Sealing of gastrointestinal anastomoses with fibrin glue coated collagen patch

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PAPERS INCLUDED IN THIS THESIS

1. Nordentoft T, Rømer J, Sørensen M. Sealing of gastrointestinal anastomoses with fibrin glue coated collagen patch: a safety study. J Invest Surg 2007; 20: 363-369.
2. Nordentoft T, Sørensen M. Leakage of colon anastomoses: Development of an experimental model in pigs. Eur Surg Res 2007: 39: 14-16.
3. Nordentoft T, Holte K. Preventing clinical leakage of colonic anastomoses with a fibrin-coated collagen patch sealing - an experimental study. Arch Clin Exp Surgery 2014; 3: 201-206.
4. Nordentoft T, Pommergaard HC, Rosenberg J, Achiam MP. Fibrin glue does not improve healing of gastrointestinal anastomoses: a systematic review. Eur Surg Res 2015;54:1-13.

INTRODUCTION

Operations involving anastomoses are commonly performed in gastrointestinal surgery. The majority of anastomoses are made on the colon or rectum and this thesis will focus on colorectal anastomoses.

Most anastomoses are performed as a part of the treatment of colorectal cancer. After resection of the cancer an anastomosis is made if it is found feasible and safe. According to the Danish Colorectal Cancer Group (DCCG), which runs a national database, the incidence of colorectal cancer in Denmark (5.5 million people) is around 4200 new cases a year [1]. This makes colorectal cancer the third most common type of cancer in Denmark for both men and women [2] and accounts for the third most common cause of cancer related death in Denmark [2]. Worldwide colorectal cancer is a very common disease as well, although the incidence of this disease varies across continents (Figure 1).

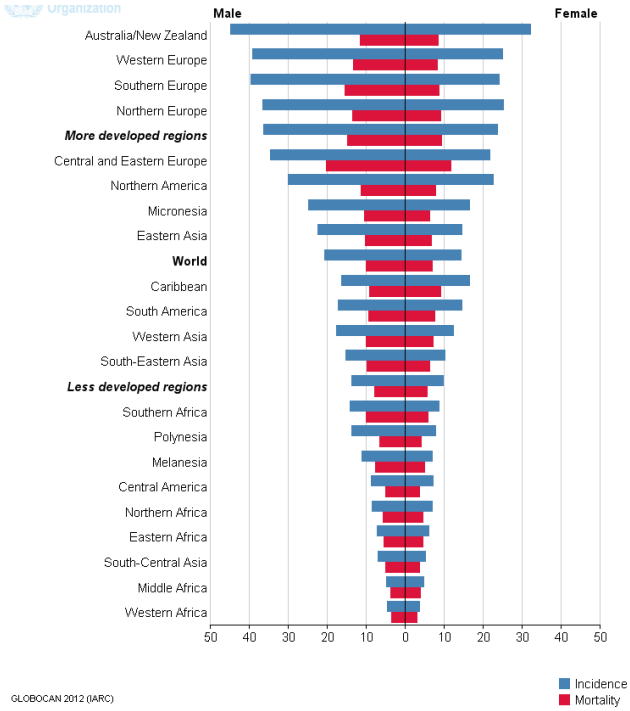

Figure 1: Estimated age-standardized rates (World) per 100,000 of colorectal cancer according to GLOBOCAN (WHO), 2012 [3]. Reproduced with permission from International Agency for Research on Cancer, World Health Organization

No worldwide database of colorectal cancer exists, but International Agency for Research on Cancer (World Health Organization) estimates the world-wide incidence to be 1,361,000 new cases/year (Table 1). Internationally, the disease is the third most common type of cancer for men and the second most common type of cancer for women [3].

Estimated numbers	Men			Women			Both sexes		
(thousands)	Cases	Deaths	5-year prev.	Cases	Deaths	5-year prev.	Cases	Deaths	5-year prev
World	746	374	1953	614	320	1590	1361	694	3544
More developed regions	399	175	1164	338	158	966	737	333	2130
Less developed regions	347	198	789	276	163	624	624	361	1414
WHO Africa region	16	11	32	15	11	31	31	22	63
WHO Americas region	125	57	362	121	55	342	246	112	705
WHO East Mediterranean region	18	12	40	15	10	33	33	21	73
WHO Europe region	255	120	686	216	108	573	471	228	1258
WHO South-East Asia region	68	48	122	52	37	93	120	85	216
WHO Western Pacific region	264	125	711	195	100	518	460	225	1229
IARC membership	418	187	1181	351	167	976	769	353	2157
United States of America	69	29	214	65	27	199	134	55	413
China	147	79	338	107	60	245	253	139	583
India	37	28	50	27	21	37	64	49	87
European Union	193	83	536	152	69	417	345	152	953

Table 1: Estimated world-wide incidence, prevalence and mortality of colorectal cancer according to GLOBOCAN (WHO), 2012 [3]. Reproduced with permission from International Agency for Research on Cancer, World Health Organization

Besides resection for colorectal cancer a large number of colorectal resections and anastomoses are made in the treatment of benign colorectal diseases such as inflammatory bowel disease, diverticular disease and benign neoplasms not suitable for endoscopic resection. The statistics for these diseases are even sparser. According to The National Patient Registry in Denmark, approximately 38% of the elective colorectal resections were due to benign diseases (data directly extracted from the registry, 2014). No similar, international register exists, but the proportion of benign resections seems to be comparable in other western countries, though the reported percentage varies [4-9].

