Tissue adhesives in colorectal surgery: a stepwise approach

Konstantinos A. Vakalopoulos
TISSUE ADHESIVES IN COLORECTAL SURGERY: A STEPWISE APPROACH

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Thesis, Erasmus University Rotterdam, The Netherlands

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TISSUE ADHESIVES IN COLORECTAL SURGERY:

A STEPWISE APPROACH

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Proefschrift

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Contents

Chapter 1: General introduction 7

Part I: Where are we now?

Chapter 2: Tissue adhesives in gastrointestinal anastomosis: a systematic review 17
Chapter 3: Critical analysis of cyanoacrylate in intestinal and colorectal anastomosis 39

Part II: Mechanical strength of tissue adhesives

Chapter 4: Mechanical strength and rheological properties of tissue adhesives with regard to colorectal anastomosis: an ex vivo study 57
Chapter 5: Reducing anastomatic leakage by reinforcement of colorectal anastomosis with cyanoacrylate glue 75

Part III: Can tissue adhesives be safely used on living tissue?

Chapter 6: Prevention of leakage by sealing colon anastomosis: experimental study in a mouse model 87
Chapter 7: Clinical, mechanical and immunohistopathological effects of tissue adhesives on the colon: an in-vivo study 99

Part IV: Prevention of colorectal anastomotic leakage with tissue adhesives

Chapter 8: Sutureless closure of colonic defects with tissue adhesives: an in-vivo study in the rat 117
Chapter 9: The prevention of colorectal anastomotic leakage with tissue adhesives in a contaminated environment is associated with the presence of anti-inflammatory macrophages 133
Chapter 10: Sealing insufficient colonic anastomoses with cyanoacrylate tissue adhesives: an in vivo study 151

Chapter 11: Summary, discussion and future perspectives 165
Chapter 12: Nederlandse Samenvatting 177

Appendices PhD Portfolio 183
About the author 185
Dankwoord 187
General Introduction
An anastomosis is a surgical connection between two hollow or tubular structures\(^1\). The basic surgical principles of the creation of a bowel (i.e. gastrointestinal) anastomosis have remained relatively unchanged since the mid 1800’s, and concentrate on manual closure by sutures\(^2\). Since their introduction in the mid 1900’s, surgical staplers have evolved to aid surgeons in the creation of bowel anastomoses in an easy and standardized fashion\(^3-7\).

One of the main complications of a gastrointestinal anastomosis is the occurrence of anastomotic leakage (AL). During AL, the bowel contents, i.e. feces, can leak into the abdominal cavity causing generalized infection of the peritoneum: i.e. peritonitis. Peritonitis is a dangerous surgical emergency necessitating immediate treatment, and may include surgical exploration and the creation of a stoma\(^8\). AL rate is especially high after the creation of an anastomosis in the most distal part of the gastrointestinal tract, which consists of the colon and the rectum, or colorectum. In these colorectal anastomoses, AL rate ranges from 5 to 25% and is linked to a mortality rate of up to 32%. AL is also linked to a high morbidity- rate, with patients requiring redo operations for the creation of a defunctioning stoma, and, longer hospital stay\(^9,10\).

Furthermore, AL has major effects on healthcare costs, as hospital stay is prolonged and requires an aggressive diagnostic and therapeutic approach\(^11\). Despite years of research on the prevention of AL and the introduction of surgical staple devices, AL rates have remained unreduced over the past decades\(^12,13\).

In the quest for ways to prevent colorectal AL, research has focused on various key elements. Firstly, knowledge on predisposing factors leading to AL has helped surgeons to identify patients at elevated risk for AL, aiding in its early detection\(^14\). Numerous risk factors have been identified, including gender, smoking, obesity, alcohol abuse, level of anastomosis, pre-existent vascular disease, corticoid therapy, distant colorectal metastases, and after-hours surgery\(^15-20\).

Secondly, research concentrating on anastomotic technique has aimed to provide consensus between surgeons, and to identify promising novel surgical techniques. Various techniques have been proposed, ranging from double layered sutured anastomoses, the addition of antitraction sutures to the sutured anastomosis and the use of surgical staplers\(^21,22\). Furthermore, techniques such as the use of magnetic anastomotic rings and tissue welding have been investigated in animal studies, but have not led to clinical implementation\(^23-25\).

Lastly, the use of a mechanical barrier around the suture/staple line has been proposed to contain leaks, giving the anastomosis time to heal without clinical complications\(^26\). Specifically, various techniques have been proposed to seal the anastomosis from the inside, that is, intraluminally. In one clinical trial, a biofragmentable plastic sheet was used that attaches to the staple line helping to seal the anastomosis in the direct postoperative period. However, the trial was not completed as no protective effects of the device could be identified at interim analysis\(^27,28\).

Another approach to AL prevention is to apply a barrier to the external surface of the anastomosis. This ‘anastomotic seal’ may be applied after the creation of a sutured or stapled anastomosis, however, the creation of sutureless anastomoses has also been described\(^29-32\). Earlier studies have evaluated numerous types of sealing materials, including bovine pericardial patches, amnionic membrane, and omental pedical patches, without convincing results\(^33\). Lastly, the use of tissue adhesives (TAs) as colorectal anastomotic sealants has been proposed to prevent AL, and will be investigated in this thesis. Our research, as described in this thesis, has been divided into several sections, which are described below.
Part I: Where are we now?

Objectives:
- To evaluate the current level of evidence of experimental and clinical research of TAs in colorectal surgery.
- To compare experimental results of TA use in colorectal surgery to other types of gastrointestinal surgery.
- To investigate the evolution of TAs in colorectal surgery.

The first studies on the use of TAs for the prevention of colorectal anastomotic leakage date back to the 1960s. The first available TAs, the cyanoacrylates, were industrial-strength ‘crazy glue’ or ‘instant glue’. Despite high adhesive capabilities, this first generation of TA was associated with poor results when used on colonic tissue, as exothermic polymerization reactions led to local tissue toxicity. Over the years, many new TAs have been developed and have been implemented as anastomotic sealants throughout the gastrointestinal tract. Especially since the early 2000s, research on the use of TAs for the prevention of AL has been gaining popularity.

In Chapter 1 as a starting point for our research, a systematic review of clinical and experimental studies that investigated the use of commercially available TAs in the sealing of gastrointestinal anastomoses will be performed.

In Chapter 2, we focus on the oldest and most investigated TA category: the cyanoacrylates (CAs). This TA category has evolved greatly throughout the years, with newer formulations being more inert and more flexible. This study helps identify the reasons why past research has not been able to establish the value of CA use, and suggests possibilities for future research.

Part II: Mechanical strength of tissue adhesives

Objectives:
- To evaluate and compare mechanical strength of TAs.
- To investigate rheological properties of TAs.
- To create a basis for the future comparison of in vivo mechanical strength, to understand effects of post-application degradation on TA mechanical strength.
- To select promising TAs for future research.

The prevention of AL by a TA seal is based on the mechanical barrier created by the TA between the leak/intraluminal contents and the free intraperitoneal space. The TA bond must be strong enough to guarantee this barrier function, while remaining flexible enough not to interfere with the peristaltic movements of the colon. Previous in vivo research on TAs reports the strength of the TA-bond directly after sacrifice, following a variable follow-up period. Little information exists on the TA-bond strength directly after its application on the colon, before TA degradation by the body’s inflammatory reaction takes place.

In Chapter 3 we evaluate the mechanical strength of a large number of TAs. Furthermore, we aim to provide the rheological profile of each TA, giving insight into the level of flexibility or ‘brittleness’ of each TA, which is crucial for understanding the differences in mechanical
strength between TAs. The importance of rheological characteristics of TA has also been highlighted in a previous study by Serrero et al.\(^{40}\).

In Chapter 4 we will perform an ex-vivo study on the use of CAs to seal standard or insufficient porcine colonic anastomoses, as previous literature has already identified the high mechanical strength of CAs\(^{41,42}\). We evaluated the mechanical strength of each anastomosis by means of bursting pressure analysis.

**Part III: Can tissue adhesives be safely used on living tissue?**

**Objectives:**
- To identify effects of TA application on tissue healing.
- To evaluate tissue toxicity associated with TA use.
- To compare clinical and histopathological profiles between TAs.
- To compare in-vivo and ex-vivo mechanical strength of TAs.

Tissue Adhesive research until now has been performed on various animal models, including canine, porcine, rat and mouse models, each with their own advantages and limitations\(^{43}\). The development of an animal model that simulates AL in a reproducible way has been an important part of recent research, and crucial for the testing of TAs\(^{44-46}\). Based on these previous studies, and while also taking into account the cost perspective of the various animal models, we will start in-vivo testing of TAs in a validated mouse model for colonic AL, as described by Komen et al.\(^{46}\).

In Chapter 5 we use this model to seal insufficient anastomoses with several TAs. Next to the mechanical strength of a TA seal described in part I, an equally important aspect of TA use is its effect on tissue healing. Previous research has focused on two types of TAs, fibrin glue and cyanoacrylate. Histopathological outcomes of fibrin glue based on several studies reveal that this TA does not elicit tissue toxicity and is relatively inert, leading only to mild inflammatory reactions\(^{48-50}\). From the early years of TA use, CAs, on the other hand, were linked to tissue toxicity and inflammation when used on the colon. Early studies point out that long-chain CA formulations elicit an exothermic reaction leading to a severe inflammatory tissue response\(^{34}\). However, present-day short-chain CA formulations have become more inert, indicating safe use intracorporeally\(^{51,52}\).

These previous studies have only evaluated TAs in the presence of a bowel defect, making it difficult to conclude whether the observed inflammatory reactions are associated only with the presence of the TA or also with the presence of the defect, and the subsequent natural healing mechanisms.

In Chapter 6 we evaluate TAs in a rat model in which two separate colonic segments will be glued together without the presence of a bowel anastomosis or defect. Using this model we will be able to evaluate the clinical and histopathological effects brought upon directly by each TA, without the added effect of the anastomotic technique and subsequent surgical complications. We will observe the clinical effects, mechanical strength and effects on healing. From clinical studies we know that AL can occur up to four weeks after the creation of the anastomosis\(^{53}\); for this reason we will include a follow-up at one week as well as at one month.
Part IV: Prevention of colorectal anastomotic leakage with tissue adhesives

Objectives:
- To assess the effect of a TA seal on the incidence of AL.
- To evaluate the effects of fecal matter on the adhesive bond.
- To investigate the possibility of TA use in a contaminated environment.
- To identify the most promising TAs for future (clinical) research.

