 2 ($P < .001$), Omnex ($P = .004$), and Histoacryl Flex ($P = .007$).

Bursting pressure

BP of the distal incision site is illustrated in Fig. 3. At 3 days, no significant differences were observed between groups [$P = .153$, $F(7,57) = 1.62$]. At 10 days, significant differences were observed [$P < .001$, $F(7,57) = 9.42$], with Histoacryl Flex, Glubran 2, and Tissucol being the 3 strongest TAs. Specifically, Tissucol was stronger than GRF ($P = .004$), Bioglue ($P = .007$), and Duraseal Xact ($P = .010$) and Glubran 2 was stronger than GRF ($P = .029$) and Bioglue ($P = .047$). Histoacryl Flex was stronger than GRF ($P = .039$).

Histopathology

Analysis of histopathologic data is illustrated in Fig. 4. At day 3, no significant differences were observed

Table 2 Synopsis of clinical outcomes

Follow-up	Study group	N	Premature mortality†	Leakage (fecal peritonitis, abscess)	Mechanical ileus
Short term (3d)	Control	10	7	9 (8, 1)	0
	Bioglue	10	0	10 (0, 10)	1
	Histoacryl Flex	10	0	7 (0, 7)	0
	Omnex	9*	0	6 (1, 5)	0
	Glubran 2	10	0	4 (0, 4)	0
	Duraseal Xact	10	1	5 (2, 3)	1
	GRF	10	0	7 (3, 4)	1
	Tissucol	10	0	7 (0, 7)	0
Long term (10d)	Control	10	4	7 (4, 3)	1
	Bioglue	10	2	5 (2, 3)	4
	Histoacryl Flex	10	1	0	0
	Omnex	9*	0	2 (0, 2)	0
	Glubran 2	10	0	2 (0, 2)	0
	Duraseal Xact	10	3	3 (3, 0)	3
	GRF	10	1	4 (2, 2)	2
	Tissucol	10	0	0	0

Numbers depict total amount of rats per specified outcome.
GRF = gelatin-resorcinol-formaldehyde.
*Died perioperatively due to anesthetic complications.
†Mortality occurring before the completion of the follow-up time (either 3d or 10d).