Not all colorectal resections end up with an anastomosis, since it is, in some cases, not technically feasible or too hazardous due to the condition of the patient. In Denmark around 78% of the colorectal resections for colorectal cancer end up with an anastomosis [1].

When the anastomoses following benign resection are added, a total of about 6800 colorectal resections and 5300 colorectal anastomoses are performed per year in Denmark. When extrapolated, according to the numbers from GLOBOCAN (WHO) given in table 1, this gives an estimate of the number of colorectal anastomoses performed in the European Union at around 435,000 a year, in United States 170,000 a year and worldwide around 1,717,000 a year.

Anastomotic leakage

In colorectal surgery, anastomotic leakage remains a feared and common complication. The frequency of this complication varies depending on many factors, primarily the localization of the anastomosis on the colon or rectum with higher rates for the lower anastomoses. The reported frequency of colorectal anastomotic leak varies from 1-51%, but comparative evaluation of the studies is difficult due to the lack of identical definitions [6-8;10-23].

Consequences of anastomotic leakage in colorectal surgery

The consequences of this feared complication can be severe, since the mortality after anastomotic leakage is as high as 6-44% [6;12-14;19;22;24-27]. Also, the morbidity is dramatically increased after anastomotic leakage [22;24;25;27]. Nevertheless, some of the leakages remain subclinical and the effects of these leakages are much less severe [15;28-30].

Non-colorectal gastrointestinal anastomoses

Besides colorectal anastomosis, gastrointestinal anastomoses are performed in many other fields of gastrointestinal surgery as well. Gastric and esophageal anastomoses are performed in the treatment of gastroesophageal cancer and obesity, pancreatico-jeonunal anastomoses in the treatment of pancreatic cancer and biliary anastomoses in the treatment of benign as well as malignant diseases in the hepato-biliary system. The number of anastomoses in these fields is widely unknown.

Anastomotic leakage is also a feared complication in these fields of surgery and, as in colorectal surgery, the consequences for the patients are severe [31;32].

Sealing of the anastomosis

In order to reduce the frequency of anastomotic leakage, and thereby the severe consequences of this, a significant number of studies have been performed on sealing of gastrointestinal anastomoses. Most of the studies have been experimental and have involved sealing with numerous different substances such as meshes, omental flaps, amniotic membrane and others. However, a few human studies have also been performed. Unfortunately, the results of these studies have been conflicting and mostly disappointing [33;34]. Because of that, sealing of colorectal anastomoses remains controversial and has not become a standard procedure.

Sealing with fibrin glue

A number of the sealing studies have been conducted with fibrin glue (FG), also called fibrin sealant. FG is forming a stable, physiological fibrin clot that assists in hemostasis and wound healing by reproducing the final steps of the blood coagulation cascade. The fibrin clots formed from FG are similar to normal blood clots and are by the body's enzymes naturally degraded after a few weeks. FG is available liquid [35;36] or bound to a mesh [37]. FG might be produced in the clinical setting from human or animal blood, but in most studies, the commercially produced FGs are used. Positive effects of FG on intestinal anastomoses have been found in both human and experimental studies [33;34]. However, none of the studies had healing as a primary endpoint. Thus, it is unclear if a positive effect is due to improved mechanical strength, protection of the anastomosis, or better healing per se.

OBJECTIVES

The overall objective of this thesis was, through a series of studies, to assess the safety and efficacy of FG for coating colonic anastomosis by:

- evaluating if sealing of gastrointestinal anastomosis with TachoSil™ is safe.
- developing a reproducible model of anastomotic leakage in pigs.
- evaluating if TachoSil™ is able to seal an anastomotic defect in an experimental model in pigs.
- evaluating if evidence exists on the ability of fibrin glue to affect healing, inflammation etc. in a positive or negative way in colorectal anastomoses.

THE STUDIES

Study 1; Safety study

Nordentoft T, Rømer J, Sørensen M. Sealing of gastrointestinal anastomoses with fibrin glue coated collagen patch: a safety study. J Invest Surg 2007; 20:363-369.

AIM

The aim of this study was to determine if it is safe to seal gastrointestinal anastomoses with a collagen patch coated with fibrin glue (TachoSil™). TachoSil™ was developed and manufactured as a hemostatic agent. Sealing properties had been described, but no previous studies had investigated the safety of this kind of sealing of gastrointestinal anastomoses.

METHODS

The study was an experimental study performed in pigs. In each pig, two end-to-end anastomoses were made at the small bowel, 50 and 100 cm from the ligament of Treitz. All the pigs had one anastomosis sealed with TachoSil™ (Figure 2) while one was left unsealed.

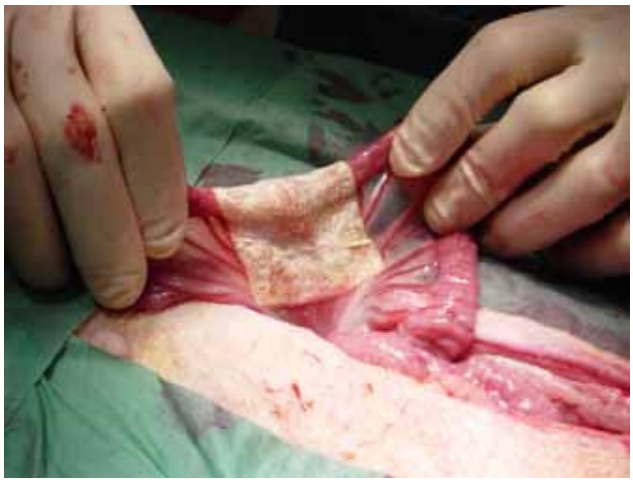

Figure 2: Sealing with fibrin glue covered collagen patch of a small bowel anastomosis in pig

In the first half of the pigs, the first anastomosis was sealed, while the second anastomosis was sealed in the other half.