In the final part of this thesis, we evaluate the sealing capabilities of TA when used to prevent leakage of bowel defects.

In Chapter 7 we make surgical incisions in the colon, which we then seal with a TA, without the use of sutures or staples to close the defect. We will include the same set of TAs as in Chapter 6, to be able to compare results in a sound manner. With this model we will hopefully be able to evaluate the protective effect of a TA barrier in terms of intraperitoneal leakage of bowel contents and healing capability, as well as mechanical strength.

Another important question regarding the use of TAs on the colon is what happens to the TA-tissue bond in the presence of an intra-abdominal infection, i.e. peritonitis. From clinical studies we know that a colorectal anastomosis created in a contaminated environment leads to a higher AL rate\textsuperscript{54,55}.

In Chapter 8, we evaluate the effects of peritonitis on the mechanical strength of TA and the efficacy of TA in the prevention of AL in a contaminated environment. In Chapter 9 based on the results of our previous studies, we will select the best performing TAs for further, clinically applicable, testing to seal insufficient colonic anastomoses in a rat model.
REFERENCES

PART I

Where are we now?
Tissue adhesives in gastrointestinal anastomosis: a systematic review

KA Vakalopoulos, F Daams, Z Wu, L Timmermans, J Jeekel, GJ Kleinrensink, AC van der Ham, JF Lange

Journal of Surgical Research 2013 Apr;180(2):290-300.

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Journal of Surgical Research 2013 Apr;180(2):290-300.
ABSTRACT

**Background:** Anastomotic leakage in gastrointestinal (GI) surgery remains a major problem. Although numerous studies have been undertaken on the role of tissue adhesives as GI anastomotic sealants, no clear overview has been presented. This systematic review aims to provide a clear overview of recent experimental and clinical research on the sealing of different levels of GI anastomosis with tissue adhesives.

**Methods:** We searched MEDLINE and Embase databases for clinical and experimental articles published after 2000. We included articles only if these addressed a tissue adhesive applied around a GI anastomosis to prevent anastomotic leakage or decrease leakage related complications. We categorized results according to level of anastomosis, category of tissue adhesive, and level of evidence.

**Results:** We included 48 studies: three on esophageal anastomosis, 13 on gastric anastomosis, four on pancreatic anastomosis, eight on small intestinal anastomosis, and 20 on colorectal anastomosis; 15 of the studies were on humans.

**Conclusions:** Research on ileal and gastric/bariatric anastomosis reveals promising results for fibrin glue sealing for specific clinical indications. Sealing of pancreatoco-enteric anastomosis does not seem to be useful for high-risk patients; however, research in this field is limited. Ileal anastomotic sealing was promising in every included study, and calls for clinical evaluation. For colorectal anastomoses, sealing with fibrin glue sealing seems to have more positive results than with cyanoacrylate. Further research should concentrate on the clinical evaluation of promising experimental results as well as on new types of tissue adhesives. This research field would benefit from a systematic experimental approach with comparable methodology.
INTRODUCTION

Each year, millions of gastrointestinal (GI) anastomoses are created worldwide. Anastomatic leakage (AL) after the creation of a GI anastomosis remains an important complication in GI surgery. Despite years of research, the incidence of AL remains high, especially after esophageal and colorectal anastomosis. Anastomotic leakage is known to have a multifactorial etiology, mostly based on ischemia of the bowel endings and/or technical failure. Many risk factors are known, and can be categorized into patient-related risk factors (i.e., comorbidity, body mass index, drug use) and operative factors (i.e., surgeon’s experience, after-hours surgery, anastomatic location and operating time). Tissue adhesives have gained popularity in various fields of surgical practice, especially in skin closure. There are various types of tissue adhesives, each with their own adhesive mechanisms and uses. Basically, a tissue adhesive forms bonds with its substrate, ensuring sufficient adhesion. These bonds can either be chemical, of which covalent bonds are the strongest, or physical, including hydrogen bonds or van der Waals forces. Furthermore, the total strength of the glue bond depends on the balance between interaction within the tissue adhesive (cohesion) and between the tissue adhesive substrate-interface (adhesion). Tissue adhesives can either be glues, intended to independently connect various structures (i.e., wound edges), or sealants, used to cover and protect an anastomosis. Except for external use, tissue adhesives can also be used intracorporeally. Various tissue adhesives are being used in cardiovascular surgery, plastic surgery, and, increasingly, surgery of the GI tract. Tissue adhesives are promising tools for wound closure. They distribute forces throughout the wound more evenly and noninvasively than sutures and staples, are strong and flexible, and do not interfere with the wound-healing process. Also, the technique of tissue adhesive application to the wound is easy and standardizable, resulting in less variation in technique between surgeons.

By using tissue adhesives as sealants of GI anastomosis, enhancing standard anastomotic techniques, AL might be prevented or reduced and its clinical symptoms ameliorated. Numerous research projects have been undertaken to assess the applicability of available tissue adhesives in GI surgery; however, no recent literature provides the surgical community with an up-to-date overview of the progress in this field. This systematic review includes recent information on tissue adhesives with regard to all types of anastomotic configurations in the GI tract and provides a means to discover similarities and make comparisons among different levels of anastomoses. An overview is provided on all available clinical and experimental research concentrating on the use of tissue adhesives around the GI anastomosis, either as suture reinforcement or in sutureless closure, presented by level of anastomosis and category of tissue adhesive used. We hypothesized that the use of tissue adhesives around a GI anastomosis is a viable concept in the prevention of anastomotic leakage and that sufficient evidence, especially clinically, has arisen in past years to justify the implementation of several types of tissue adhesives for routine use.

METHODS

Search strategy
We performed this systematic review according to the PRISMA guidelines. We performed a literature search including all relevant articles from January 1, 2000, until May 12, 2011. The search was performed using the Embase and MEDLINE databases. We included only English
articles and excluded review articles and meta-analyses. For the study selection process, see Figure 1.

![Flowchart showing study selection process]

**Study selection**

We included articles only if they addressed a tissue adhesive applied around a GI anastomosis to prevent AL or to decrease leakage-related complications. The definition of tissue adhesive used for the purpose of this review was arbitrarily described as any liquid or gelled substance capable of adhering directly to the outer gastrointestinal tissue surface, without the need for an extra matrix layer. We excluded studies on the use of tissue adhesives with regard to the treatment of GI perforations and GI fistulas, or studies using artificial matrix or patch mounted tissue adhesives in GI anastomosis.

We extracted the following data for the clinical studies:

- First author and year of publication
- Level of evidence (following the Centre of Evidence Based Medicine, University of Oxford)
- Study design
- Number of subjects
- Location of anastomosis in gastrointestinal tract
- Anastomotic technique
- Tissue adhesive used and mode of application
- Definition of outcome by the authors (AL, clinical AL, radiological AL, complication rate)
- Results and statistical analysis

The following data were extracted for the experimental studies:

- First author and year of publication
• Study design
• Number of animals per group
• Anastomotic technique
• Type of tissue adhesive used and mode of application
• Species
• Outcome parameters for anastomotic healing (AL, anastomotic bursting pressure (ABP), breaking strength, histology, or collagen-concentration)
• Results and statistical analysis


RESULTS
Table 1 provides an overview of all types of tissue adhesives, as mentioned in the included articles. Results are summarized below according to the level of GI anastomosis, and are grouped by type of research (experimental or clinical) and by tissue adhesive category; tissue adhesive categories are mentioned only if they were used in at least one included study.

Tissue adhesives in esophageal surgery (Table 2)

a) Experimental studies
   Fibrin glue/ Cyanoacrylate
   The role of sealing in esophageal surgery has been investigated experimentally by Yurtcu et al.\(^\text{10}\). In this rabbit study three tissue adhesives, including fibrin glue (FG) and cyanoacrylate glue (CA), were applied on an esophago-gastric anastomosis. No AL was observed in any of the study groups and CA showed superior histological scores and higher bursting pressure when compared to the other groups (Table 2).

b) Clinical studies
   Fibrin glue
   Two clinical trials were conducted to study the use of FG in esophageal surgery in infants. Level 1b evidence is provided in a randomized controlled trial (RCT) performed by Upadhyaya et al.\(^\text{11}\). That study investigated the application of FG (Tisseel) to end-to-end esophagostomies for esophageal atresia. The Tisseel group showed significantly less leakage and strictures compared with the control group. A case-control study by Saldana-Cortes et al.\(^\text{12}\) showed significant reduction of AL after FG sealing of 14 esophagectomies with colonic interposition. No clinical studies could be found on regular esophagectomies in adults.
Table 1. Available commercial tissue adhesives per category

**Fibrin glues:**

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>Trade name</th>
<th>Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethicon (J&amp;J, USA)</td>
<td>CROSSEEL (USA)/Quixil (EU), EVICEL</td>
<td>Fibrin glue, no aprotinin, with transhexamic acid (Crosseel/Quixil)</td>
</tr>
<tr>
<td>Baxter (USA)</td>
<td>Tisseel (USA)/TissuCol (EU)</td>
<td>Fibrin glue, with aprotinin</td>
</tr>
<tr>
<td>Angiotech (USA)</td>
<td>Hemaseel APR</td>
<td>Fibrin glue, with aprotinin</td>
</tr>
<tr>
<td>CSL Surgery (USA)</td>
<td>Beriplast</td>
<td>Fibrin glue, with aprotinin</td>
</tr>
<tr>
<td>Guanzhou Bio Seal co (CHI)</td>
<td>Guanzhou Bio Seal</td>
<td>Fibrin glue, no aprotinin</td>
</tr>
<tr>
<td>Green cross P.D. co. (KOR)</td>
<td>Greenplast</td>
<td>Fibrin glue, with aprotinin</td>
</tr>
</tbody>
</table>

