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Decreased Leakage Rate of Colonic Anastomoses by Tachosil Coating: An Experimental Study

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Colorectal anastomotic coating has been proposed as a means to lower the leakage rate. Prior to clinical testing, coating materials need thorough experimental evaluation to ensure safety and efficacy. The aim of this study was to evaluate Tachosil as an anastomotic coating agent. Technically insufficient colon anastomoses were created in 80 C57BL/6 mice, and in half of the animals the anastomoses were covered with Tachosil. The animals were examined for clinical signs of anastomotic leakage, and the breaking strength of the anastomoses was evaluated. The number of leakages was reduced by Tachosil coating (10/40 versus 20/40 in controls; $P = 0.037$). However, more cases of large bowel obstruction were found in the Tachosil group (12/40 versus 0/40 in controls; $P < 0.0005$). Breaking strength was comparable between the Tachosil and control groups (0.49 N versus 0.52 N, respectively; $P = 0.423$). Clinical studies are needed to clarify the efficacy of Tachosil anastomotic coating.

Key words: Colon anastomotic leakage – Anastomotic dehiscence – Anastomotic failure – Animal model – Experimental – Coating – Tachosil – Sealing

Anastomotic leakage remains a feared and frequent complication in gastrointestinal surgery, especially for anastomoses on the colon and rectum, with leakage rates of 3% to 7% and 13%, respectively,[1–5] leading to a mortality rate of up to 27%.[1,6]

In addition to existing interventions used to decrease the risk of anastomotic leakage, such as altered surgical techniques, preoperative optimization, and different postoperative regimens, anastomotic coating has been suggested as a potential method.[7] Numerous experimental studies evaluating anastomotic coating have reported positive effects; however, contradictory results were found when the same coating material was evaluated by other researchers or in a different study design. Furthermore, a few coating materials have been evaluated in humans, but without convincing

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results.[7] In order to establish a rationale for conducting large, clinical, randomized, controlled trials in which specific coating agents are evaluated, positive results have to be reproduced in several animal studies to ensure the safety and efficacy of the materials and methods. Tachosil (Hakeda, Osaka, Japan) has been evaluated in a technically insufficient anastomosis in a mouse model by Pantelis *et al*.[8] This study reported the positive result that Tachosil reduced the anastomotic leakage rate from 36.7% to 5.9%. These results, together with the findings of other studies,[9,10] suggest that Tachosil is one of the most promising agents for colorectal anastomotic coating.

The aim of this study was to evaluate the use of Tachosil to lower the anastomotic leakage rate in a mouse model of the technically insufficient colon anastomosis. Furthermore, we aimed to investigate if the results produced by Pantelis *et al*[8] were reproducible in our setting.

Materials and Methods

End-to-end colonic anastomoses were created in 80 male C57BL/6 mice (23–30 g). The animals were anesthetized with isoflurane (isoflurane, 2%; O_2, 1000 mL/min). Initially, the abdomen was shaved and excess hair was removed with a piece of tape. The skin was disinfected, and midline incisions (1.5–2.0 cm) of both the skin and muscle layer were performed. With 2 sterile swabs, the coecum was identified and pulled forward, exposing only the part of the bowel that was used for the anastomosis. The colon and a small part of the mesentery 1 cm distal to the coecum were divided by microscissors. We constructed a technically insufficient end-to-end colo-colic anastomosis using 4 extramucosal, interrupted, equidistant, coated Vicryl 8-0 sutures (Ethicon, Somerville, New Jersey) with a taper-point needle; a prior study[8] showed that this technique produces a reproducible leakage rate of 36.7%. The abdominal wall was closed using a Vicryl 4-0 running suture in the muscle layer and 3 Vicryl 4-0 interrupted sutures with inverted knots in the skin. After surgery, the animals received 0.2 ml saline subcutaneously as fluid therapy. Microsurgical instruments (Opitek, Glostrup, Denmark) and an operation microscope (Wild Heerbrugg, Heerbrugg, Switzerland) were used. The temperature of the animals was held at a constant of 36.5°C by a temperature control unit (HB 101/2; Panlab Harward Apparatus, Barcelona, Spain) during the operation, and for the first 24 hours after operation the mouse cages were placed on heat plates (30°C).