The pigs were observed for 1-6 weeks, after which the pigs were examined in general anesthesia. Examination of the abdominal cavity was performed by looking for macroscopic signs of surgical complications such as anastomotic leakage, abscesses or bowel obstruction. In order to examine the degree of stenosis at the anastomotic site, X-ray of the anastomosis was performed (Figure 3).

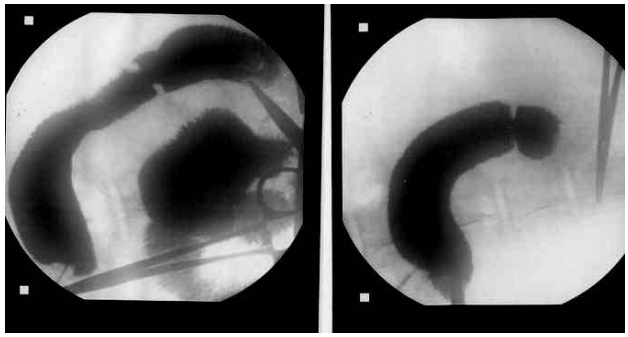

Figure 3: X-Ray comparing the degree of stenosis in sealed (left) and unsealed (right) anastomoses

After this, the in-vivo bursting pressure was measured at the anastomotic site. The pigs were finally sacrificed and the anastomoses were microscopically examined.

RESULTS

There were no difference between the sealed and the unsealed anastomoses with respect to abdominal pathology, in-vivo bursting pressure, or degree of stenosis. Microscopically there was no difference in healing or signs of infection.

CONCLUSION

It was safe to seal gastrointestinal anastomoses with a collagen patch coated with fibrin glue components.

STRENGTHS AND LIMITATIONS

The strength of the study is that it was the first study to evaluate the safety of sealing of gastrointestinal anastomoses with TachoSil™. The study was performed in a standardized and well-described way, which makes the results somewhat generalizable despite the lack of randomization.

The main limitation of this study was that the anastomoses were performed on small bowel, while colonic anastomoses are much more frequent in humans. The reason for the chosen design was that the pig colon differs from the human in being partly fixed in a spiral. If two anastomoses should be made in a systematic way, it would involve varying degrees of dissection, which might have influenced the outcome [38]. Another limitation was the non-randomized design. Instead, we selected a model in which the pigs served as their own controls in that they had two anastomoses each. The reason for this decision was to reduce the number of experimental animals required. In half of the pigs oral anastomoses were sealed, in the other half the anal anastomoses were sealed. This was non-randomized, which might be considered a limitation. One more limitation is the small sample size, causing a risk of type II error with respect to the macroscopic findings, especially anastomotic leakage and abscesses. The reason for choosing this design was again to reduce the number of experimental animals.

Study 2; Model study

Nordentoft T, Sørensen M. Leakage of colon anastomoses: development of an experimental model in pigs. Eur Surg Res 2007; 39:14-16.

AIM
The aim of this study was to develop a model of a significantly leaking colonic anastomosis in the pig.

METHODS
Colonic anastomoses were made in pigs. The anastomoses were made on the free part of the transverse colon, not involved in the colon-spiral. In all anastomoses, a standardized rupture in the anastomotic line was made. The rupture ranged from 5 mm in the first group of pigs, increasing subsequently to 21 mm in the last group. The pigs were sacrificed after seven days or earlier if any signs of illness were presented. The endpoints were macroscopic leakage and fecal peritonitis. The defect in the anastomotic line was considered too small to induce significant leakage if two consecutive animals in the group did not develop signs of leakage of peritonitis. Until this goal was reached, new groups of pigs were operated with increasing defects in the anastomotic line.

RESULTS
Groups of pigs with defects of 5, 6, 7, 10, 15, 18 and 21 mm of the anastomotic line were investigated. In the 5-18 mm groups 0-33% of the pigs developed macroscopically leakage or peritonitis. In the group of pigs with a 21 mm defect of the anastomotic line 100% of the pigs developed macroscopic leakage and peritonitis.

CONCLUSION
In an animal model of anastomotic leakage of pig colon, a 21 mm defect will result in a reliable and reproducible clinically significant leakage from the anastomosis.

STRENGTHS AND LIMITATIONS
The strength of the study was that it was the first study trying to develop a validated model of an incomplete and significantly leaking colonic anastomosis in pigs [38]. Up to this study most experimental sealing studies had been made on random models. The main limitation of this study was the study design. The ideal study would be an RCT randomizing the pigs to different sizes of leak in the colonic anastomoses. To avoid type 1 and 2 errors this design would involve 10-20 pigs in each group, i.e. around 100 pigs in total, which would be very demanding, due to both economical and ethical considerations. Another limitation is that all of the endpoints were macroscopic findings. A leakage might occur as a micro-leakage, not causing visible leakage or fecal peritonitis, but microscopically inflammation in the abdominal cavity. The reason for the decision of only focusing on macroscopic findings as endpoints were that some degree of inflammation of the peritoneum would be expected 7 days after a laparotomy thus raising the risk of false positive results.