**Cyanoacrylate glues:**

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>Trade name</th>
<th>Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethicon (J&amp;J; USA)</td>
<td>Dermabond</td>
<td>2-octyl-cyanoacrylate</td>
</tr>
<tr>
<td></td>
<td>Omnix</td>
<td>2-octyl-cyanoacrylate</td>
</tr>
<tr>
<td>B.Braun (GER)</td>
<td>Histoacryl Blue</td>
<td>n-butyl-2-cyanoacrylate</td>
</tr>
<tr>
<td>GEM Italia (IT)</td>
<td>Glubran 2</td>
<td>n-butyl-2-cyanoacrylate and methacryloxsulfolane</td>
</tr>
<tr>
<td>Adhezion medical (USA)</td>
<td>Surgiseal</td>
<td>2-octyl-cyanoacrylate</td>
</tr>
<tr>
<td>GluStitch Inc. (CAN)</td>
<td>GluSeal</td>
<td>2-octyl-cyanoacrylate</td>
</tr>
<tr>
<td>Henkel (GER)</td>
<td>Pattex</td>
<td>Ethyl-2-cyanoacrylate</td>
</tr>
</tbody>
</table>

**Polyethylene glycol:**

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>Trade name</th>
<th>Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Covidien (FR)</td>
<td>Duraseal</td>
<td>Polyethylene glycol, trilisine amine and blue dye</td>
</tr>
<tr>
<td></td>
<td>Duraseal Xact</td>
<td>Idem, with N-hydroxy succinimide</td>
</tr>
<tr>
<td>Baxter (USA)</td>
<td>Coseal</td>
<td>Polyethylene glycol, hydrogen chloride and sodium phosphate-sodium carbonate</td>
</tr>
<tr>
<td>Genzyme Biosurgery Inc. (USA)</td>
<td>Focalseal-L</td>
<td>Polyethylene glycol, acrylate-capped poly-L-lactide and polytrimethylene carbonate</td>
</tr>
</tbody>
</table>

**Other categories:**

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>Trade name</th>
<th>Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardial SA (FR)</td>
<td>GRF glue</td>
<td>Gelatin-resorcinol-formaldehyde glue</td>
</tr>
<tr>
<td>Geister GmbH (GER)</td>
<td>Gluetiss glue</td>
<td>Gelatin-resorcinol-glyoxal glue</td>
</tr>
<tr>
<td>Biomet (USA)</td>
<td>GPS system for PRP glue</td>
<td>Platelet rich plasma (PRP)</td>
</tr>
<tr>
<td>Cryolife (USA)</td>
<td>BioGlue</td>
<td>Glutaraldehyde-albumin glue</td>
</tr>
<tr>
<td>Mundipharma GmbH, (GER)</td>
<td>Polydione-liposome (PVP-1)</td>
<td>Elemental iodine and polyvinylpyrrolidone (polydione) + liposome hydrogel</td>
</tr>
<tr>
<td>Cohera medical Inc. (USA)</td>
<td>TissuGlu</td>
<td>Urethane adhesive (lysine derived)</td>
</tr>
</tbody>
</table>

*not marketed for medical use
Tissue adhesives in gastrointestinal anastomosis (Table 3)

No studies evaluating the use of tissue adhesives in patients undergoing gastrectomy were found (Table 3).

a) Experimental studies

**Fibrin glue**

Two studies evaluated FG. In a pig model of insufficient gastrojejunoanastomosis, in which a defect was created in the anastomotic line, Bonanomi et al.\(^{13}\) and Nguyen et al.\(^{14}\) independently showed improvement in the AL rate (resp. from 100% to 0% and from 83% to 0%) after FG sealing compared with unsealed controls.

**Cyanoacrylate**

Cyanoacrylate was tested in one study. Weiss and Haj\(^{15}\) reported that sealing of the gastrojejunal anastomosis in a rat model with CA was not inferior to an unsealed anastomosis with regard to AL rate, stricture, peritonitis, and mortality rate.

**Other categories**

In an ex vivo pig study, Nandakumar et al.\(^{16}\) reported that the use of glutaraldehyde-albumin glue (BioGlue) to reinforce complete and incomplete circular stapled gastrojejunostomies resulted in significantly increased ABP compared with an unsealed control group.

b) Clinical studies

**Fibrin glue**

Clinical evidence for the effectiveness of FG is derived from nine studies, including one level 1b RCT and six level 2b prospective cohort studies. Silecchia et al.\(^{17}\) performed the only RCT. In that multicenter, prospective RCT, FG was applied to both the gastrojejunal and jejunojejunal anastomoses during laparoscopic Roux-en-Y gastric bypass (LRYGB). The differences in AL between the two groups (three of 160 in the control group and one of 160 in the FG group) were not significant; however, the overall reoperation rate for specific early complications (AL, internal hernia, and gastrojejunal anastomotic bleeding) was lower in the FG group (P = 0.016). The incidence of major late complications was similar in both groups. In a nonrandomized, case-control study, Liu et al.\(^{18}\) found that patients in whom the gastrojejunal anastomosis was sealed with FG after RYGB developed significantly less AL than the unsealed controls. One prospective study by Efthimiou et al.\(^{19}\), in which 474 patients undergoing LRYGB received FG sealing of gastrojejunal anastomosis and gastric staple line, showed no effect of sealing on the incidence of AL. However, the authors found that FG is associated with an increased clinical inflammatory response mimicking AL. Four observational uncontrolled studies showed a low prevalence of AL after the use of FG in LRYGB (Sapala et al.\(^{20}\): 0% [0 of

<table>
<thead>
<tr>
<th>LOE*</th>
<th>Author / year</th>
<th>Model</th>
<th>N</th>
<th>Tissue adhesive</th>
<th>Methods</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>Yurtcu / 2010(^{10})</td>
<td>Rabbit</td>
<td>24</td>
<td>CA (Glubran 2) FG (Beriplast)</td>
<td>Esophageal anastomosis</td>
<td>+</td>
</tr>
<tr>
<td>1b</td>
<td>Upadhyaya / 2007(^{11})</td>
<td>Clinical (RCT)</td>
<td>52</td>
<td>FG (Tisseel)</td>
<td>Esophageal anastomosis</td>
<td>+</td>
</tr>
<tr>
<td>3b</td>
<td>Saldana / 2009(^{12})</td>
<td>Clinical</td>
<td>38</td>
<td>FG (Quixil)</td>
<td>Colonic interposition</td>
<td>+</td>
</tr>
</tbody>
</table>

*LOE: level of evidence
738]; Cottam et al.\textsuperscript{21}: 1.6% [two of 126]; Raquel et al.\textsuperscript{22}: 2% [two of 100]; and Nguyen et al.\textsuperscript{14}: 0% [0 of 66]. Retrospectively, Fullum et al.\textsuperscript{23} reported three leaks in 760 (0.39%) LRYGBs performed by a single surgeon using FG.

Other categories
One case report on the use of autologous platelet gel in 10 morbidly obese patients undergoing LRYGB reported positive effects of autologous platelet gel\textsuperscript{24}. A contrast study on the first postoperative day showed no AL in any patient, and no AL was seen during the follow-up period (7 days).

Table 3. Synopsis of results; gastric/bariatric anastomosis

<table>
<thead>
<tr>
<th>LOE</th>
<th>Author/ year</th>
<th>Model</th>
<th>N</th>
<th>Tissue adhesive</th>
<th>Methods</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>Bonanomi / 2004\textsuperscript{13}</td>
<td>Pig</td>
<td>20</td>
<td>FG (Tisseel)</td>
<td>Gastrojejunal anastomosis</td>
<td>+</td>
</tr>
<tr>
<td>-</td>
<td>Nguyen / 2004 a\textsuperscript{14}</td>
<td>Pig</td>
<td>16</td>
<td>FG (Tisseel)</td>
<td>Gastrojejunal anastomosis</td>
<td>+</td>
</tr>
<tr>
<td>-</td>
<td>Weiss / 2011\textsuperscript{15}</td>
<td>Rat</td>
<td>64</td>
<td>CA (Histoacyl Blue)</td>
<td>Gastrojejunal anastomosis</td>
<td>+/-</td>
</tr>
<tr>
<td>-</td>
<td>Nandakumar / 2010\textsuperscript{16}</td>
<td>Pig</td>
<td>30</td>
<td>BioGlue</td>
<td>Gastrojejunal anastomosis</td>
<td>+</td>
</tr>
<tr>
<td>1b</td>
<td>Silecchia / 2006+2008\textsuperscript{17,70}</td>
<td>Clinical (RCT)</td>
<td>340</td>
<td>FG (Tisseel)</td>
<td>LRYGB\textsuperscript{**}</td>
<td>+/-</td>
</tr>
<tr>
<td>3b</td>
<td>Liu / 2003\textsuperscript{18}</td>
<td>Clinical</td>
<td>480</td>
<td>FG (Tisseel)</td>
<td>RYGB</td>
<td>+</td>
</tr>
<tr>
<td>2b</td>
<td>Sapala / 2004\textsuperscript{20}</td>
<td>Clinical</td>
<td>738</td>
<td>FG (Hemaseel APR, Tisseel)</td>
<td>Gastrojejunal anastomosis</td>
<td>+</td>
</tr>
<tr>
<td>2b</td>
<td>Cottam / 2006\textsuperscript{21}</td>
<td>Clinical</td>
<td>126</td>
<td>FG (Tisseel)</td>
<td>Laparoscopic sleeve gastrectomy</td>
<td>+</td>
</tr>
<tr>
<td>2b</td>
<td>Raquel / 2009\textsuperscript{22}</td>
<td>Clinical</td>
<td>100</td>
<td>FG (Tissucol)</td>
<td>LRYGB/Laparoscopic sleeve gastrectomy</td>
<td>+</td>
</tr>
<tr>
<td>2b</td>
<td>Fullum / 2009\textsuperscript{23}</td>
<td>Clinical</td>
<td>760</td>
<td>FG (not specified)</td>
<td>LRYGB</td>
<td>+</td>
</tr>
<tr>
<td>2b</td>
<td>Nguyen / 2004 b\textsuperscript{14}</td>
<td>Clinical</td>
<td>66</td>
<td>FG (Tisseel)</td>
<td>Gastrojejunal anastomosis</td>
<td>+</td>
</tr>
<tr>
<td>2b</td>
<td>Efthimiou / 2010\textsuperscript{19}</td>
<td>Clinical</td>
<td>474</td>
<td>FG (Tisseel)</td>
<td>LRYGB</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>Brady / 2006\textsuperscript{24}</td>
<td>Clinical</td>
<td>10</td>
<td>APG (autologous platelet gel)</td>
<td>Gastrojejunal anastomosis</td>
<td>+</td>
</tr>
</tbody>
</table>

\*2 part publication, discussed separately.
\**(L)RYGB: (laparoscopic) Roux-en-Y gastric bypass

Tissue adhesives for pancreatic anastomosis (Table 4)

a) Experimental studies

Other categories

Argya et al.\textsuperscript{25} performed a study on 10 pigs in which a sutureless pancreatico-jejunal anastomosis with polyethylene glycol glue (PEG) was created (Table 4). They concluded
that the use of PEG was technically feasible, prevented AL and did not interfere with the wound healing process.

b) Clinical studies

*Fibrin glue*

Clinically, we derived level 1b evidence from Lillemoe et al.\(^{26}\), who presented an RCT with 124 patients undergoing pancreatico-duodenal resection in which the pancreatico-jejunostomy was sealed with FG. In that study on high-risk patients (i.e., soft normal texture gland and a non-dilated pancreatic duct), FG did not reduce the incidence of pancreatic fistula, length of hospital stay, total complications, or death. Oida et al.\(^{27}\) reported a prospective series of 26 patients undergoing pancreatico-duodenectomy and subsequent sealing of the pancreatico-gastrostomy with FG and round ligament. In that study, no leakage was seen in any patients.

