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Serosal abrasion of bowel ends does not enhance anastomotic healing

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ABSTRACT

Background: Anastomotic leakage rates remain unacceptably high, warranting reconsideration of current anastomotic technique. Anastomotic healing may improve by abrading the serosal surface of bowel ends that are invertedly anastomosed, based on the concept that serosal damage evokes inflammatory adherent processes. It is studied if local abrasion leads to stronger anastomoses and reduces leakage.

Methods: Ninety-eight Wistar rats were allocated to six groups. Either a regular anastomosis (RA) or abraded anastomosis (AA) was constructed in the proximal colon. Animals were sacrificed at day 3 (groups RA3 and AA3, $n = 2 \times 17$) or day 5 (groups RA5 and AA5, $n = 2 \times 17$). Groups RA-Dic and AA-Dic ($n = 2 \times 15$) received diclofenac from day 0 until sacrifice on day 3 to impair anastomotic healing. Outcomes were leakage, bursting pressure, breaking strength, adhesions, and histological appearance.

Results: Both in abraded (AA3 and AA5) and control (RA3 and RA5) groups without diclofenac, 1 of 17 anastomoses leaked (6%). Leak rate was 9 of 15 (60%) in group AA-Dic and 8 of 15 (53%) in RA-Dic ($P = 1.0$). The bursting pressure in group RA3 (127 ± 44 mm Hg) was higher ($P = 0.006$) compared with group AA3 (82 ± 34 mm Hg), breaking strength was comparable ($P = 0.331$). Mechanical strength was similar between groups RA5 and AA5. Abrasion did not increase mechanical strength in the diclofenac groups. Adhesion formation was not different between groups. Histology showed dense interserosal scar formation in abraded groups, compared with loose connective tissue in control anastomoses.

Conclusions: Abrasion of serosal edges of large bowel ends invertedly anastomosed does not improve anastomotic strength, neither does it reduce leakage in anastomoses compromised by diclofenac.

1. Introduction

Despite increased knowledge of anastomotic healing, leakage rates have not declined in the past decades and remain between 3 and 14% [1–3]. Leakage is a significant cause of increased morbidity and mortality after visceral surgery [4]. Attempts to reduce leakage by mechanical stapling, external sealants (e.g., fibrin glue), biological stimulants (e.g., growth factors), internal conduits, or various suture techniques have failed [3,5,6].

The current standard for constructing an end-to-end or side-to-side handsewn or stapled anastomosis is an inverting

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anastomosis [7]. This means that the largest contact area of the two bowel parts is formed by the opposing serosal surfaces. However, the physiological function of the thin epithelial layer of mesothelial cells covering the serosa is to provide a lubricant surface and not to adhere. Injury of the relatively large sero-serosal contact area might provide a way to optimize anastomotic healing. When serosa is damaged, fibrous attachments may form between viscera or the abdominal wall because of the inflammatory process [8]. Serosal abrasion is the most common method to induce adhesion formation in experimental adhesion research [9]. Also for other mesothelial tissue layers, like the parietal and visceral pleura, chemical injury and promotion of the inflammatory process is used to achieve proper adherence (e.g., treatment of relapsing pneumothorax). Several methods to achieve serosal abrasion and adhesion have been described. It is mostly done by sterile gauze rubbing, but dental brushes are also used [10]. A study in dogs showed that complete removal of the mesothelium before making an inverted anastomosis accelerated and improved the healing process without an increased risk of stenosis [11]. Based on pathophysiological principles of inflammation and wound healing, it is hypothesized that isolated injury to the serosal edges of connecting bowel parts may increase anastomotic strength and reduce leak rates by stimulating fibrous adhesions between bowel ends. In the first experiment of the present study, the effect of serosal abrasion on anastomotic strength as the primary outcome was assessed. In the second experiment, it was studied if abrasion can reduce leakage of anastomoses compromised by diclofenac administration. A rat anastomosis model was used because of extensive experience with this model in our laboratory and the consistent findings of leak rates and strength over the years [12,13]. Previous research showed that the administration of diclofenac provides a reliable model to study leakage of ileal and proximal colon anastomoses [13–15]. Diclofenac causes 60%–100% leakage in the ileum and proximal colon when given from day 0 until sacrifice on day 3 [13,15].