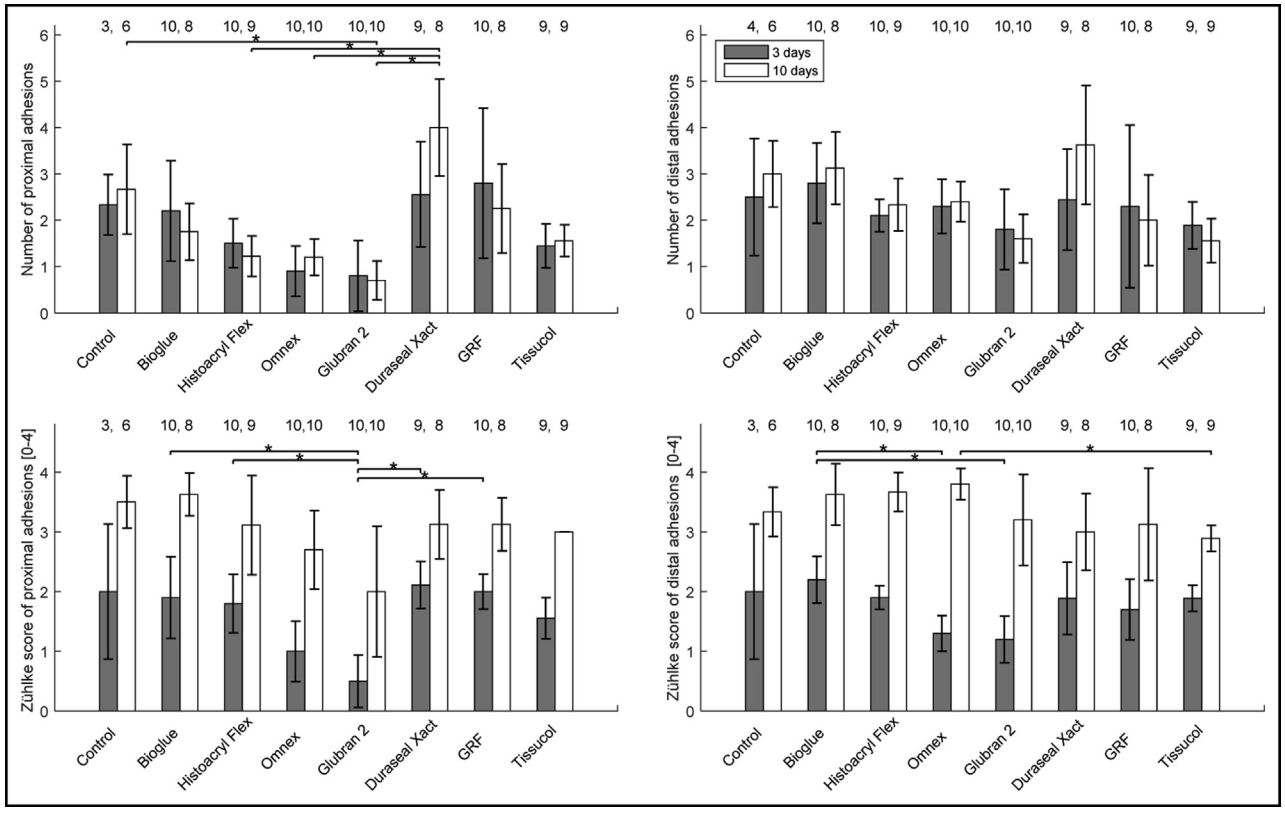

Figure 2 Amount and strength of adhesions at the proximal and distal bowel defect at days 3 and 10. Error bars represent 95% confidence interval. Asterisks annotate statistical significance between the connected groups. Numbers at the top of each bar indicate the amount of rats used for each analysis.

between groups for any of the analyzed parameters, except that Bioglue exhibited a lower inflammation rate than Tissucol ($P = .030$). At day 10, Histoacryl Flex exhibited a higher amount of collagen formation than Duraseal Xact ($P = .002$), Bioglue ($P = .034$), and Tissucol ($P = .040$).

Comments

The sealing of colonic anastomoses with TAs has been proposed as a promising new method for preventing the leakage of intraluminal contents into the abdominal cavity through a (technically) insufficient anastomosis. In this

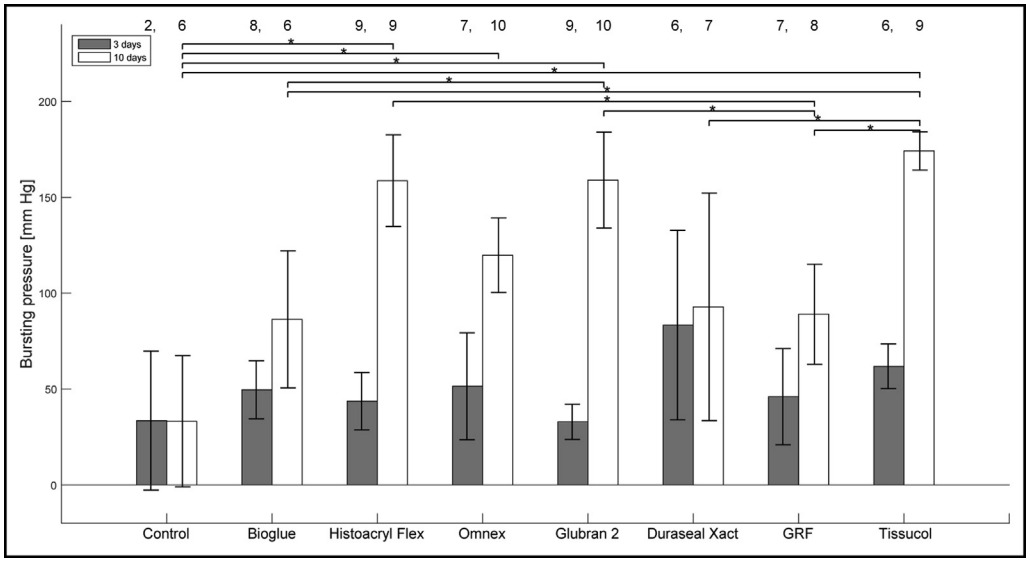

Figure 3 Mean BP at days 3 and 10. Error bars represent 95% confidence interval. Asterisks annotate statistical significance between the connected groups.