*Other categories*

One retrospective case-control study on 64 patients in which the pancreatico-jejunostomy was sealed with BioGlue reported 12 leaks and subsequent fistula formation in the control group and 13 in the BioGlue group. This difference was not statistically significant\(^{28}\).

### Table 4. Synopsis of results; pancreatic anastomosis

<table>
<thead>
<tr>
<th>LOE*</th>
<th>Author/ year</th>
<th>Model</th>
<th>N</th>
<th>Tissue adhesive</th>
<th>Methods</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>Argyra / 2009(^{25})</td>
<td>Pig</td>
<td>10</td>
<td>PEG (Focalseal L)</td>
<td>Pancreaticojejunostomy</td>
<td>+</td>
</tr>
<tr>
<td>1b</td>
<td>Lillemoe / 2004(^{26})</td>
<td>Clinical (RCT)</td>
<td>125</td>
<td>FG (Not specified)</td>
<td>Pancreatico-duodenectomy</td>
<td>+/-</td>
</tr>
<tr>
<td>4</td>
<td>Oida / 2009(^{27})</td>
<td>Clinical</td>
<td>26</td>
<td>FG (not specified)</td>
<td>Pancreatico-duodenectomy</td>
<td>+</td>
</tr>
<tr>
<td>3b</td>
<td>Fisher / 2008(^{28})</td>
<td>Clinical</td>
<td>64</td>
<td>BioGlue</td>
<td>Pancreatectomy</td>
<td>-</td>
</tr>
</tbody>
</table>

*Tissue adhesives in small intestinal anastomosis (Table 5)*

a) Experimental studies

*Fibrin glue*

Li et al.\(^{29}\) performed two rat studies in which they combined FG with human derived growth hormone (Table 5). They found that FG benefited anastomotic healing up to 5 d, and FG combined with growth hormone worked synergistically to improve anastomotic healing up to 14 d. Wang et al.\(^{30}\) also reported that FG combined with growth hormone decreased AL and improved anastomotic healing in a pig model of traumatic shock. Another study on canine jejunal anastomoses compared hemostatic and adhesive effects of two types of FG (Greenplast and Tisseel). One case of AL was apparent in the control group and none in the glue groups. It was reported that both glues had similar hemostatic and adhesive properties and may be useful as anastomotic sealants\(^{31}\).

*Cyanoacrylate*

Elemen et al.\(^{32}\) used industrial-grade cyanoacrylate (Pattex) for the creation of ileal anastomosis in a rat model. In that study, the glue caused less tissue damage and the
glued anastomoses healed better than the controls. Another cyanoacrylate (Glubran 2) was evaluated by Ensari et al.\textsuperscript{33}. In that study, jejunal anastomoses were sealed with Glubran 2 in 40 rats and ischemia reperfusion was induced before anastomosis creation. The authors reported that Glubran 2 significantly increased ABP either with or without existence of ischemia-reperfusion, and also increased adhesion formation around the anastomosis.

Other categories
In a study by Sweeney et al.\textsuperscript{34}, PEG sealant (FocalSeal-L) was tested in a rabbit model for incomplete ileal anastomosis. According to the study, an incomplete ileal anastomosis sealed with FocalSeal-L was not inferior to a sutured anastomosis in terms of adhesion formation, stenosis, and ABP.

b) Clinical studies

\textit{Fibrin glue}
One clinical study was performed, a level 2b cohort study by Wang et al.\textsuperscript{35}, in which patients with intra-abdominal sepsis underwent primary anastomosis and FG sealing. The authors concluded that FG protected the primary anastomosis in patients with intra-abdominal sepsis, therefore preventing the need for stoma construction.

Table 5. Synopsis of results; small intestinal anastomosis

<table>
<thead>
<tr>
<th>LOE*</th>
<th>Author/year</th>
<th>Model</th>
<th>N</th>
<th>Tissue adhesive</th>
<th>Methods</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>Li / 2006\textsuperscript{21}</td>
<td>Rat</td>
<td>360</td>
<td>FG (Guanzhou bio seal)</td>
<td>Ileal anastomosis</td>
<td>+</td>
</tr>
<tr>
<td>-</td>
<td>Li / 2007\textsuperscript{29}</td>
<td>Rat</td>
<td>300</td>
<td>FG (Guanzhou bio seal)</td>
<td>Ileal anastomosis; Incomplete</td>
<td>+</td>
</tr>
<tr>
<td>-</td>
<td>Wang / 2009\textsuperscript{30}</td>
<td>Pig</td>
<td>63</td>
<td>FG (Guanzhou bio seal)</td>
<td>Ileal anastomosis; gunshot wound model</td>
<td>+</td>
</tr>
<tr>
<td>-</td>
<td>Park / 2002\textsuperscript{31}</td>
<td>Dog</td>
<td>18</td>
<td>FG (Greenplast, Tisseel)</td>
<td>Jejunal anastomosis</td>
<td>+</td>
</tr>
<tr>
<td>-</td>
<td>Elemen / 2008\textsuperscript{32}</td>
<td>Rat</td>
<td>96</td>
<td>CA (Pattex)</td>
<td>Ileal anastomosis</td>
<td>+</td>
</tr>
<tr>
<td>-</td>
<td>Ensäri / 2010\textsuperscript{33}</td>
<td>Rat</td>
<td>40</td>
<td>CA (Glubran 2)</td>
<td>Jejunal anastomosis</td>
<td>+</td>
</tr>
<tr>
<td>-</td>
<td>Sweeney / 2002\textsuperscript{34}</td>
<td>Rabbit</td>
<td>24</td>
<td>PEG (FocalSeal)</td>
<td>Ileal anastomosis</td>
<td>+</td>
</tr>
<tr>
<td>2b</td>
<td>Wang / 2007\textsuperscript{35}</td>
<td>Clinical</td>
<td>48</td>
<td>FG (Guanzhou bio seal)</td>
<td>Ileal anastomosis</td>
<td>+</td>
</tr>
</tbody>
</table>

\textit{Tissue adhesives in colorectal anastomosis (Table 6)}

a) Experimental studies

\textit{Fibrin glue}
In this field, most research has been performed on rats (Table 6). Several authors performed a 1- to 2-cm resection and end-to-end anastomosis of the descending colon. Akgun et al.\textsuperscript{36} and Girgin et al.\textsuperscript{37} independently reported that FG sealing of the anastomosis significantly increased ABP after 3 and 7 d, respectively, compared with a control group. In a study by Giuratrabocchetta et al.\textsuperscript{38}, three different colonic
anastomoses in each rabbit were randomized for sealing with FG or PEG sealant. No differences were found between these adhesives and the control group, regarding ABP and AL, after 15 d. In the other included studies, anastomosis was performed at the level of the transverse colon. Kanellos et al.\(^3\) found that FG sealing resulted in significantly higher ABP compared with a control group; however, no significant differences were found in AL rate or histopathology after 8 d. In later studies by the same authors, FG protected anastomotic healing from the adverse effects of 5-fluorocil\(^4\), interferon-a2a\(^4\), and leucovorin application\(^5\). Another study showed that FG significantly increased ABP and also resulted in fewer adhesions, more fibroblast production, and increased neovascularization\(^6\). Furthermore, two studies were included on the creation of a sutureless colonic anastomosis. In one report, the tensile strength of the anastomosis was lower than the sutured anastomosis, and it caused stricture in 8.57% of cases. However, FG resulted in lower inflammation, minor edema, and quick fibrous healing, and did not result in AL\(^7\). In another study, Tingstedt et al.\(^8\) compared three-suture ileocolic with sutureless FG anastomosis. After 3 and 5 d, no differences were found in ABP or mortality rate.