2. Material and methods

2.1. Ethics

This experiment was conducted according to the Dutch "Experiments on Animals Act" and European Federation of Laboratory Animal Science Associations guidelines and was approved by the Institutional Animal Ethics Committee of the Central Animal Laboratory of the Radboud University Nijmegen (AEC-number 2012-290). Humane end points were defined to avoid unnecessary suffering of animals during the study.

2.2. Animals

Adult male Wistar rats (Harlan, Horst, The Netherlands) were accustomed to laboratory conditions for 1 wk and weighed 307 grams (standard deviation ± 19) at the start of the experiment. The rats were housed two per cage at 22°C–23°C with a 12 h day cycle and had free access to standard rodent chow (Ssniff R/M-H; Bio Services BV, Uden, Netherlands) and acidified tap water throughout the experiment. The cages were enriched with a shelter and nesting material and were randomly placed on the shelves.

2.3. Groups

Ninety-eight male Wistar rats were randomly allocated to one of the six groups. Either a regular anastomosis (RA) or an abraded anastomosis (AA) was constructed in the proximal colon.

In experiment 1, animals were sacrificed at day 3 (group RA3, $n = 17$ and group AA3, $n = 17$) or at day 5 (group RA5, $n = 17$ and group AA5, $n = 17$). On day 3, anastomotic strength is at its lowest and thus most important to improve [16,17]. Day 5 was chosen as an additional sacrifice day to study if the effect of abrasion needs additional time for wound healing. Postponing sacrifice to day 7 or longer would not be useful when assessing bursting strength because anastomotic strength exceeds that of normal intestine after this period [12,13]. Three animals per group were used for histologic analysis and fourteen animals for mechanical strength testing.

In experiment 2, two groups of rats were given diclofenac (3 mg/kg/d by oral gavage; Cayman Chemical Company, Ann Arbor, MI) from day 0 until sacrifice on day 3 to induce leakage (group RA-Dic, $n = 15$ and group AA-Dic, $n = 15$) [13,15]. All animals in experiment 2 were sacrificed at day 3 because diclofenac-induced leakage occurs mostly before day 3 and postponing sacrifice would increase animal discomfort [13,15]. Twelve rats per group were used for anastomotic strength measurements and three for histologic analysis.

2.4. Intervention and surgical technique

The rats were anesthetized by inhalation of 3% isoflurane (Abbott, Hoofddorp, The Netherlands) mixed with pressurized air and oxygen. They were shaved, disinfected, and operated under sterile conditions using an operation microscope. By a 3 cm midline laparotomy, the cecum was visualized and carefully placed outside the abdomen in wet gauzes. Two centimeters distal from the cecum, the place for anastomosis was determined. In the abrasion groups, the complete circumference of the colon was abraded over a length of 2 cm with 10 soft strokes of a dental brush (Oral-B 1 2 3 Indicator Medium, Kruidvat, Nijmegen, The Netherlands) to create a precise and superficial damage of the serosal surface (Fig. 1) [10]. The middle 10 mm segment was resected, leaving 5 mm of abraded colon on both sides. In the control animals group, a 10 mm segment was removed at the same location in thee colon without abrasion. The end-to-end anastomoses were all constructed under a microscope (Wild M650; Heerbrugg, Switzerland, at ×10 magnification) by a trained researcher (S.T.K.Y.) using a single layer of eight interrupted, inverting sutures (Ethilon 8-0; Ethicon, Norderstedt, Germany). A monofilament synthetic suture was chosen because it causes little inflammatory reaction, it is available in 8-0 size, it has produced consistent results in previous experiments, and rats are sacrificed before absorption is expected to play a role in the healing process [13,18]. The abdominal wall was closed with a running suture (Vicryl