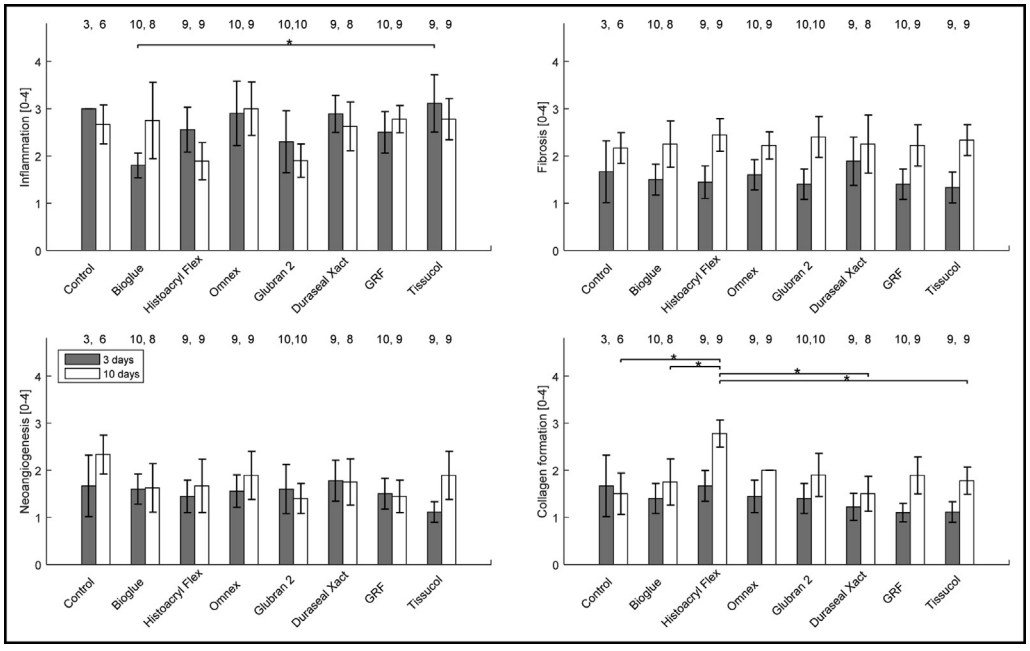

Figure 4 Histopathologic analysis at days 3 and 10. Error bars represent 95% confidence interval. Asterisks annotate statistical significance between the connected groups. Numbers at the top of each bar indicate the amount of rats used for each analysis.

study, we included 7 clinically available TAs to seal iatrogenic colonic defects, in which the TA acts as a protective barrier against intra-abdominal leakage of bowel contents, preventing fecal peritonitis. We evaluated the effectiveness of each TA as a colonic sealant by assessing clinical effects, mechanical strength, and histologic profile. By applying the TAs directly to the defect in a sutureless fashion, it was possible to evaluate the protective effects of the TA without confounding factors such as operative technique or TA application.

Clinical effects

Overall, total leakage rates at day 3 were higher than reported in previous rat studies where a sutureless colonic anastomosis was created using TA.[22–24] TAs with the lowest leakage rate in our study showed up to 40% leakage at day 3. This difference may be attributed to our definition of leakage, which was not limited to the onset of fecal peritonitis, but also included subclinical abscess formation. When focusing only on fecal peritonitis, leakage rates in this study were in line with previous studies in which FGs and CAs were used for the sutureless sealing of a colonic anastomosis.[22,25] At day 10, the best performing TAs in the present study showed neither signs of fecal peritonitis nor abscess formation implying that local abscess formation and leakage-related complications at day 3 are reversible as healing of the colonic defect progresses. Most important, the control group and the TA groups were associated with different presentation of bowel leakage. In the control group, as expected, most rats developed fecal peritonitis, while rats treated with a TA mostly developed subclinical local abscess formation directly at the glue site, leading to a significantly lower mortality rate than that of the control group (Table 1). This finding suggests a protective role of TA sealing.

Bioglue, GRF, and Duraseal Xact led to the most clinical complications when compared to the other TA groups. At day 3, these TAs showed high leakage rates, with the highest incidence of fecal peritonitis and mechanical ileus. This finding persisted at day 10 with highest mortality, leakage, and mechanical ileus and is line with previous research.[26] CAs, on the other hand, showed low rates of leakage and mechanical ileus. This was especially apparent at day 10, where no fecal peritonitis was seen in any rats treated with CAs. In the case of Histoacryl Flex, no clinical complications at all were seen in any rats at this time point. At day 10, no rats treated with FG (Tissucol) showed any clinical complications. This finding is in line with previous research in which FG was used around colonic anastomoses.