\textbf{Tissue adhesives in gastrointestinal anastomosis} 

\textit{Cyanoacrylate}  
In two rat studies, a 1-cm resection was performed in the transverse colon followed by an end-to-end anastomosis. Bae et al.\(^9\) used Histoacryl Blue both as an anastomotic sealant and for sutureless anastomosis. They found no AL in any groups, but reported that the use of CA resulted in significantly higher strictures, lower ABP, and a strong inflammatory response. Kanellos et al.\(^10\) created sutureless anastomosis with Dermabond and reported no difference in AL rate between controls and CA. Furthermore, they found no significant differences for the amount of adhesions, ABP, and wound-healing scores. In three rat studies, the anastomosis was created in the descending colon, after a 0.5- to 1-cm resection. Irkorucu et al.\(^11\) studied the effect of GluSeal as an anastomotic sealant and for sutureless anastomosis after ischemia. No significant differences were found in the AL rate, and in the ischemia groups no differences were found in ABP. The CA groups had significantly less inflammatory cell presence and fibroblast infiltration in the granulation tissue than the controls. Histoacryl Blue was also evaluated in a similar model for sutureless anastomosis\(^12\). The authors found no effect on the AL rate, but reported more stricture formation, adhesions, and lower ABP compared with the control group. Glubran 2 was also evaluated in a clean-contaminated or bacterial peritonitis environment\(^13\). The authors concluded that Glubran 2 caused increased inflammatory reaction, necrosis, and adhesion formation, especially in the bacterial peritonitis group. No differences in AL rate were reported between groups. Nursal et al.\(^14\) used Dermabond to seal a high-risk, three-suture anastomosis crushed by forceps. The authors reported no differences in AL, but found that CA decreased ABP at 7 d. Also, both at 3 and 7 d, an ongoing inflammatory reaction and more necrosis were seen in the CA group. Finally, Paral et al.\(^15\) compared two types of CA (Glubran 2 and Dermabond) for the creation of sutureless sigmoidal anastomoses in a pig model. They reported no AL in the Glubran 2 group and two cases in the Dermabond group. Also, Dermabond was linked to higher foreign body reaction and more fibrosis of the anastomosis.
Other categories
Ustek et al.\textsuperscript{53} reported that the use of polydione-liposome (PVP-1) hydrogel around a (descending) colon anastomosis in the rat improved wound healing after 7 d, reflected by significantly higher ABP and hydroxyproline levels in the PVP-1 group. Another study, by Yol et al.\textsuperscript{54}, compared the use of platelet-rich plasma (PRP) or glutaraldehyde-albumin glue (BioGlue) around a six-suture anastomosis. The authors reported that PRP sealing resulted in significantly higher ABP and hydroxyproline levels compared with the BioGlue and control groups after 7 d. Furthermore, the BioGlue group showed higher infiltration of inflammatory cells, collagen, and fibroblasts.

b) Clinical studies
\textit{Fibrin glue}
In a prospective cohort study, Huh et al.\textsuperscript{55} reported on 223 patients who underwent laparoscopic low anterior resection for rectal cancer, without the use of a defunctioning stoma. Stapled anastomoses were created intracorporeally and one of two types of FG were applied around the anastomosis via a catheter. In that study, the use of FG was not associated with a decrease in AL (5.8% versus 10.9%; \(P = 0.169\)).

DISCUSSION
Anastomotic leakage remains an important complication in GI surgery. It is a significant cause of morbidity and mortality, necessitating redo operations and increasing length of hospital stay.\textsuperscript{56-57} AL occurs in every level of GI surgery. In this review we have addressed recent tissue adhesive research for all levels of GI anastomosis.

\textit{Esophageal}: The extraperitoneal anastomosis created in the esophagus is associated with a high incidence of AL, ranging from 10% to 27%.\textsuperscript{58,59} Factors involved include poor blood supply to the esophagus, the absence of a protective omentum and the lack of a supporting serosal layer on the esophagus.\textsuperscript{12} Animal research supports the use of sealing end-to-end esophageal anastomosis with CA or FG; however, this finding is based on a single rabbit study. Two RCTs were included, which focused only on specific clinical problems (atresia and colon interposition in the infant), and reported positive results. We found no studies on adults or on oncologic esophagectomies. Although the included RCTs showed that the use of FG reduced AL, conclusions may be drawn only for this small subset of clinical problems, which reveals that sealing of esophageal anastomosis with FG may be helpful in esophageal atresia or colonic interposition in infants. Future research should focus on the use of FG sealing after (oncologic) esophagectomies or colon interposition in adults.
### Table 6. Synopsis of results; colorectal anastomosis

<table>
<thead>
<tr>
<th>LOE*</th>
<th>Author/year</th>
<th>Model</th>
<th>N</th>
<th>Tissue adhesive</th>
<th>Methods</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>Akgun / 2006</td>
<td>Rat</td>
<td>38</td>
<td>FG (Tisseel)</td>
<td>Colonic anastomosis</td>
<td>+</td>
</tr>
<tr>
<td>-</td>
<td>Capitan Morales / 2000</td>
<td>Rat</td>
<td>105</td>
<td>FG (Tisseel)</td>
<td>Colonic anastomosis</td>
<td>+</td>
</tr>
<tr>
<td>-</td>
<td>Girgin / 2009</td>
<td>Rat</td>
<td>28</td>
<td>FG (Tisseel)</td>
<td>Colonic anastomosis</td>
<td>+</td>
</tr>
<tr>
<td>-</td>
<td>Subhas / 2011</td>
<td>Rat</td>
<td>70</td>
<td>FG (Tisseel)</td>
<td>Colonic anastomosis</td>
<td>+</td>
</tr>
<tr>
<td>-</td>
<td>Kanellos / 2003</td>
<td>Rat</td>
<td>36</td>
<td>FG (TissuCol)</td>
<td>Colonic anastomosis</td>
<td>+</td>
</tr>
<tr>
<td>-</td>
<td>Kanellos / 2004</td>
<td>Rat</td>
<td>64</td>
<td>FG (TissuCol)</td>
<td>Colonic anastomosis</td>
<td>+</td>
</tr>
<tr>
<td>-</td>
<td>Kanellos / 2007</td>
<td>Rat</td>
<td>60</td>
<td>FG (TissuCol)</td>
<td>Colonic anastomosis</td>
<td>+</td>
</tr>
<tr>
<td>-</td>
<td>Giuratrabocchetta / 2011</td>
<td>Rabbit</td>
<td>10</td>
<td>FG(TissuCol) PEG (CoSeal)</td>
<td>Colonic anastomosis</td>
<td>+/-</td>
</tr>
<tr>
<td>-</td>
<td>Tingstedt / 2007</td>
<td>Rat</td>
<td>132</td>
<td>FG (Tisseel)</td>
<td>Iliocolic anastomosis</td>
<td>-</td>
</tr>
<tr>
<td>-</td>
<td>Bae / 2010</td>
<td>Rat</td>
<td>60</td>
<td>CA (Histoacryl Blue)</td>
<td>Colonic anastomosis</td>
<td>-</td>
</tr>
<tr>
<td>-</td>
<td>Irkorucu / 2009</td>
<td>Rat</td>
<td>40</td>
<td>CA (GluSeal)</td>
<td>Colonic anastomosis</td>
<td>-</td>
</tr>
<tr>
<td>-</td>
<td>Kanellos / 2002</td>
<td>Rat</td>
<td>40</td>
<td>CA (Dermabond)</td>
<td>Colonic anastomosis</td>
<td>+/-</td>
</tr>
<tr>
<td>-</td>
<td>Kayaoglu / 2009</td>
<td>Rat</td>
<td>80</td>
<td>CA (Glubran 2)</td>
<td>Colonic anastomosis</td>
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</tr>
<tr>
<td>-</td>
<td>Nursal / 2004</td>
<td>Rat</td>
<td>90</td>
<td>CA (Dermabond)</td>
<td>Colonic anastomosis</td>
<td>-</td>
</tr>
<tr>
<td>-</td>
<td>Ozmen / 2004</td>
<td>Rat</td>
<td>40</td>
<td>CA (Histoacryl Blue)</td>
<td>Colonic anastomosis</td>
<td>-</td>
</tr>
<tr>
<td>-</td>
<td>Paral / 2011</td>
<td>Pig</td>
<td>12</td>
<td>CA (Glubran 2) CA (Dermabond)</td>
<td>Colonic anastomosis</td>
<td>+ (Glubran2)</td>
</tr>
<tr>
<td>-</td>
<td>Ustek / 2005</td>
<td>Rat</td>
<td>70</td>
<td>Povidone-liposome (PVP-I)</td>
<td>Colonic anastomosis</td>
<td>+</td>
</tr>
<tr>
<td>-</td>
<td>Yol / 2008</td>
<td>Rat</td>
<td>30</td>
<td>BioGlue PRP (Autologous)</td>
<td>Colonic anastomosis</td>
<td>+ (PRP)</td>
</tr>
<tr>
<td>2b</td>
<td>Huh / 2010</td>
<td>Clinical</td>
<td>223</td>
<td>FG (Tissucol, Greenplast)</td>
<td>Rectal cancer surgery</td>
<td>+/-</td>
</tr>
</tbody>
</table>

**Gastric/bariatric:** We found no studies evaluating the use of tissue adhesives with regard to gastrectomy. In bariatric surgery, (laparoscopic) Roux-en-Y gastric bypass staple line reinforcements and tissue adhesives are increasingly being used to protect from staple line dehiscence and bleeding [60]. Roux-en-Y gastric bypass dehiscence rates vary from 0.7% to 6% [13,14,61,62]. In two large pig studies, FG was used to cover a defect in the gastrojejunal anastomosis, and resulted in a dramatic decrease in AL. These pig studies were well performed and provide evidence of the positive effects of FG sealing in this field. BioGlue also proved useful in a single ex vivo study; however, this was not repeated in an in vivo model. Cyanoacrylate glue was evaluated in a single rat study, which deemed it equal to conventional techniques. Of the nine included clinical studies on LRYGB, clinical evidence is derived from one large RCT that evaluated the effect of FG sealing. The authors of that study reported more AL in the control group; however, the difference was not significant. They did report benefits of FG use with regard to the prevention of several early complications. That multicenter,
prospective trial was methodologically sound, but the sample size was not sufficient to provide firm conclusions on statistical differences between end points. Furthermore, three large prospective cohorts provided inconclusive results on the use of FG sealing after RYGB: two reported positive results on the AL rate and the other reported no difference when FG was applied. Interestingly, the latter study also reported that FG was associated with an increased clinical inflammatory response mimicking AL, a finding not seen in the other studies. Several smaller series also found that FG sealing prevents AL. One case control study showed positive results with the use of FG. However, in that study one surgeon performed all of the FG operations and two other surgeons performed the controls. One pilot study on 10 patients deemed the use of PRP to be helpful. However, it is questionable how much statistical power can be derived. Thus, most of the included studies showed that FG may protect the staple line from AL after LRYGB. The only RCT did not verify this but that may have been because of the lack of statistical power. A new multicenter, prospective RCT may provide the answer.