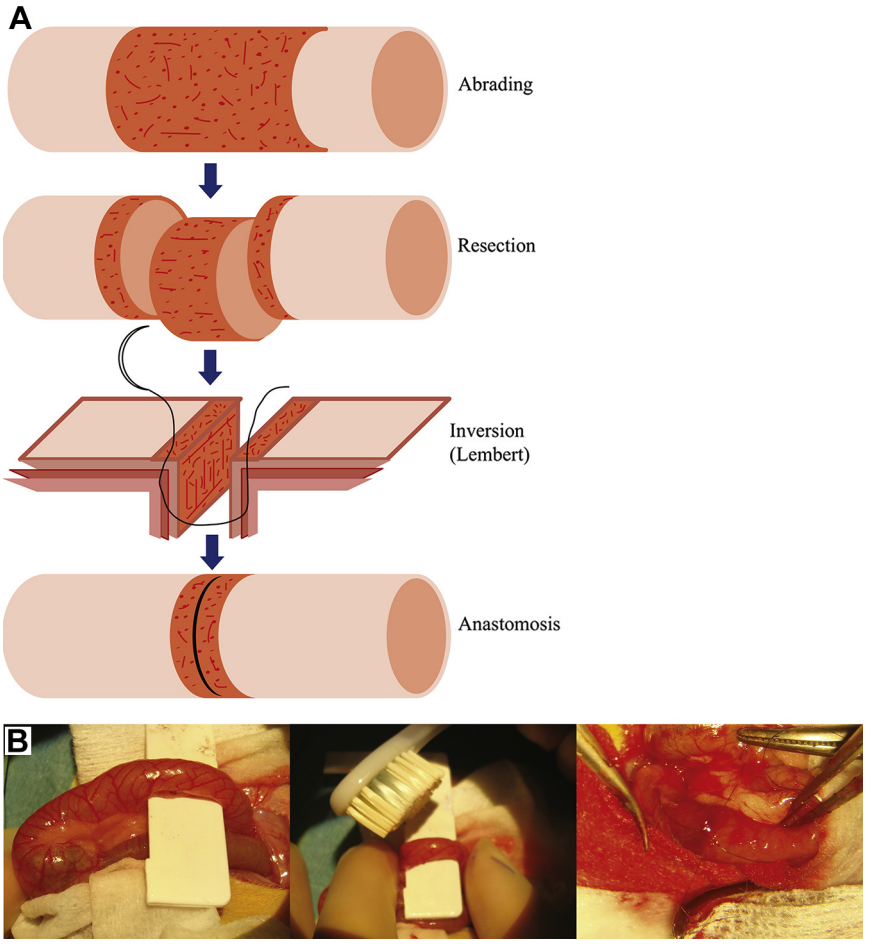

Fig. 1 – Anastomotic abrasion technique. Serosa is abraded by dental brush. A 10 mm segment is resected, leaving approximately 5 mm abraded tissue on each side. These were then invertedly anastomosed by eight interrupted sutures. (A) Schematic drawing, (B) intraoperative view. (Color version of the figure is available online.)

3–0; Ethicon), and the skin was closed with staples. During the operation, body temperature was kept at 38°C using a heating pad and a lamp. To prevent postoperative dehydration, 10 mL of 0.9% normal saline was administered subcutaneously. For analgesia in all groups, buprenorphine (Temgesic; Schering Plough, Houten, the Netherlands), 0.02 mg/kg was administered subcutaneously every 12 h, starting at least 15 min before the operation until 48 h postoperatively. All animals were weighed once daily and inspected twice daily for signs of reduced wellbeing, including dirty nose, dirty eyes, piloerection, aberrant behavior, distended abdomen, increased respiration activity, and diarrhea.