Bursting pressure

We found no significant differences between TAs in mechanical strength at day 3. Mechanical strength was higher at day 10 than at day 3 for all TAs. Duraseal Xact showed relatively high mechanical strength at day 3 and an incremental increase from 3 to 10 days. This finding, taken together with the comparatively high fecal peritonitis rate at both time points infers that the adhesive bond in Duraseal Xact may erode when it comes into contact with fecal matter, following the chemical breakdown process, or hydrolysis, of polymer bonds of the TA by acid content in fecal matter.[27,28] Based on the results of the present study

and previous literature, Duraseal Xact does not seem suitable as a colonic sealant.[26]

Tissucol was the strongest TA at day 10. In our previous ex vivo research on TA application on intact colon, Tissucol exhibited the lowest mechanical strength of all included TAs.[8] This finding implies that the curing process of Tissucol is altered when applied on an in vivo surgical wound, most probably due to the presence of blood, which may act as a catalyst in the fibrin clotting cascade which FG depends on for curing.[27,28] This high strength of Tissucol has also been reported in previous research, in a rat model where it was used to seal leaking colonic anastomosis.[29]

We found that CAs with short chain polymeric carbon chains, especially Histoacryl Flex and Glubran 2 (n-butyl CAs), exhibited a trend toward a higher mechanical strength than long chain CAs such as Omnex (a n-octyl CA), which showed lower BP than Histoacryl flex and Glubran 2. This finding remains unclear as, generally, the longer the polymeric carbon chain of a CA, the stronger its bond.[30]

Histopathology

Naturally, the histopathologic data in this study were influenced by a combination of the foreign body reaction of each TA after tissue application and the inflammation brought on by the leakage of bowel content through the defect. It is worth noting that Bioglue, which showed the highest leakage rate at day 3 and 10 of all TAs, was associated with the lowest rate of inflammation at day 3. This finding suggests that Bioglue has either low or negligible toxicity in the direct postoperative period.

Omnex and Tissucol showed the highest rates of inflammation at day 3. Despite being clinically inert, Tissucol showed the highest levels of inflammation at day 10. This finding is not in line with previous research in which Tissucol was used to seal colonic tissue in a contaminated environment and remained inert until 14-day follow-up.[31] Overall, at day 10, CAs were the most inert TAs, showing the highest scores on the included healing parameters (neoangiogenesis, collagen formation, and tissue fibrosis), which suggests that the presence of these TAs do not interfere with wound healing mechanisms after a bowel defect. It should be noted that histopathologic results in this study are not fully comparable to the situation of an actual colonic anastomosis, in which the bowel edges are completely discontinuous.

Limitations

This model enabled us to answer the question if a TA is capable of stopping leakage of a colonic defect, as would be the case in a leaking sutured/stapled anastomosis in which the last defense is a TA bond. We opted for a sutureless approach as to evaluate the pure sealing potential of the TA and the effects of the fecal contents on the TA bond in a controlled setting without confounding factors associated with variations in surgical technique and TA application. Furthermore, the present model enabled us to compare results to our previous work, enabling selection of promising TAs for future research. Naturally, in clinical practice the objective would be to use TA as an adjuvant to the sutured lesion and the sutured or stapled anastomosis. Therefore, we recommend a follow-up study on the interaction of staples or suture material with the TA bond, in a model using a (insufficient) colonic anastomosis. Furthermore, in this study, we encountered high mortality rates in the control group. Although this finding was expected, it should be taken into account that when comparing the control group to the TA groups. One should take into account that a small part of control-group rats were included in the full-statistical analysis as they did not reach the end of the follow-up period. Concerning TA use on the colon, which remains a relatively novel application, we recommend further research on the effects of TA dosage on the colon.

At last, it was chosen not to include a second control group with a primary suture of the defect based on ethical considerations, as vast previous surgical literature has already reported on the leakage rate, inflammatory reaction, and mechanical strength of the simple suture closure of a colonic wall defect in the rat.[32–34]

Conclusions

Before the clinical use of TA sealing of a colonic perforation or anastomosis, a stepwise approach, evaluating the efficacy of multiple TAs using the same experimental model is needed. In this study, we used TAs from various surgical fields for the sutureless closure of colonic defects, to study the effect of a colonic perforation or leaking anastomosis on the TA bond. We showed that the sealing of a leaking colonic defect is a viable and promising technique to decrease leakage-related complications. Results point out that differences exist in the sealing capability of various clinically used TAs. CA TAs generally seem to prevent the onset of fecal peritonitis by stopping bowel leakage from spreading into the abdominal cavity. Biological (Bioglue, GRF) and PEG adhesives (Duraseal Xact) were associated with the most leakage-related complications and low mechanical strength, making these TAs unsuitable for use as colonic sealants. In this study, the CAs Histoacryl Flex, Glubran 2 and Omnex, as well as the FG Tissucol showed the most promising results, combining fewer leakage-related complications compared to the other TAs, while maintaining high mechanical strength and an inert histologic profile. These TAs should be further evaluated in future research, which should focus on the prevention of AL in experimental colonic anastomotic models, as a final step before clinical implementation.

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