Study 3; Efficacy study

Nordentoft T, Holte K. Preventing clinical leakage of colonic anastomoses with a fibrin-coated collagen patch sealing - an experimental study. Arch Clin Exp Surg 2014; 3:201-206.

AIM
The aim of this study was to determine if a collagen patch coated with fibrin glue (TachoSil™) was able to seal a leaking colonic anastomosis and thereby preventing clinical leakage and peritonitis.

METHODS
A colonic anastomosis with a 21 mm defect in the anastomotic line, according to the model described in Study 2, was created in 20 pigs. The anastomoses were made on the free part of the transverse colon, not involved in the colon-spiral. The animals were randomized to sealing or no sealing with the anastomotic line being covered with TachoSil™ in the sealing group. The pigs were observed for seven days after which they were sacrificed. However, the pigs were sacrificed earlier if any signs of illness were presented. The endpoints were visible leakage at the anastomotic site, fecal peritonitis and sacrificed/death before end of the observation period.

RESULTS
A significant reduction in macroscopic anastomotic leakage and fecal peritonitis were observed in the sealing group (Fischer's exact test, p=0.0055). A non-significant reduction of death and early sacrifice was found in the sealing group (Fischer's exact test, p=0.3034).

CONCLUSION
A collagen patch coated with fibrin glue components efficiently sealed leaking colonic anastomoses in the pig.

STRENGTHS AND LIMITATIONS
The main strengths of this study were the validated experimental model used, the randomized design, and the uniform and thereby convincing results.

This study has some limitations. The key aim of the present thesis, and thereby the studies included, was to evaluate if the fibrin glue collagen patch was able to seal a colonic anastomosis and thus prevent clinical anastomotic leakage. Nevertheless, it was not a study on anastomotic leakage, but on sealing of incomplete anastomoses. The ideal study would be a study design randomizing complete colonic anastomoses to sealing or not, but since the frequency of spontaneous anastomotic leakage in healthy pigs is very low, a very large number of animals would have to be included to prevent the risk of Type 2 error. The number of pigs would be very demanding, due to both economical and ethical considerations. Another limitation is that the study was made on pigs and not on humans, since pig colon is not completely similar to human colon. This raises the question whether the results might be reproducible in humans or not. However, the colon of the pig is very similar to the human colon in regards to anatomy, physiology, digestive function and splanchnic blood flow. On the other hand, the anatomy differs from man since the pig colon consists of a spiral, in which most of the colon is coiled together [38]. Thus, this limitation must be considered minor.

Study 4; Systematic review

Nordentoft T, Pommergaard H-C, Rosenberg J, Achiam MP. Fibrin glue does not improve healing of gastrointestinal anastomoses: a systematic review. Eur Surg Res. 2015, 54(1-2), 1-13.

AIM
The aim of this systematic review was to investigate if fibrin glue has a histological or biochemical effect on the healing of gastrointestinal anastomoses.

METHODS

A systematic review was performed according to the PRISMA guidelines [39]. To be included in the quantitative synthesis of the review, a study had to fulfill all of the three following criteria: 1) A study on gastrointestinal anastomoses. 2) Anastomosis sealed with fibrin glue. 3) A control group to the sealed anastomosis must exist in the study design. For further details on the search strategy, study selection criteria please see the manuscript attached to this thesis.

RESULTS

Twenty-eight studies were included in the qualitative synthesis. All of the studies were experimental, since no human study fulfilled the above-mentioned criteria. The results of the studies were conflicting, but predominantly negative or neutral, since only seven out of the 28 studies revealed a positive effect of fibrin glue on the healing of the anastomosis.

Among the studies with positive results 3 of 7 (43%) of the anastomoses were made on small intestine, whereas this was only the case in 11% and 18% studies with negative results and no effect, respectively. No differences were found relating to of type of fibrin glue used or kind of experimental animal.

CONCLUSION

It is unlikely that fibrin glue has a positive influence on the healing of gastrointestinal anastomosis. Consequently, it is likely that a positive effect of sealing with fibrin glue on the overall outcome might be due to mechanical properties of the sealing.

If fibrin glue has any positive effect on healing of anastomoses, this effect might be impaired by an infected environment such as in the colon.

STRENGTHS AND LIMITATIONS

The strength of the review is that it is made as a systematic review according to the PRISMA guidelines [39]. Furthermore, this is the first review on this subject.

This study has certain limitations too. Bias assessment of the individual studies was not performed. Suitable tools for evaluation of studies exist for observational and interventional clinical studies [40]. However, no validated methods for experimental studies exist. Another important limitation of this review is the high degree of heterogeneity between the studies both with regard to evaluation of healing, study design, and outcome measures. Macroscopically healing was the primary endpoint in all of the studies. Hence, microscopically healing was sparsely and occasionally unsystematically described, and a meta-analysis was therefore not possible. Such heterogeneity is known to limit comparability of the studies, which may be a partly explication to the inconsistent results of the studies [40]. Publication bias is existing for all types of studies, but are almost four times more common in experimental studies compared with randomized controlled trials [41]. Therefore, the experimental design of the included studies may be subject to a high degree of publication bias, which may overestimate the effect of FG. Selection bias is less common in experimental studies due to a high degree of similarity between the animals compared with humans in clinical trials. Furthermore, 61% of the included studies were randomized, which further reduces the risk of selection bias. Finally, none of the studies were performed in humans, which must be considered a limitation for clinical applicability.