2.5. *Outcome assessment*

The rats were euthanized by CO_2 asphyxiation. A relaparotomy was performed to inspect for signs of anastomotic leakage, defined as anastomotic abscess, pus, fecal peritonitis, or visible dehiscence. The inspection was done by two blinded researchers (S.T.K.Y. and R.M.L.M.L.) and only if both agreed on the leakage aspect, this was scored accordingly. The leakage severity was scored as "0" for no signs of leakage, "1" for anastomotic abscesses, "2" for free pus or large abscesses, and "3" for fecal peritonitis or visible dehiscence, as previously reported [15]. Adhesions between the anastomosis and other viscera were scored as "0" if absent, as "1" if detachment was possible by light traction, as "2" if blunt dissection was needed, or as "3" if sharp dissection was needed to detach the adhesive organ.

2.6. *Mechanical strength*

Except for the samples used for histologic analysis ($n = 3$ and $n = 2$ per group in experiment 1 and 2, respectively), all other anastomoses were subjected to mechanical strength testing. The anastomotic bowel parts were carefully resected *en bloc* with 2 cm of bowel on each side, and any scar tissue or adhesions covering the anastomosis was left in place. To measure bursting pressure (BP), the segments were infused (2 mL/min) with water containing methylene blue, determining the strength of the weakest spot within the anastomosis [13,19].

Table – Anastomotic leak, leakage severity, and premature death in the six groups.

Outcome	Group					
	RA3	AA3	RA5	AA5	RA-Dic	AA-Dic
Rats per group, *n*	17	17	17	17	15	15
Anastomotic leak, *n* (%)	1 (6)	1 (6)	1 (6)	1 (6)	8 (53)*	9 (60)*,†
Leak severity score 0–3 (SEM)	0.1 (0.1)	0.1 (0.1)	0.1 (0.1)	0.1 (0.1)	1.2 (0.3)	1.1 (0.2)
Premature death, *n*	0	0	0	2	0	0

SEM = standard error of the mean.
Groups: RA3/RA5: regular anastomosis, sacrifice at day 3 or 5. AA3/AA5: abraded anastomosis, sacrifice at day 3 or 5. RA-Dic/AA-Dic: regular or abraded anastomosis, compromised by diclofenac administration from day 0 until sacrifice on day 3.
* $P = 0.000$ compared with groups without diclofenac.
† $P > 0.05$ compared with RA-Dic.

Dehiscent anastomoses were scored as "0 mm Hg." The site of the rupture was noted as at the anastomosis or outside the anastomosis. To determine the maximal suture holding capacity, the segments were attached to a tensiometer (Aikoh 500; Aikoh Engineering CO. LTD., Tokyo, Japan) and pulled apart at 3 cm/min [13,20]. The highest force measured before rupture was recorded as breaking strength (BS).

2.7. Histology

The segments of 1 cm of normal bowel, resected at the initial operation, were collected to check if proper abrasion was performed. After sacrifice, 1-cm long segments containing the anastomosis were collected. All samples were opened at the mesenterial side. After gentle washing with saline, the samples were fixed in 4% buffered formaldehyde before paraffin embedding. From these paraffin embedded samples, 4 -μm sections were prepared and stained with hematoxylin and eosin. Sections were analyzed using a binocular light microscope.

2.8. Statistics

The required sample size for experiment 1 was determined to detect an absolute reduction of 30 mm Hg in "BP" as the primary outcome. With an estimated standard deviation of 25 mm Hg, an α of 0.05 and β of 0.80, anticipating analysis of two groups (regular *versus* abraded) with an independent *t*-test, the group size was determined at 14. Adding three animals per group for histologic analysis of the anastomosis, group size in experiment 1 was 17. In experiment 2, sample size was calculated with leak rate as the primary outcome. With an expected leak rate in the positive control between 70 and 80% and anticipating analysis with Fisher exact test, 15 animals per group were needed to detect a risk reduction to 25%–35%. As leakage assessment does not interfere with histologic analysis, no extra animals were added to these groups.

Fisher exact test was used for analyzing leak percentages and adhesion percentages. The two-tailed unpaired *t*-test was used for analysis of BP, BS, leak severity, and adhesion severity. One-way analysis of variance was used for comparing weight loss among all groups. Results were considered statistically significant at $P < 0.05$.