**Pancreatic:** Pancreatic leakage and subsequent fistula formation occurs in 5%-25% of patients undergoing pancreatice-digestive anastomosis, depending on the etiology and definition used\(^63\). PEG use was promising in one well-performed pig study; however, no clinical studies have been performed to assess PEG in humans. This may be a target for future research. One well-performed RCT concluded that the use of FG sealing of the pancreatice-jejunal anastomosis provided no benefits. In that study, only high-risk pancreatice-jejunal anastomoses were included, as judged by the surgeon. Although a higher rate of AL (postoperative fistula) was observed in the control group (24% versus 14%), there were no statistical differences between this and any other complications. A small prospective study on a similar high-risk patient population reported that FG reduced the AL rate to 0%. Nevertheless, that was a small study and cannot provide as much evidence as the RCT. These studies have focused only on patients known to have a high risk for AL; these patients had a soft texture gland. Considering the working mechanism of a tissue adhesive, which also relies on the texture of its substrate for a sufficient bond, it can be imagined that these high-risk patients may be less susceptible to a strong FG bond at the anastomotic site. Future research should focus on a broader spectrum of patients, not only high-risk ones, to test the hypothesis that FG can adhere better to a normal texture gland. Furthermore, a small, retrospective case-control study showed more pancreatic fistulas when BioGlue was used around the pancreatic anastomosis. It seems that FG sealing provides no benefit for patients with high-risk pancreatice-enteric anastomosis. However, research may also focus on other subsets of patients in this field.

**Small bowel:** Small bowel anastomosis is primarily performed after resection for inflammatory disease, after small bowel obstruction or abdominal trauma, or as a part of bariatric surgery. In addition, another important indication for ileal anastomosis is closure of defunctioning loop ileostomy, which is associated with an AL incidence of 3.0%\(^3\). We found mostly experimental studies in this field. Three rat studies used FG to seal a primary anastomosis either after formation of an intestinal fistula or in a leaking anastomosis model. It was observed that FG glue protects the anastomosis in both demanding environments, with effects apparent after day 5. Interestingly, it was reported that combining FG with growth hormone may enhance its protective effects. This may be a path for future research. Fibrin glue was also used in a pig model, which showed that FG may be used to seal perforated bowel after an abdominal gunshot wound. Because of their ease of application and speed, tissue adhesives may indeed be useful after major trauma or even in a battlefield setting, and future research should focus
on this use. Furthermore, PEG was also used in a single rabbit study of insufficient anastomosis, and was reported to improve wound healing. More studies should focus on PEG in this field to verify these findings. Cyanoacrylate glue sealing proved positive in two large rat studies, both in ischemia-reperfusion as in a leaking anastomosis model. Only one human study was included, a small prospective study in which septic patients undergoing ileal resection were sealed with FG. Patients were also given human derived growth hormone, and a relatively low rate of AL (8.3%) was seen. Although well performed, this study does not provide substantial clinical evidence and may be a target for future research aiming at a large RCT. All included studies in this field showed positive results when tissue adhesives were used to protect the ileal anastomosis. Despite these promising results, more clinical studies, especially level 1, are needed before these techniques can be recommended for clinical practice.

**Colorectal:** In colorectal surgery, the reported incidence of anastomotic leakage ranges from 5% to 25%, with a mortality rate of up to 32%\textsuperscript{5,64}. This may be because of the anatomical positioning of the colorectum, which is partly located extra peritoneally, and is not accessible to the sealing function of the greater omentum. Furthermore, ultralow anastomoses may be affected by their lack of perfusion, as a result of minimal and fragile arterial supply, and their subjection to high intraluminal pressures and peristaltic forces\textsuperscript{65}. Along with other known risk factors such as male gender, obesity, smoking, and anastomosis under tension, the colorectum is a fragile and precarious location for the creation of an anastomosis\textsuperscript{2}. Until now, research has almost exclusively been performed in animals, and there is little variation in studied tissue adhesives. Of the numerous experimental studies, most were small rat studies focusing on the use of either FG or CA. Interestingly, all nine rat studies on FG showed a decrease in AL. In these studies, only Tisseel/Tissucol FG was used, and mostly sealing of a sufficient anastomosis was performed. There were some notable differences between these studies: Some models consisted of a 1- or 2-cm resection, and others only transection and anastomosis. Also, there were differences in sutures and numbers of sutures used, and in one study a sutureless anastomosis was created using FG, which was not inferior to the control. Cyanoacrylate glue sealing was also evaluated in experimental studies. Again, six of the seven studies used rat models and in none of the rat studies were positive effects of CA use reported. In the only pig study included, two CA adhesives were compared, and a decrease in AL was only seen when Glubran 2 glue was used. One clinical study was included, a prospective study on 223 patients undergoing laparoscopic rectal surgery. The authors used two types of FG, with no systematic randomization for type of FG. Also, there was a large variation in the amount of FG used, varying from 1 to 2 mL, and the exact application technique was not described. Using little amounts of FG laparoscopically applied through a catheter seems difficult, especially with regard to the posterior side of the anastomosis. This study found no benefit in the use of FG. A new level 1 study might shed light on the use of FG in this field. In conclusion, the future of research in this field will entail a wider palette of tissue adhesives and more uniformity in animal testing methodology. Only thereafter may clinical studies provide enough evidence for clinical use.

The field of tissue adhesives in surgery is relatively new and the adhesive market has changed substantially throughout the years. Because of improvements in surgical adhesive composition and characteristics, especially in the new millennium, we decided to include only studies that were published after 2000 in this review. More detailed information on the early period of adhesive research in GI surgery is addressed in several older reviews\textsuperscript{5,66}.
Despite all the research that has been performed to date in the field of surgical adhesives, it remains difficult to draw conclusions on the effects of the tested tissue adhesives on each level of GI anastomosis. The reason for this is that there is too much heterogeneity in experimental methods among research groups. Most authors use ABP as a major end point, a test that is useful, in our opinion, because it reflects the strength of the intact anastomosis. However, although it is popular, this test has also been scrutinized, and it has been said that ABP might not be correlated with the integrity of the anastomosis and clinical outcome. We observed great differences in ABP test methods ranging from ABP on intact in vivo colon with air insufflation to the use of dyed saline on resected colon. Furthermore, when using a tissue adhesive, it is imperative to provide details on its application; such as the amount used, layer thickness and width, and curing time. Only a minority of authors stated these parameters, which makes repetition of results difficult if not impossible. Also, anastomotic technique should be further standardized to make results more comparable. In particular, the amount of sutures used for the sutured anastomosis varied greatly per animal model, and in the case of the sutureless (glued) anastomosis, there were variations in the use of guide sutures at the (anti)mesenteric edges. The most popular animal model for the creation of anastomosis is the rat. Within this model, there are great differences, with some authors devitalizing the wound edges by selective devascularization or by crushing, and others making a sufficient anastomosis, with variable amounts of sutures. Our recommendation would be to standardize the methodology of this type of research, including the use of a standard animal model. To date, FG and CA seem to be the most popular categories of tissue adhesive. Within these categories, different formulations have produced contradicting results. In the case of CA, the use of shorter chain lengths such as n-butyl-cyanoacrylate tend to implicate more tissue toxicity and tissue damage based on the degree of exothermic reaction. New CAs are less histotoxic and more flexible than older formulations. Differences between FG are seen in the use of antifibrinolytic agents (i.e., aprotinin), which have also been associated with adverse effects on experimental colonic healing, as well as hypersensitivity reactions in humans. We found no remarkable differences between outcomes of FG with and without aprotinin. For all levels of anastomosis, we have seen that research focused mostly on FG and CA. Clinical studies have almost exclusively been performed on FG, with the occasional exception of a biologically based tissue adhesive. It seems that surgeons are still wary of chemically based adhesives such as CA and PEG, despite positive reports from animal research. With the new generation of CA and other new adhesive formulations on the horizon, we may also see the first human applications. In the future, we think that research in this field should also provide a better perspective on the biomechanical mechanisms of adhesiveness for the different anastomotic locations. This may enable the development of tissue adhesives, custom made, for the various anatomic locations, or better yet, a universal glue for use in various anastomotic locations. This will provide a better understanding of why certain tissue adhesives tend to be more effective than others, and may provide insight into the next steps of development of tissue adhesives for GI anastomosis.

In conclusion, the field of tissue adhesives is gaining ground in GI surgery. Despite years of research, the ideal tissue adhesive is yet to be found; however, the benefits of using adhesives are becoming more apparent. The use of FG and CA has been the main focus of research for the sealing of GI anastomosis to date, especially in clinical research. Currently, it seems that FG may be an effective anastomotic sealant for specific types of esophageal and bariatric surgery; however, contrary to our hypothesis, recommendations for
the general population are premature. Results for the sealing of pancreatic, ileal, and colorectal anastomoses remain inconclusive and are based mostly on animal studies. Future research should concentrate on evaluating a wider palette of tissue adhesives, and more level 1 studies are needed to implement tissue adhesives on a larger scale. Current animal research on tissue adhesives may benefit from a more systematic approach, based on basic adhesiveness mechanisms and evaluation of existing tissue adhesives. This interesting field is becoming increasingly popular in surgery, and this trend will continue in future research and in clinical practice.

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Critical analysis of cyanoacrylate in intestinal and colorectal anastomosis

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ABSTRACT

Background: Although cyanoacrylate glue (CA) has been widely used in various kinds of medical applications, its application in gastrointestinal anastomosis remains limited, and outcomes of experimental studies have not been satisfactory. This systematic review summarizes research regarding CA application in intestinal and colorectal anastomosis, and correlates methodological aspects to experimental outcomes.

Methods: A systematic literature search was performed using Medline, Embase, Cochrane and Web-of-Science libraries. Articles were selected when CA was applied to intestinal or colorectal anastomoses. Included articles were categorized according to CA molecular structure; the method details in each study were extracted and analyzed.

Results: Twenty-two articles were included. More than half of the inclusions reported positive outcomes (7 articles) or neutral outcomes (8 articles). Analysis of the methods revealed that methodological details such as CA dosage, time of polymerization are not consistently reported. Porcine studies, inverted anastomosis, and n-butyl-cyanoacrylate studies showed more positive outcomes; everted anastomosis, and oversized sutures may negatively influence the outcomes.