DISCUSSION

Basic findings

The present thesis has three major conclusions: It was feasible and safe to seal gastrointestinal anastomoses with a fibrin-coated collagen patch, the fibrin-coated collagen patch was able to seal an incomplete anastomosis in an experimental model, and the effect of this sealing was probably due to mechanical/physical properties of the fibrin glue or the patch and not due to healing properties. Furthermore, an experimental model of a leaking colonic anastomosis has been developed.

Anastomotic leakage in colorectal surgery

Anastomotic leakage remains the most serious complication in colorectal surgery. The reported leakage range is 1-39%, but comparison of studies is difficult due to lack of standardized definitions [13;14;16;21;42]. According to the national database of the Danish Colorectal Cancer Group (DCCG), to which all operations for colorectal cancer in Denmark are reported, the overall leakage frequency is 8.4% with 6.0% on the colon anastomoses and 15.8% on the rectum anastomoses [1]. Thus, the number of anastomotic leakages is around 275/year after operation for colorectal cancer and around 450/year, if all colorectal anastomoses are included. When the Danish numbers are extrapolated according to the numbers given in table 1, approximately 37,000 leakages would occur per year in the Europe Union, 14,500 per year in USA and around 146,000 worldwide.

The pathophysiological mechanisms for anastomotic leakage are widely unknown, although compromised blood supply and thereby impaired healing of the anastomosis are considered central in this mechanism [43-46].

Risk factors for anastomotic leakage have been intensively studied. The demographic factors male gender, obesity and abuse of tobacco have been shown to be independent risk factors [10;18;20;22;47-50], while alcohol abuse do not seems to be an independent risk factor [51]. Severe comorbidity, such as cardiovascular pulmonary disease and use of steroid as well as general malnutrition and low s-protein or s-albumin, have been shown to be risk factors [5;9;13;22;24;48;52-54]. Preoperative chemotherapy, American Society of Anesthesiologists (ASA) score > 2, acute operation, long duration of surgery, intraoperative complications and blood loss increases the risk of anastomotic leakage too [13;20;23;24;47;50;53-55].

The localization of the anastomosis is essential to the risk of anastomotic leakage too, since the highest incidence of anastomotic leakage is at the low rectum where the incidence might be as high as 20% falling gradually to 2-5% at the right colon [6;11;13;20;23;26;50;54] (Figure 5).

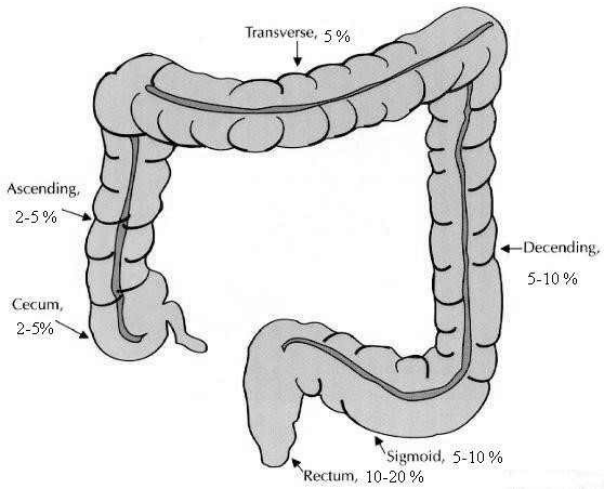

Figure 4: General risk of anastomotic leakage depending on localization of the anastomosis

The reason for the influence of the anastomoses localization is probably the differences in blood supply, since rectum has a more vulnerable blood supply than the right colon. Similarly, the lack of sufficient blood supply is probably the reason for the increased risk for anastomotic leakage in patients with severe cardiopulmonary or vascular disease. Furthermore, immunological differences between colonic and rectal anastomoses might be another explication of higher frequency of leakage on rectum [56].

Perioperative interventions such as bowel preparation, temporary stoma creation or drain insertion do not influence the incidence of anastomotic leakage [14;57;58], and until now no operative intervention studies has demonstrated the ability to reduce the leakage rate.

Clinical consequences

The immediate consequences of anastomotic leakage are dramatic. The mortality of this complication is reported to be as 6-44% [6;12-14;19;22;24-27]. According to the calculations in the previous paragraphs this indicates that anastomotic leakage in colorectal surgery causes between 30-200 deaths in Denmark per year and around 9000-64000 deaths per year worldwide. Moreover, anastomotic leakage is a cause of severely increased morbidity and prolonged hospital stay [22;24;25;27], and thereby significantly increased economical expenses.

Anastomotic leakage has significant long-term consequences as well. Patients operated for a colorectal cancer and surviving an anastomotic leakage have a significant increased risk of cancer related death and thereby reduced five-year survival [11;19;21;25;59-63]. The reduction in survival is highly significant, e.g. one study reports a reduction in five-year survival is reported from 63.7% to 25% after anastomotic leakage [19]. Also, the frequency of local recurrence [12;59-62] and distant recurrence [63] is increased after anastomotic leakage. The reason for these findings is not fully understood, but the delay or cancellation of chemotherapy caused by the leakage might contribute [63]. Immunological effects induced by peritonitis and septicemia caused by the leakage might be another explication [64].
Subclinical leakages

Anastomotic leakage can manifest as fulminant peritonitis, local abscess or be subclinical. If the condition includes clinically or paraclinically recognized peritonitis, it always requires acute re-operation. However, if the only manifestation is a smaller, local abscess, an option might be percutaneous or trans-bowel drainage, possibly combined with a diverting stoma [15;28-30;62;65]. The subclinical anastomotic leakages are most often not recognized and are considered to be less dangerous and to have less impact on the short-term and long-term outcome [62]. Thereby, the frequency of subclinical leakage is widely unknown, but more, radiological studies on leakage after operation for rectal cancer revealed that the number of subclinical leakages was 2-3 times as high as the clinically identified leakages [15;28;30].