3. Results

3.1. Animal welfare and mortality

All rats showed minor signs of discomfort during the first 2 d after surgery visible by a dirty nose, dirty eyes, pillow erection, distended abdomen, or diarrhea. One animal in the AA5 group died from an unknown cause and one from the same group reached the humane end point and was taken out of the experiment showing severe inactivity, low body temperature, and aberrant shape. No signs of leakage were observed in these two animals; they were not used for strength analysis. All rats had weight loss in the first 2–3 d after surgery, but rapidly regained normal weight thereafter. The percentage of weight loss in group AA-Dic (6.2 ± 2.9%) was significantly less than in the groups AA3 (10.4 ± 2.0%; $P = 0.010$), AA5 (10.9 ± 2.4%; $P = 0.047$), and RA5 (9.9 ± 3.5%; $P = 0.035$), probably due to diclofenac administration and slight tissue edema. Differences among other groups were not significantly different.

3.2. Anastomotic leakage

In experiment 1, 1 rat in each group of 17 rats without diclofenac administration (AA3, RA3, AA5, and RA5) showed macroscopic signs of leakage (6%) (Table). In experiment 2, signs of leakage were present in 9 of 15 rats (60%) in the AA-Dic group and 8 of 15 rats (53%) in the RA-Dic group. The overall leak incidence in experiment 2 (57%) was significantly higher than in experiment 1 (6%; $P = 0.000$) (Table). The mean leakage severity score was not significantly different between the AA-Dic group (1.0 ± 0.9) and the RA-Dic group (1.2 ± 1.2; $P = 0.740$) (Table).

3.3. Anastomotic strength

The BP in the RA3 group (127 ± 44 mm Hg) was significantly higher ($P = 0.006$) compared with the AA3 group (82 ± 34 mm Hg; Fig. 2A). There were no differences in BP between the groups sacrificed at day 5 (AA5, 207 ± 71 mm Hg; RA5, 205 ± 64 mm Hg; $P = 0.941$). Both in groups AA5 and RA5, 5 out of 14 anastomoses burst outside the anastomotic line, all other anastomoses burst within this line. There were no differences in BP between the AA-Dic (85 ± 28 mm Hg) and the RA-Dic (83 ± 51 mm Hg) groups (Fig. 2A).

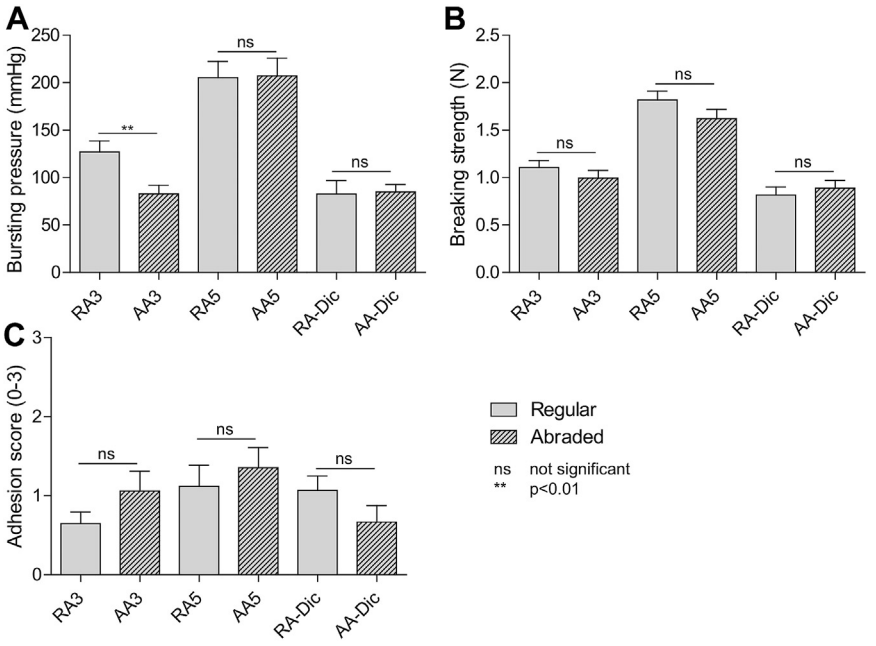

Fig. 2 – BP (A), BS (B), and adhesion score (C) per group. Groups: RA3/RA5: regular anastomosis, sacrifice at day 3 or 5. AA3/AA5: abraded anastomosis, sacrifice at day 3 or 5. RA-Dic/AA-Dic: regular or abraded anastomosis, compromised by diclofenac administration from day 0 until sacrifice on day 3.