The intervention and control groups consisted of 40 mice each. In the intervention group, the anastomoses were covered with Tachosil, whereas the anastomoses of the control group were left untreated. Both groups had the same anastomoses performed with 4 sutures each at 90° distances to simulate a technically insufficient anastomosis. In the control group, half of the animals from a prior experiment were used, since the exact same procedure was performed in that experiment.[11]

Completely dry instruments were used for the application of Tachosil. A piece of Tachosil of the size 0.5 × 1 cm was cut with microscissors, after which the patch was compressed to minimize the thickness. A piece of gaze was placed on the skin just beside the incision, where the piece of Tachosil was placed. With sterile swabs, the backside of the anastomosis was placed on the Tachosil, covering the anastomosis line from the mesenteric border. The Tachosil was then wrapped around the anastomosis to cover the entire anastomotic line using dry instruments. Sterile water was dropped onto the anastomosis to activate the Tachosil while compressing Tachosil onto the anastomosis using sterile swabs to ensure complete contact with anastomotic tissue in the whole circumference. The piece of Tachosil was left untouched for 3 minutes to allow complete adherence. Only the piece of bowel containing the anastomosis was left outside the abdomen for the coating procedure, and after 3 minutes the anastomosis and Tachosil patch were gently pushed back into the abdomen using a sterile swab. During this procedure it was ensured that the Tachosil patch stayed *in situ*.

Anastomotic leakage, manifested as fecal peritonitis or abscess formation, was the primary outcome measure. The definition of fecal peritonitis was feces in the abdomen leaking from the anastomosis, whereas an abscess was defined as a cavity close to the anastomosis containing purulent matter with communication to the anastomotic line and lumen.

The animals were observed for 7 days postoperatively, and weight and wellness scores were recorded daily. Wellness score (1–12, 12 being the best clinical condition) described the wellbeing of the animals.[12] After 7 days, or before if the mice were considered too ill (based on clinical evaluation or a wellness score of 6 or lower), the mice were reanesthetized and sacrificed. By performing relaparotomy, the abdomen was examined for signs of abscesses or fecal peritonitis. In the mice that

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completed the experiment, the anastomoses were resected. The anastomoses were examined for breaking strength using a material testing machine (LF+; Lloyd Instruments, Fareham, UK) with an XLC10n loading cell as previously described.[13]

Statistics and ethics

In the study by Pantelis *et al*[8], the leakage rate was reduced from 36.7% to 5.9% by applying Tachosil to 4 suture anastomoses. The minimal relevant difference was set to 30% as a conservative measure, assuming that Tachosil application would at least lower the leakage rate by 30%. Thus, 40 animals in each group were needed ($\alpha = 5\%$, $\beta = 20\%$).

For categorical data, the Fisher exact test was used. For breaking strength, the Mann-Whitney test was used; absolute values are expressed as median (range). In Fig. 1, weight loss is presented as median values. The Friedman test for differences in medians was used for differences in wellness score and weight loss during the 7 postoperative days. SPSS version 19 (IBM, Armonk, New York) was used for the statistical analyses. $P < 0.05$ was regarded as statistically significant.

This study was approved by The Danish Council of Animal Experiments before initiation (license 2011/561-1977).

Results

There were significantly fewer leakages in the Tachosil group compared with the control group. However, at the same time, there were significantly more cases of anastomotic stenosis leading to large bowel obstruction in the Tachosil group compared with controls (Table 1). Eight of the 12 animals with large bowel obstruction were sacrificed prior to the end of the experiment because of signs of illness after 5 (median) postoperative days (range, 4–6). The remaining 4 animals did not show any signs of clinical illness and lived to the end of the experiment. However, upon autopsy, the mice had signs of large bowel obstruction. Thus, none of the mice with large bowel obstruction died from the condition, and only 8 mice had clinical symptoms.