The reason why some leakages remain subclinical is widely unknown. It might be hypothesized that these leakages are bounded by omentum, other peritoneal surfaces etc., and thereby sealed by natural factors.

Sealing of anastomoses

Sealing or coating of colorectal anastomoses with a variety of materials has been investigated in both clinical and experimental studies as an attempt to reduce the frequency of anastomotic leakage or to reduce the consequences of these. Unfortunately, many of these attempts have failed to show convincing results [66;67]. Several experimental studies have shown promising results, but these results were often not reproducible, especially in human studies.

Sealing with omentum has been tried in experimental as well as human studies. One study on dog colon anastomoses found no beneficial effect on clinical outcome or bursting pressure compared to controls [68]. Another study on rat showed a positive effect on clinical leakage and death as well as healing factors after omental sealing of incomplete anastomoses compared to controls. After microscopically examination, the authors concluded that the omental sealing is providing a biologically viable plug to prevent early leakage and is a source of granulation tissue and neovasculature for later wound repair [69]. A human randomized controlled trial (RTC) on 126 colorectal anastomoses found significant reduced frequency of anastomotic leakage in the sealing-group [70] similar to another RTC on sealing 112 rectal anastomoses [71]. Nevertheless, in a third human RCT on 712 colorectal anastomosis, no difference in the clinical outcome between sealing-group and controls were found [72].

Three experimental studies have investigated peritoneal graft for sealing colonic anastomoses. One study on dogs found significant less anastomotic leakage and improved healing in the sealing group [73], while two studies on rats and dogs were not able to demonstrate improvement in anastomotic leakage compared to controls [68;74]. In one of these studies, impaired bursting pressure and anastomosis healing were demonstrated in the sealing group, and on basis of the microscopical findings the authors hypothesized that this was due to aggravated adhesions creating a favorable environment for bacteria leading to decreased healing [74]. No human studies on sealing with peritoneal grafts have been published.

Sealing of colonic anastomoses with small intestinal submucosa has been investigated in two, experimental studies by the same authors [75;76]. No benefit in anastomotic leakage and similar was found, but in one of the studies the sealing was found to have a positive effect on bursting pressure and healing in the early phase since neovascularization, fibroblast ingrowth and collagen deposition were significantly increased after 4 days compared to controls [76]. No human studies on sealing with small intestinal submucosa have been published.

Two experimental studies on sealing colonic anastomoses in rat with amniotic membrane have been published by the same authors. In one study normal and high-risk anastomoses were sealed [77]. This study failed to demonstrate beneficial effect on anastomotic leakage but found increased bursting pressure in the sealed group. Also, healing was improved since fibroblast activity, collagen deposition, and hydroxyproline concentration were all significantly higher. In the other study the anastomotic sealing was studied in a model of peritonitis [78]. Bursting pressure and microscopical healing was improved in this study too, and the authors concluded that the sealing physically protected the anastomoses from the negative effect of the intraperitoneal sepsis on the healing process. No human studies on sealing with amniotic membrane have been published.

Sealing of colonic anastomoses in rats with platelet-rich plasma has been shown to improve bursting pressure. Furthermore, less inflammation and improved healing factors such as hydroxyproline level and collagen production were found in the sealing group [79]. No human studies on sealing with platelet-rich plasma have been published.

Two experimental studies on sealing colonic anastomoses in rats with hyaluronic acid/carboxymethylcellulose (Seprafilm®) showed no benefit of the sealing on bursting pressure, anastomotic strength or histopathologic healing [80;81]. However, in a human RTC anastomotic leakage, fistula formation, abscess formation, peritonitis, and sepsis were significantly more frequent in the sealing group [82].

Three experimental studies on sealing colonic anastomoses with polyglycolic acid mesh have been published, although with conflicting results. In a study on dogs, no effect on anastomotic leakage and healing was found in a septic environment [73]. A study on complete and incomplete colonic anastomoses in rats found increased leakage, decreased bursting pressure and impaired microscopical healing in the sealing group and concluded that the impaired healing was probably due to reduced peritoneal or omental contact [83]. In contrast to this, another study on rabbit colon found increased bursting pressure and no difference in histological healing, concluding that the increased bursting pressure was a result of the external mechanical support to the anastomoses [84]. No human studies on sealing with polyglycolic acid mesh have been published.

Polypropylene mesh has been used for sealing colonic anastomoses in two experimental studies. One study on dogs found significant less anastomotic leakage in the sealing group [68], while another study on rabbits showed in increased bursting pressure in the sealing group, but no difference in anastomotic leakage [85]. No human studies on sealing with polypropylene mesh have been published.

Different kinds of cyanoacrylate have been use for sealing colonic anastomoses in six experimental studies. None of these studies were able to demonstrate any difference in anastomotic leakage compared to controls, but some of the studies found decreased bursting pressure, more strictures and increased inflammatory reaction in the sealed groups [86-91]. No human studies on sealing with cyanoacrylate have been published.