Abrasion did not affect BS (AA3 group, 1.0 ± 0.3 N; RA3 group, 1.1 ± 0.3 N; $P = 0.331$). No differences were found between groups sacrificed at day 5 (AA5 group, 1.6 ± 0.4 N; RA5 group, 1.8 ± 0.4; $P = 0.157$) and between both groups of rats treated with diclofenac (AA-Dic group, 0.9 ± 0.3 N; RA-Dic, 0.8 ± 0.3 N; $P = 0.540$) (Fig. 2B).

3.4. *Adhesions*

Adhesion scores were low and not significantly different between groups AA3 and RA3 (1.1 ± 0.2 and 0.7 ± 0.2; $P = 0.165$), and between groups AA5 and RA5 (1.4 ± 0.3 and 1.1 ± 0.3; $P = 0.532$) and groups AA-Dic and RA-Dic (0.7 ± 0.2 and 1.1 ± 0.2; $P = 0.162$, Fig. 2C).

3.5. *Histology*

Because of one technical failure (group AA3), two dehiscent anastomoses (groups RA-Dic and AA-Dic) and two premature deaths (group AA5), a reduced number of anastomotic samples could be analyzed. Histologic analysis of the bowel pieces resected at the initial operation showed a moderate degree of mesothelial damage in all samples of both abraded ($n = 7$) and non-abraded ($n = 7$) groups (Fig. 3A–C). An intact mesothelial segment was only seen in one control sample (Fig. 3A). The connective tissue layer beneath the mesothelium was more frequently injured in the abraded groups, as visible by microscopic hematomas (Fig. 3C).

In 6 out of 7 anastomotic control samples (RA3 $n = 3$, RA5 $n = 3$ and RA-dic $n = 1$), the connection of the opposing serosal layers of both bowel parts appeared incomplete (Fig. 3D, F and H), whereas dense connective tissue was seen between the serosal layers in 3 out of 4 anastomoses in the abraded groups (AA3 $n = 2$, AA5 $n = 1$, and AA-Dic $n = 1$) (Fig. 3E, G and I).

4. Discussion

The results from the present study show that surgical abrasion of the bowel serosal layer does not result in stronger anastomoses and does not reduce leakage rate of compromised anastomoses in a rat model despite histologic evidence of more dense interconnective tissue. The assumed benefit of serosal abrasion of bowel ends would be an increased adhesive and thus fibrous reaction between the two inverted serosal edges resulting in a stronger anastomosis. An increased response on abrasion was suggested by analysis of the histologic samples, in which loose connective tissue was seen between the two opposing serosal layers in most non-abraded anastomoses, compared with marked scar tissue in most abraded anastomoses. Apparently, such increase in fibrous connections does not lead to a stronger anastomosis at two relevant time points in the process of anastomotic wound healing of rats [13,15].

Possible explanation for the findings is that the healing process of normal intestinal anastomoses in the rat is near optimal, and improvement is difficult to achieve and to assess with additional interventions. Notably, it is difficult to induce leakage in a normal rat anastomosis and even incomplete rat anastomoses can heal well in less than a week [21]. On the opposite, the impairment of the healing process by administration of diclofenac might have been too strong to find a beneficial effect of abrasion on the leak rate in a compromised anastomosis. In addition, the relative contribution of scar tissue formation because of serosal abrasion might have been low compared with the scar formation by the sutures or the