Conclusions: Due to the positive outcome from the porcine studies, application of CA in GI anastomosis still seems promising. To achieve a better consistency, more methodological details need to be provided in future studies. Optimizing the dosage of CA, choice of animal model, inverted anastomosis construction, and other method details may improve intestinal and colorectal anastomoses with CA application in future studies.
INTRODUCTION

Cyanoacrylate (CA) was invented more than 60 years ago for industrial applications [1, 2]. Famous for its strong adhesiveness, various commercial names such as “crazy glue” or “instant glue” are well known in daily life. Moreover, the strong adhesiveness of CA also made it an ideal candidate for replacement of conventional sutures in medical use, such as wound closure. In addition to a strong bond, a fully and evenly sealed anastomosis can be created with CA, avoiding excessive tissue approximation that can induce disturbances in the microcirculation [3, 4]. In 1998, the Food and Drug Administration (FDA) approved Dermabond (2-octyl-cyanoacrylate) for topical skin wound closure [5], which was the first FDA approved CA for medical use. Ever since then, more and more medical-use CA have appeared on the market for different indications [6, 7].

Except for skin wounds, the gastrointestinal anastomosis is another important type of wound closure. However, the use of CA in this field is still limited, and no clearly documented clinical attempts have been made so far. Though substantial experimental efforts have been made, the results of animal studies have not yet been encouraging. Some experimental studies reported anastomoses could be well constructed with CA [8, 9]; while others reported a mortality rate as high as 30-40% [10, 11]. Besides large variations in results, inconsistencies with regard to the methodology were also noticed in those experiments. As it has been demonstrated that the anastomotic technique used in clinical gastrointestinal surgery influences the outcomes, we hypothesized that the inconsistent results of experimental studies are partly due to differences in their methods. Thus, the purpose of this systematic review is to summarize the experimental studies regarding CA application in intestinal and colorectal anastomosis, correlating the methodological details to the experimental outcomes.

METHODS

Search methods

This systematic review was performed according to the PRISMA (Preferred Items for Reporting of Systematic Reviews and Meta-Analyses) guidelines [12]. The systematic literature search was performed on the 5th of November 2012. The systematic search of literature was performed using the databases of Medline, Embase, Cochrane and Web-of-Science libraries. The same search strategy was used in all the databases. The search strategy encompasses the following:

(cyanoacrylate/de OR 'cyanoacrylate derivative'/de OR 'cyanoacrylic acid octyl ester'/de OR enbucrilate/de OR bucrilate/de OR 'poly (ethyl 2 cyanoacrylate)'/de OR (cyanoacryl* OR 'cyano acrylate' OR 'cyanoacrylic acid' OR 'octylcyano acrylate' OR enbucrilat* OR bucrilat* OR enbucrilat* OR bucrylat* OR butylcyanoacryl* OR fimomed OR histacryl OR histocryl OR sicomet OR isobutylcyanoacryl* OR ocrilat OR ocrylate OR octylcyanoacrylat* OR dermabond OR omnex OR glubran OR surgiseal OR floraseal OR 'derma flex qs' OR gluseal OR octyseal OR wormglu OR periacyrl OR indermil OR liquiand OR xion):ab,ti ) AND ('gastrointestinal surgery'/exp OR ('gastrointestinal tract'/exp OR 'digestive system'/exp) AND (surgery/exp OR (surg* OR operat* OR preoperat* OR postoperat* OR perioperat* OR intraoperat*):ab,ti)) OR (((gast* OR digestiv* OR intestine* OR anal OR anus OR anorect* OR rect* OR bariatr* OR pancrea* OR stomach* OR antireflux* OR colon* OR colorect* OR bowel* OR duoden* OR esophag* OR oesophag*):ab,ti)) NEAR/3 (surg* OR operat* OR postoperat* OR preoperat* OR perioperat* OR intraoperat* OR anastom* OR suture* OR adhesi* OR glue* OR
sealant* OR hemosta* OR coat* OR lesion* OR wound* OR dehis* OR disattach* OR attach*)) OR vagotom* OR colectom* OR gastrostom* OR stoma* OR appendectom*).ab,ti

Study selection
Two independent researchers (Z.W. and G.B.) screened all the articles (the title and the abstract) in a standardized manner. Articles were included only if the CA glue was applied in an intestinal or colorectal anastomosis. The search was restricted to publications in English. Presentations, reviews and letters to editor were not included. All references from the selected articles were screened for further possible inclusions.

Data extraction
For all selected studies, a standard data extraction form was filled in, and the following data were extracted: year of publication, first author, subject (animal species), number of animals, glue (chemical name), glue (commercial name), usage (CA sutureless anastomosis / sealant), dosage, curing time, anastomotic material (additional material to create the anastomosis other than CA), suture material (chemical component), suture size, suturing technique, GI level, and outcome (positive / negative, judged according to conclusions of the articles).

RESULTS
A total number of 962 articles were found, from which 22 studies were included for final data analysis (Figure 1). Among these, seven articles had positive outcomes; eight had neutral outcomes; the others had negative outcomes. As is listed in Table 1, CA with different molecular structures produced by different manufacturers were used and tested. The included articles were divided according to the chemical structure of the CA used, and their chemical names (commercial names if applicable) were listed. Further subdivisions were made according to the use of CA with regard to anastomosis (sutureless anastomosis or sealant).

Metho-cyanoacrylate (MCA)
Four studies were included that report the use of MCA [2, 10, 11, 13]. A sutureless anastomosis was created in all of them, and none of these studies had positive outcomes (Table 2).

In 1962, O’Neill et al. used MCA (Eastman 910) to create a sutureless anastomosis in canines’ small intestine or colon. In this model, a clamp was used to construct an everted anastomosis [11]. They found that most of the intestinal anastomoses (11 / 12) were satisfactory and no death occurred, but 28.6% (4 / 14) of canines died when CA anastomoses were created in the colon [11]. A similar clamp was also used by Weilbaecher et al., who performed the intestinal anastomosis with a greater number of canines. Mortality rate as high as 34% (34/101), and no advantage of MCA were found when compared with conventional suture methods [10]. A high mortality rate of 22% (8 / 35) was also found when Gennaro et al. used an intraluminal gelatin stent to create a colonic MCA anastomosis in a rat model [2]. Different from those experiments, Linn et al. reported a canine study [13], in which no anastomosis-related mortality occurred. Anastomoses with MCA had less inflammation than the conventional group, but stricture occurred in 40% of the anastomoses when a new invagination technique to construct the MCA anastomoses was used [13].

Etho-cyanoacrylate (ECA)
Only one study used ECA to create the CA sutureless anastomosis [3], and no study used MCA as an anastomotic sealant (Table 2).

**Figure 1. Study selection for relevant articles.**

**Table 1. Cyanoacrylate adhesives used in the included studies**

<table>
<thead>
<tr>
<th>Chemical structure</th>
<th>Abbreviation</th>
<th>Trade name</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metho-cyanoacrylate</td>
<td>MCA</td>
<td>910 Eastern</td>
<td>Ethicon (Somerville, New Jersey, USA)</td>
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<tr>
<td>Etho-cyanoacrylate</td>
<td>ECA</td>
<td>Pattex</td>
<td>Henkel (Dusseldorf, Germany)</td>
</tr>
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<td>N-butyl-cyanoacrylate</td>
<td>NBCA</td>
<td>Histoacryl (blue)</td>
<td>B. Braun (Melsungen, Germany)</td>
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<td></td>
<td>NBCA</td>
<td>Glubran 2</td>
<td>GEM Italia (Via reggio, Italy)</td>
</tr>
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<td>2-octyl-cyanoacrylate</td>
<td>OCA</td>
<td>Dermabond</td>
<td>Ethicon (Norderstedt, USA)</td>
</tr>
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<td></td>
<td>OCA</td>
<td>Gluseal</td>
<td>GluStitch, Inc (Delta, BC, Canada)</td>
</tr>
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</table>
### TABLE II. Synopsis of Results: Cyanoacrylate Application in Intestinal and Colorectal Anastomosis

<table>
<thead>
<tr>
<th>Year</th>
<th>Author</th>
<th>Subject</th>
<th>n</th>
<th>Glue (Chemical Name)</th>
<th>Glue (Trade Name)</th>
<th>Usage</th>
<th>Dosage</th>
<th>Curing Time</th>
<th>Anastomotic Material</th>
<th>Suture Material</th>
<th>Suture Size</th>
<th>Anastomotic Pattern</th>
<th>GI Level</th>
<th>Outcome</th>
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<td>1962</td>
<td>O'Neill et al.</td>
<td>Canine</td>
<td>26</td>
<td>MCA</td>
<td>910 Easterman</td>
<td>Anastomosis NS</td>
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<td>Clamp</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Evert</td>
<td>Intestine Colon</td>
<td>+/-</td>
</tr>
<tr>
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<td>Weilbacher et al.</td>
<td>Canine</td>
<td>101</td>
<td>MCA</td>
<td>910 Easterman</td>
<td>Anastomosis NS</td>
<td>3 min</td>
<td>Clamp</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Evert</td>
<td>Intestine</td>
<td>–</td>
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<td>Linn et al.</td>
<td>Canine</td>
<td>30</td>
<td>MCA</td>
<td>910 Easterman</td>
<td>Anastomosis NS</td>
<td>NS</td>
<td>Invaginate</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Invaginate</td>
<td>Intestine</td>
<td>+/-</td>
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<td>Rat</td>
<td>35</td>
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<td>910 Easterman</td>
<td>Anastomosis NS</td>
<td>NS</td>
<td>10-20s</td>
<td>Gelatin suture</td>
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<td>–</td>
<td>Evert</td>
<td>Colon</td>
<td>–</td>
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<td>Eleftheos et al.</td>
<td>Rat</td>
<td>96</td>
<td>ECA</td>
<td>Polyglactin</td>
<td>Anastomosis NS</td>
<td>NS</td>
<td>Holding suture</td>
<td>Polyglactin 910</td>
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<td>NS</td>
<td>Invert</td>
<td>Intestine</td>
<td>+</td>
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<td>Porcine</td>
<td>12</td>
<td>NBCA vs. OCA</td>
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<td>Anastomosis 1.0ml</td>
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<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Invert</td>
<td>Colon</td>
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<td>Weiss and Haj</td>
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<td>Histoacryl</td>
<td>Anastomosis NS</td>
<td>NS</td>
<td>3-4min</td>
<td>Holding suture</td>
<td>Vicryl sutures</td>
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<td>Stomach-Jejunal</td>
<td>+/-</td>
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<td>NS</td>
<td>Holding suture</td>
<td>Polypropylene</td>
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<td>NS</td>
<td>Invert</td>
<td>Colon</td>
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<td>Suture</td>
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<td>Invert</td>
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<td>Suture</td>
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<td>NS</td>
<td>Suture</td>
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<td>Invert</td>
<td>Intestine</td>
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*aShown on the picture of the inclusion.
Abbreviation of different cyanoacrylate is specified on Table I. NS = not specified.
In 2009, Elemen et al. used ECA (Pattex) to construct end-to-end, side-to-side, or side-to-end intestinal anastomoses in a rat model. No deaths occurred during follow up, and no differences in bursting pressure were found between the CA anastomosis and sutured anastomosis, while higher hydroxyproline levels (a parameter of anastomotic wound healing) and shorter operating time were found in the CA groups [3].