There were no significant difference in breaking strength between the Tachosil and the control anastomoses (Table 1).

As seen in Fig. 1, the weight loss for the Tachosil group was larger compared with that of the control group ($P < 0.001$), and the wellness score was lower in the Tachosil group compared with that of the control group ($P < 0.001$).

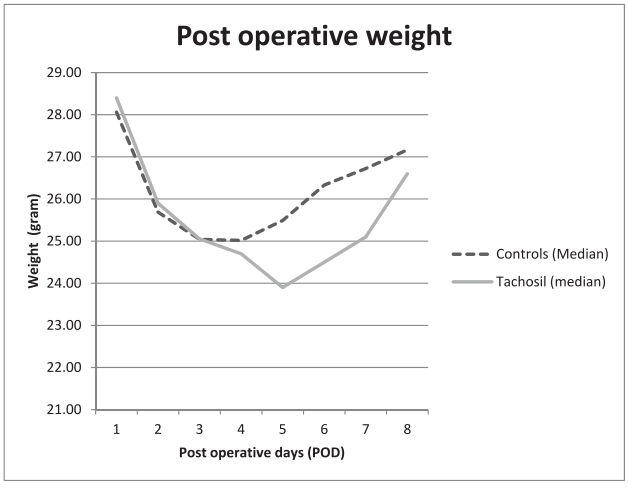

Fig. 1 Median postoperative weight of the Tachosil and control groups.

Discussion

This study found that the application of Tachosil on a technically insufficient anastomosis significantly lowered the anastomotic leakage rate. However, at the same time the rate of large bowel obstruction was increased. The application of Tachosil did not affect the anastomotic breaking strength. The findings of this study are in agreement with the results produced by Pantelis *et al*,[8] in which Tachosil was evaluated in a similar mouse model.

We were able to reproduce the results of a previous study in a different setting, but with a comparable design. Besides Pantelis *et al*,[8] 2 other studies evaluating Tachosil have confirmed the feasibility and safety of Tachosil when used for anastomotic coating. One study[9] evaluated the use of Tachosil on small bowel anastomoses in pigs and found that the risk of anastomotic leakage and stenosis was comparable to that of controls. A clinical study[10] evaluated the use of Tachosil on anastomoses in 25 patients treated for rectal cancer with anterior resection and found that the application of Tachosil was technically feasible in 96% of the patients. No complications associated with Tachosil, neither leakage nor stenosis, were recorded. All together, this suggests that Tachosil can be used safely in humans and may be suitable for preventing anastomotic leakage. Furthermore, the results of the present study show that breaking strength seems to be a poor surrogate measure to evaluate the risk of clinical leakage.

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Table 1 Summarized results

	Controls (n = 40)	Tachosil (n = 40)	*P*
Clinical leakage rate, %	50	25	**0.037**[a]
Anastomotic stenosis/obstruction (large bowel obstruction) rate, %	0	30	**<0.0005**[a]
Breaking strength, N [median (range)]	0.52 (0.22–0.84)	0.49 (0.28–0.66)	0.423[b]

Significant results are in bold.

[a]Compared with controls, Fisher exact test.

[b]Compared with controls, Mann-Whitney test.

Among the animals with anastomotic leakage, the ones with fecal peritonitis quickly became very ill. Conversely, the mice with abscesses were less clinically affected. As the animals with fecal peritonitis were quickly removed from the experiment due to illness, the differences in wellness score and weight loss between the control and Tachosil group probably were a result of large bowel obstruction in the Tachosil-coated animals.