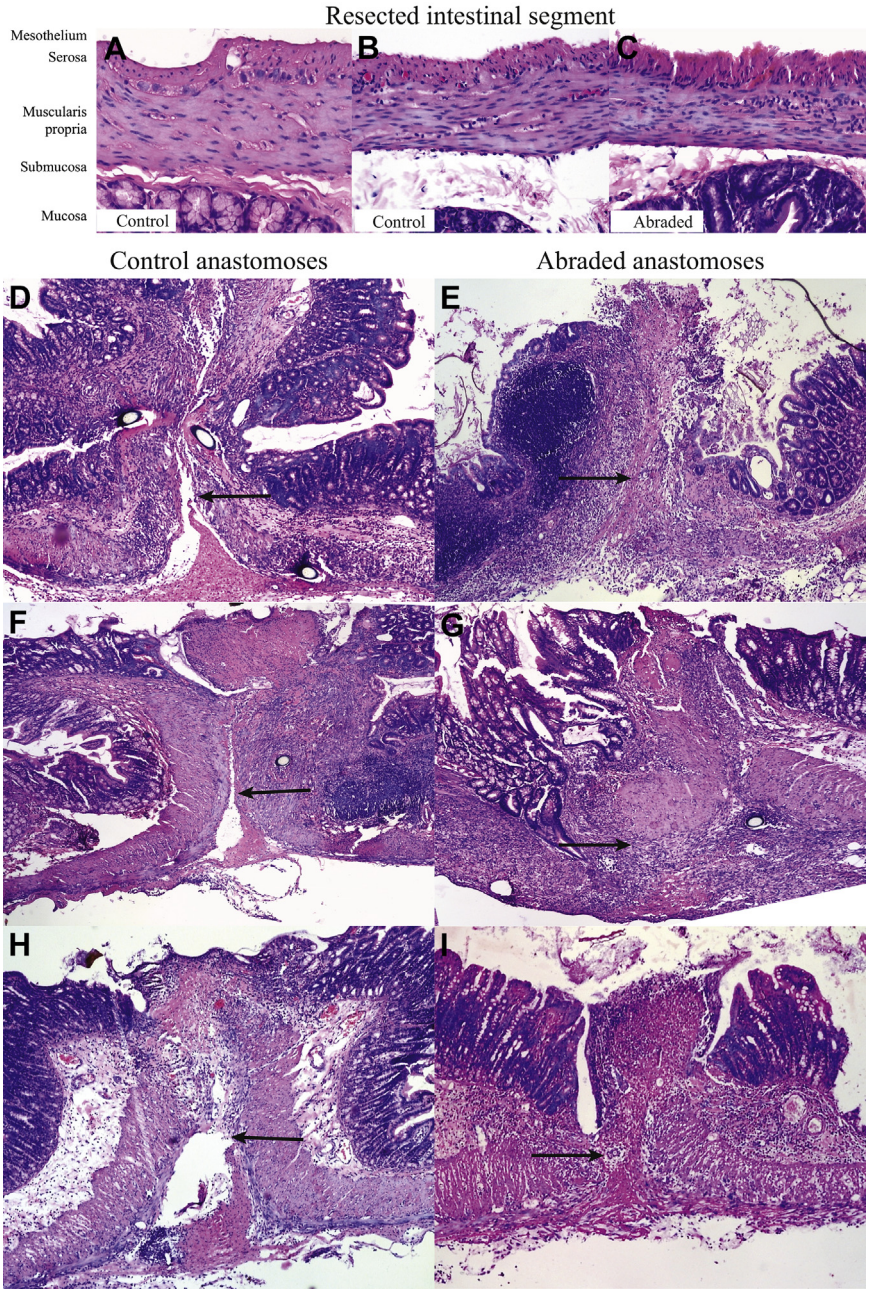

Fig. 3 – Hematoxylin and eosin-stained samples of resected intestinal segments (A–C) and anastomoses (D–I). Examples of intact mesothelium in control group (A), damaged mesothelium and serosa in control groups (B), and more severely damaged serosa in abrasion groups (C). Examples of incomplete connection of the opposing serosal layers in control anastomoses (D, F, H) and dense connective tissue in abraded anastomoses (E, G, I). Arrows indicate interserosal area. (Color version of the figure is available online.)

strength provided by the repair of the submucosal layer, which is mostly responsible for the anastomotic strength [16,22,23]. Abrasion may even have delayed healing as indicated by lower BPs after 3 d. An excessive inflammatory response and edema formation on injury both negatively interfere with normal healing [17,24]. Histology, however, did not show edema whereas fibrosis, as a product of an excessive inflammatory response, was present in the specimens of AA already at day 3.