Numerous other coating materials have been tried in experimental studies without positive results [66;67].

Sealing with fibrin glue

Most promising and consistent results have been seen in sealing with fibrin glue. Sealing of anastomoses with fibrin glue has been studied in both human and experimental studies. The sealing studies included in this thesis all comprise of sealing with fibrin glue.

From Study 1 we conclude that sealing of gastrointestinal anastomoses with a fibrin glue coated collagen patch (TachoSil™) was safe [92]. No similar safety studies on humans have been published and none of the previously published human studies with fibrin glue have reported safety problems such as stenosis, abscess or anastomotic leakage. In a resent feasibility- and safety study, 25 patients had a rectal anastomosis sealed by TachoSil™. The patch was found feasible and safe, since no adverse events were considered related to TachoSil™ [93]. In another non-randomized, human study, 24 colorectal anastomoses was sealed with TachoSil™ and compared to similar, non-sealed patients. In this study, like the others, no adverse events was related to the sealing was found [94].

In contrast to the human studies, some safety problems with sealing colorectal anastomoses with TachoSil™ in experimental studies have been reported. In a recently published study, in which incomplete colonic anastomoses in mice were sealed with TachoSil™, an increased rate of bowel obstruction in the sealing-group was found [95]. This finding is in line with the results of a study by Chmelnik et al. [96], which reported a high frequency of stenosis at the anastomotic site after similar sealing of small bowel anastomoses in rats. Consequently, they concluded that this kind of sealing could not be recommended in small-diameter anastomoses. No other studies on sealing with fibrin glue have revealed similar results, and therefore it is likely to believe that it is rather the patch than the fibrin glue per see that causes the stenosis. The results from these studies indicate that TachoSil™ is unsuitable for sealing small-diameter anastomoses, while it is safe to use is for sealing of other anastomoses.

The effect of fibrin glue sealing of colorectal anastomoses has been investigated as well. In a human study by Huh et al. [97], 223 patients with rectal cancers were randomized for sealing or no sealing after stapled anastomosis. The clinical leakage rate was 10.9% in the non-sealed group and 5.8% in the sealed group. However, this difference was not significant probably due to a Type 2 statistical error. The study is the only human RCT on sealing colorectal anastomosis with fibrin glue. One recent, non-randomized study on sealing colonic anastomoses with TachoSil™ found that the sealing was associated with shorter postoperative stay compared with a similar, non-sealed group [94]. The efficacy of sealing colorectal anastomosis with both liquid and mesh-bound fibrin glue has been investigated in experimental studies as well. The results according to the macroscopically endpoints such as anastomotic leakage, abscess formation and bursting pressure or anastomotic strength are varying, but mainly positive. A recent study on mice found a decreased rate of leakage after sealing of insufficient anastomoses with TachoSil™, but no difference in breaking strength [95]. The same study reported an increased rate of bowel obstruction as described in the previous paragraph. In a similar study Palentis et al. found significantly lower mortality and leakage rates, as well as significantly higher bursting pressure values and histopathologic scores [98]. Several experimental studies on sealing colorectal anastomoses with liquid fibrin glue have been conducted as well. Most studies were made on rat colon under different conditions. A majority of studies reported a positive effect on the macroscopically endpoints anastomotic leakage, bursting pressure or anastomotic strength [80; 99-108]. Nevertheless, a few studies found a negative effect of the sealing [109-111].

The results of both human and experimental studies are in line with the results of the efficacy study included in this thesis, Study 3 [112]. While the effects of fibrin glue sealing for the macroscopic factors anastomotic leakage, bursting pressure, and anastomotic strength are predominantly positive, this is not the case for histological/biochemical healing of the anastomosis sealed with fibrin glue. In Study 4 [113], 28 studies on sealing of gastrointestinal anastomoses with fibrin glue were included [80;92;96;98;99;101-105;107;108;110;111;114-127]. Only seven of the studies were positive on the microscopic healing of the anastomoses after fibrin glue was added [98;106-108;116;119;126]. On the other hand, in the same 28 studies only two studies revealed a negative effect on bursting pressure or macroscopic properties [96;110], while 14 studies reported a positive effect on these macroscopically parameters. From these findings we conclude, that a positive effect of fibrin glue sealing of gastrointestinal anastomosis most likely is due to a mechanical influence and not due to improved healing. From the results of the systematic review and from the literature discussed above, we find it likely that the positive effects of sealing colorectal anastomosis with fibrin glue is due to a mechanical barrier. Furthermore, we believe that the positive effect of fibrin glue sealing primarily is by avoiding subclinical leakages to develop into clinical leakages.

CONCLUSION

The studies included in this thesis have shown that:

- Sealing of colonic and small bowel anastomoses with TachoSil™ is both safe and feasible.
- The developed pig-model of clinically significant anastomotic leakage is both feasible and reproducible.
- TachoSil™ is able to seal an anastomotic leakage using this model.
- Fibrin glue does not seem to have a positive effect on healing of gastrointestinal anastomoses according to the current evidence. Thus, we find it likely that a positive effect of anastomotic sealing with fibrin glue is due to mechanical/psychical properties and not due to increased healing per se.

PERSPECTIVES/FUTURE RESEARCH

The present studies showed that sealing of anastomoses in a pig-model of defect, colonic anastomoses is safe and efficient. Since FG probably has no additional effects on anastomotic healing, the mechanism of the sealing must be mechanical. If these results can be transferred to humans, it would be a major step in the development of colorectal surgery by hindering the development of sub-clinical to clinical leakages.