Many different coating materials have been evaluated for prevention of anastomotic leakage. Animal studies have generally found disappointing as well as contradictory results.[7] In human studies, fibrin sealant, omental pedicle grafts, and hyaluronic acid/carboxymethylcellulose have been evaluated. The use of fibrin sealant lowered the anastomotic leakage rate from 10.9% to 5.8% in a nonrandomized study of 223 patients operated on for rectal cancer.[14] However, this difference was not significant. The effect of an omental pedicle graft was evaluated in a randomized study with 712 patients operated on for colonic or rectal cancer.[15] Neither anastomotic leakage nor mortality was improved. A study evaluating hyaluronic acid/carboxymethylcellulose for anastomotic coating found a higher leakage rate associated with the treatment; thus, this coating material should not be used in the clinical setting.[16] None of the above-mentioned studies in humans found increased rates of stenosis or large bowel obstruction, as we did in our study.

The main difference between the results of this study and the ones produced by Pantelis *et al*[8] was the occurrence of large bowel obstruction in our animals. The large bowel obstruction rate of that study was not reported; thus, one must assume that the condition was absent. In that study, only animals that died from illness were recorded. No animals died of large bowel obstruction in our experiment. Of the 12 cases with large bowel obstructions, 8 were sacrificed due to clinical illness and 4 had no clinical symptoms but were only diagnosed with large bowel obstruction upon autopsy. Compared to their study, we may have had a lower threshold when clinically diagnosing the animals with large bowel obstruction. According to protocol, that study sacrificed animals on postoperative days 2, 5, and 14. Thus, two thirds of the animals were sacrificed by postoperative day 5, which was the same point in time at which we diagnosed most of the cases with large bowel obstruction. Therefore, it cannot be ruled out that the two thirds of the animals in that study were sacrificed before they had clinical signs of large bowel obstruction and, thus, before they might have died of the condition. Furthermore, we cannot rule out that the large bowel obstruction in the animals in our study may have been self-limiting, if the animals had not been sacrificed due to clinical symptoms.

This study has some limitations. Half of the control group was used from a previous experiment; however, this was not considered a problem for the experimental design, since the two experiments were conducted in close, timely relation to one another. Moreover, all operations were conducted by the same surgeon who, with much experience in this experimental procedure, may be considered on a learning-curve plateau. Thus, little improvement, if any, in technique may separate the two experiments. Large bowel obstruction may have occurred in the mouse, and not in pig or human, due to the small diameter of the bowel. Moreover, too much pressure may have been put on the Tachosil patch when applying it to the anastomosis, which may have led to stenosis. Compared to the small size of the mouse colon, the patch was relatively oversized. This is not the case in humans, where the proportions between the size of Tachosil patch and the bowel diameter are different. Furthermore, the presence of a stapler in the lumen of the human bowel may ensure counter pressure from the inside, which may diminish the risk of stenosis. However, in laparoscopic surgery where stapled anastomoses are commonly used, instrument handling of the Tachosil patch may require some practice. Also, an immunologic reaction to the patch may be larger in

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mice because of the human origin of the coagulation factors, although this is purely hypothetical.

In light of existing knowledge about the beneficial effects of Tachosil, the next step could be a large, randomized, clinical trial evaluating Tachosil coating of colorectal anastomoses compared with no coating in humans. Due to the relatively low leakage rate in colorectal anastomosis, a large number of patients would be needed in order to show a clinically relevant reduction in the leakage rate. Therefore, the study may have to be a multicenter and multinational study to include a sufficient number of patients.

In conclusion, we found that Tachosil applied to technically insufficient mouse colon anastomoses lowered the leakage rate significantly. However, in this model Tachosil created large bowel obstruction, which was likely due to technical difficulties or shortcomings of the specific mouse model. In light of existing evidence and due to the differences between animals and humans, large bowel obstruction may not be a problem in humans. Thus, sufficient safety and efficacy data exist to design and conduct a randomized clinical trial evaluating Tachosil for coating of colorectal anastomoses in humans.

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