It was expected that adhesions to other surrounding structures and organs would be more extensive in the intervention groups, but no difference in adhesion severity was observed. Notably, assessment of adhesion formation and severity is typically done after 1 wk or even longer, and not after 3 or 5 d [10]. Particularly, adhesions at day 3 may be fibrinous in nature and are still susceptible for fibrinolysis by a plasminogen activator in the abdominal cavity [25]. Days 3 and 5 were chosen in this study because at these times,

anastomotic healing disturbances are most likely to occur. Comparable adhesion formation further indicates that the consequences of abrasion seem limited when considering the healing processes evoked by the surgical resection or the suturing. From several animal studies it has been suggested that nonsteroidal anti-inflammatory drugs reduce abdominal adhesion formation [26,27]. Intraperitoneal administration of cyclooxygenase 2 inhibitors showed anti-adhesive effects in several animal studies [28–30]. In our study, we did not find a reduction of adhesions after diclofenac administration in the second experiment. The frequent occurrence of anastomotic leakage in these groups is a confounding factor when assessing adhesion formation because intraperitoneal infection strongly induces adhesion formation [31,32].

A validated and frequently used animal model with a sufficient number of animals was used to assess the effects of abrasion on anastomotic strength and leakage ruling out a type 2 error. The number of histologic samples, however, was not sufficient to allow for conclusive interpretation.

Potential disadvantage of the rat model is the small size of the intestine, which may have hampered adequate abrasion. A larger bowel size may be the reason why a beneficial effect of abrasion was reported in a dog study [11]. Therefore, the negative results of the present study do not exclude a beneficial effect in humans. Another disadvantage of the small bowel size is the relative damage done by handling the bowel ends while creating the anastomosis. Though trying to avoid damage, swabs and forceps may have already induced serosal injury comparable with that in abraded groups, as was also observed in some histologic samples (Fig. 3B). A possible concern regarding outcome assessment involves the diagnosis of leakage. The clinical definition often involves the need for reintervention, which is not applicable to animals [33]. Taking differences in leakage definitions into account, macroscopic signs were scored according to severity to allow for a more accurate discrimination of the adverse effect on the animal. The severity score was used in a previous study in which mean severity scores corresponded with leak rates and strength. However, the score was not statistically validated and thus should be interpreted with caution [15]. To minimize observer bias, scoring was done by two observers in a blinded fashion. The compromised anastomosis model that was used is more appropriate to study healing processes compared with models using extensive ischemia or large suture defects, which are unrealistic in the clinical situation [21]. Leakage was successfully induced by diclofenac administration with rates comparable with previously obtained results, making it a consistent model of anastomotic leakage [15]. The increased leak rate after diclofenac administration in proximal rat colon adds to the increasing evidence that nonsteroidal anti-inflammatory drugs impair anastomotic healing [34].

5. Conclusions

Abrasion of the serosal layers of large bowel ends that are invertedly anastomosed does not improve strength and does not reduce leak rate in a rat model of normal and compromised anastomotic healing.

Acknowledgment

Authors' contributions: S.T.K.Y. and H.V.G. contributed to the conception. S.T.K.Y., R.M.L.M.L., and H.V.G. did the design. S.T.K.Y., A.H., and R.M.L.M.L. conducted the experiment, did the analysis and interpretation, and wrote the article. R.M.L.M.L. and H.V.G. did the critical revision of article. H.V.G. did the supervision.

The data from this study were not presented elsewhere.

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Disclosure

The authors reported no proprietary or commercial interest in any product mentioned or concept discussed in the article.

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