To answer this question human RCTs must be designed and conducted. To recruit patients and surgeons for such studies would properly not be a problem, since colorectal anastomoses are preformed frequently, both in Denmark and worldwide, and since many specialized centers for colorectal surgery exist, especially in Europe and USA. Nevertheless, such RCTs will lead to two major challenges, one technical and one economical. The technical challenge comes from a correct application of the mesh around the anastomosis in the area of laparoscopic surgery. The studies in this thesis were all performed as open surgery. Since the start of these studies, laparoscopic colorectal surgery has gained increasing interest and is today the gold standard in many centers. Although efforts in developing a laparoscopic application tool for TachoSil™ have been substantial, none of these devices have yet been useful. This challenge must be solved.

The economical challenge comes from the huge number of patients that needed to be included in such a trail. A RCT where patients are randomized to sealing or no sealing after colorectal anastomoses should be performed. This kind of study might be performed on either colonic or rectal anastomosis, but both have advantages and disadvantages: The obvious advantage of a study on colonic anastomoses is the easier application compared to rectal anastomoses, especially the low ones. The disadvantage of a colonic study is the lower leakage rate on colonic anastomoses compared with rectal anastomoses, and thereby the higher sample size required to avoid a Type II error.

The hypothesis in such an RCT might be that sealing with TachoSil™ will reduce the leakage frequency with 50%. Provided an $\alpha = 0.05$ and $\beta = 0.20$ the minimal required sample size would be 2 x 800 in a study on all colon anastomoses, 2 x 400 in a study on left colon anastomoses, or 2 x 150-200 in a study on rectal anastomoses. These calculations are made from the leakage rates given in figure 5. Such a study has to be done as a multicenter study, probably international and it will therefore require considerable amount of work and significant economic support.

On the other hand, if such an RCT succeeded to reproduce the results of the present studies in humans, a huge step would have been taken in solving the most important problem in colorectal surgery.

SUMMARY

Background
Colorectal cancer (CRC) is the most common cancer of the gastrointestinal tract. In Denmark is CRC the 3. most frequent form of cancer and the 3. leading cause of cancer-related death.

Anastomoses
Surgical resection is the only curative treatment of CRC and in Denmark about 85% of patients with CRC therefore operated. An anastomosis will be established in most cases. Colorectal anastomoses are established in the treatment of benign diseases too, i.e. as part of the surgical treatment of inflammatory bowel disease and in acute surgery. Furthermore anastomoses are conducted in other parts of the gastrointestinal tract. i.e. esophagus, stomach, small bowel and bile system.

Anastomotic leakage (AL)
AL is the most serious complication of gastrointestinal surgery with a 30-day mortality of 13-27%. The reported AL rate ranges from 1 to 39%. In addition to immediate clinical consequences AL is an independent predictor of reduced general and cancer-specific survival. Leakage can manifest as generalized peritonitis, requiring acute re-surgery or as a more localized accumulation/abscess or as a subclinical leakage.

Sealing of anastomoses
Numerous studies on anastomotic sealing have been conducted with the aim of reducing the number of AL's. The results of these are conflicting and predominantly disappointing. The drug TachoSil® (TS) consists of a collagen patch, which on the one side is coated with Fibrin Glue (FG), which gives it an adhesive property. TS is registered for use in surgical hemostasis.

Animal Models
Spontaneous AL in animals is infrequently. It is therefore necessary to use a model of AL. No such model exists and must be developed.

Objective
- To clarify if the sealing of anastomoses with TS is feasible and safe in an experimental design.
- To develop a standardized model of AL in pigs.
- To clarify if sealing of colon-anastomoses with TS can reduce the number of clinical ALs in an experimental design.
- To clarify whether there is evidence that FG influences healing of gastrointestinal anastomosis.

Studies
Safety Study, that examines whether it is safe to seal anastomoses with a TS. Experimental study on pigs. Two anastomoses on each pig, one sealed with TS. After 1-6 weeks of observation the anastomosis were examined for AL, stenosis, strength and compared microscopic.
Results: No difference between sealed and un-sealed anastomosis.
This study is completed and published [92].

Model Study, to develop model of AL on pigs. A total of 22 pigs had an anastomosis of colon. All anastomoses were left with a standardized defect on 5-21 mm. The pigs were observed in order to assess how big the defect should be to the pigs developed visible leakage and/or fecal peritonitis.
Results: Model developed. 21 mm defect significant.
This study is completed and published [128].

Efficacy study, testing if TachoSil® can seal an AL and thus prevent that this becomes clinically significant. A total of 20 pigs had a colon-anastomoses with a standardized defect of 21 mm. The pigs were randomized to sealing with TS or no sealing. Re-laparotomy after 7 days examining for visible leakage and/or fecal peritonitis.
Results: TachoSil® able to seal the defect (p=0.0055).
This study is completed and published [112].

Systematic review, with the purpose to study whether there is evidence that FG influence the healing of gastrointestinal anastomosis.
Results: Conflicting. FG does not seem to have an effect.
This study completed and published [113].

Conclusions
- Sealing of GI-anastomosis with TachoSil is safe and feasible
- A defect of at least 21mm must be left in an colon anastomosis to induce clinical peritonitis
- Sealing of defect colon-anastomosis in pigs with TachoSil can prevent clinical leakage and peritonitis
- FG has no positive effect on microscopically healing of GI-anastomosis.

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