**N-butyl-cyanoacrylate (NBCA)**

Nine studies regarding NBCA were included [8, 9, 14-20] (Table 2). Among these, three studies focused on the sutureless anastomosis [8, 16, 17], three studies looked into NBCA sealant [9, 18, 20], and the other three tested both applications [14, 15, 19]. In the NBCA studies, all four large animal studies had positive results [8, 9, 14, 15], while of the other five rat studies, only one had positive outcomes [20].

Matsumoto et al. reported a comparison between CA in different molecular structures (N-butyl-, Amyl-, Heptyl-cyanoacrylate) in a canine model of intestinal anastomosis. Only NBCA showed good wound healing without stenosis after four or twelve weeks [21]. Another comparison between NBCA (Glubran 2) and OCA (Dermabond) was performed in a porcine model [8]. The CA sutureless anastomoses were constructed in the colorectum with a modified stapling device, in which all the staples were taken out in advance. All the NBCA anastomoses were satisfactory, while two leakages occurred in the OCA group; NBCA was also superior to OCA regarding to the adhesion and stenosis severity [8]. Tebala et al. also tested NBCA with different suturing techniques in a porcine model [15]. They performed 11 different types of anastomosis. Good wound healing was observed in macroscopic, histological and angiographic examinations; foreign body reaction was even less in the sutureless anastomosis group than the sealant group [15]. Tebala et al. also created an insufficient anastomosis in a pig model by removing 1/5 of the sutures or staples from a normal anastomosis [9]. NBCA was then used to seal the defect. Anastomotic healing was sufficient, and no ileus occurred during the follow up [9].

Positive results of CA use were reported in a rat study by Ensari et al. [20]. In this study, the authors constructed an ischemic-reperfused intestinal anastomosis and used NBCA (Glubran 2) to reinforce it. Higher bursting pressures were found after the CA reinforcement with or without the initial ischemic intervention, while more adhesions were found in the CA groups [20]. Weiss et al. tested another NBCA (Histoacryl) and created gastrojejunal anastomoses in a rat model, comparing it with resorbable sutures. In this study, anastomotic healing regarding leakage rate, stricture, peritonitis, and mortality were similar between both groups. The only significant difference was a shorter operating time in the NBCA group [16]. Bae et al. tested the same glue in a rat model, in which they created NBCA (Histoacryl) reinforced anastomoses and the NBCA sutureless anastomoses in the rat colon. No leakage occurred in any of these groups, but more strictures, lower bursting pressure and more severe inflammation was found in the CA reinforced group and the CA sutureless group [19]. Similarly, a lower bursting pressure was also reported by Ozmen et al. in a CA sutureless colonic anastomosis with two holding sutures [17]. NBCA has also been tested in high-risk animal models. Kayaoglu et al. used 0.2 ml NBCA (Glubran 2) as sealant to reinforce the anastomosis in a fecally contaminated environment. Similar macroscopic wound healing and bursting pressure were found on day 3 and day 7 in both the CA group and the suture group; however, more inflammation and necrosis were found in the CA group [18].
Iso-butyl-cyanoacrylate (isoBCA)
Four studies regarding isoBCA were included [22-25], of which no study had positive results. Dating back to 1980, Kirkegaard et al. used isoBCA to create the sutureless anastomosis with a gelatin stent [25]. They found more stenosis and inflammation in the CA group, however these complications were significantly reduced when the CA anastomosis was covered with an omental tag [25].

High mortality was reported by all the other isoBCA studies. Stirling et al. used isoBCA to create the sutureless everted anastomosis, which resulted in a mortality rate of 27.0% (10/37) of canines [22]. In 1968, Hale et al. first used a rat model to compare the influence of isoBCA as sutureless anastomosis or as suture reinforcement. Twelve of 16 canines (75%) died in the sutureless anastomosis group, while conventional anastomoses or CA reinforced anastomoses were mostly satisfactory [23]. In 1971, Uroskie et al. used a canine model and performed two intestinal anastomoses in each animal, in which the distal anastomosis was sealed with isoBCA. Sixty percent (9/15) of the animals died during the follow-up due to anastomosis-related complications, mostly due to AL in the CA reinforced anastomoses [24].

2-octyl-cyanoacrylate (OCA)
Three studies on OCA were included [26-28]. None reported additional advantages in anastomotic healing when OCA was applied.

Kanellos et al. resected a segment of 1.0 cm in the rat transverse colon, and randomly chose OCA (Dermabond) or sutures to create the sutureless anastomosis. Similar leakage rates, bursting pressures and histological results were found between the CA and suture groups [26]. In 2009 Ikorucu et al. also used OCA (Gluseal) to seal or construct rat colonic anastomoses after inducing wound ischemia. Similar bursting pressure and hydroxyproline concentrations were found between groups, while more adhesions were found in the CA reinforced and the sutureless groups than the conventionally sutured groups [28]. However, in an ischemic anastomosis model by Nursal et al., the mechanical strength of the OCA (Dermabond) anastomosis was significantly lower on day 7 than the conventionally sutured groups; furthermore, a higher inflammatory response and necrosis were found in the OCA group [27].

Other
Galvao et al. used CA to assist a cuff apparatus to create an invaginated anastomosis on rat intestine. The chemical structure of the used CA was not described in this study, but satisfactory anastomoses were still found in both macroscopic and histological evaluations, the CA anastomosis also cost much less time. However, after one and three days, tissue lesions due to CA toxicity were observed [29].

Method Details
As is shown in Table 2, methodological details of each included study were listed. These details mainly focused on the material and technique used for the anastomosis construction.

CA dosage and curing time:
Of all 22 included studies, only four studies specified the amount of CA used in each anastomosis. One study used 1.0 mL CA to create the sutureless anastomosis in a pig model [8], obtaining positive outcomes. 0.5 mL and 0.2 mL CA were also used in three rat models for creating sutureless anastomoses or as an anastomotic sealant [18, 20, 27]. In these rat studies,
only one reported positive conclusions [20]. Only eight studies listed the curing time after CA application, which varied from 10 seconds to 4 minutes [2, 10, 11, 16, 22, 24, 29].

Animal species:
Three different animal species were used in the included studies. Most studies used animal was the rat (14 studies), and four of them had positive outcomes [3, 20, 25, 29]. Six canine studies were included. All of them were performed in the 1960’s and 1970’s, while only one had positive conclusions [14]. Only three porcine studies were included, all showing positive conclusions [8, 9, 15].

Anastomotic construction:
Fourteen studies described or had figures demonstrating the anastomotic pattern such as inverted (serosa to serosa), everted (mucosa to mucosa) or invaginated (mucosa to serosa) anastomosis. Six studies employed an inverted anastomosis [8, 9, 15, 16, 18, 24], among which three had positive outcomes [8, 9, 15]. Five studies used an everted anastomosis [2, 10, 11, 19, 22]; none of these had positive results. Three studies constructed an invaginated anastomosis [13, 14, 29], and two of them showed positive outcomes [14, 29].

Sutureless anastomosis constructed with CA was tested in 18 studies, of which five reported positive outcomes [3, 8, 14, 15, 29]. Different materials such as clamps, stents, modified staplers or holding sutures were used to approximate the two cutting edges, as is shown in Table 2. Within those materials, none of the studies that used an anastomotic clamp (3 studies [10, 11, 22]) showed positive outcomes. In the other studies which used holding sutures or a modified stapler to create CA anastomosis, mostly the canine and porcine studies (3/4) had positive results [8, 14, 15]. In the contrast, only one rat study (1/8) with holding sutures had positive results [3].

Nine studies tested CA as a sealant after construction of a primary anastomosis; among these, four reported positive results (two porcine studies [9, 15], one rat study [20]). Most of these studies used different suture materials (silk, polypropylene or glycolic acid) and varying suture techniques for the construction of the primary anastomosis. Except for materials, different suture sizes were tested as well. Two porcine studies used 3/0 sutures, both of these having positive outcomes [9, 15]. Five studies used 5/0 or 6/0 sutures, mostly in rat models [18, 19, 23, 24, 28], and none of them conclude positively. One rat study used 7/0 sutures, and it had positive outcomes [20].

DISCUSSION

Substantial efforts have been made to test the feasibility, effect and safety of the use of CA in intestinal and colorectal anastomosis. Using CA as suture-replacement, early experiments in the 1960s and the 1970s failed to create a successful sutureless anastomosis [10, 30], some recent results, though promising, still vary from one to another. Previous opinions mainly put the blames on the chemical characteristics of CA [2, 7]. Indeed, intra-abdominal (actually intra-peritoneal) application of CA is distinct from its topical use such as skin wound closure, because intra-abdominally applied CA can only be absorbed, metabolized, and degraded by the body instead of falling off by itself. However, this still does not explain everything, as most current available CA contain longer molecular chain, which are less toxic than short length CA [7]. Creating anastomoses with artificial materials not only requires a good mechanical strength,
but should also induce a good physiological wound healing which eventually supports the bowel continuity and biomechanical strength by itself. All these influences indicate the importance to investigate methodological details in CA application, such as selection of CA molecular structure, dosage, animal model, and anastomotic technique. With this aim, this review summarizes the studies regarding application of CA in intestinal and colorectal anastomosis, linking the method details to the outcomes. We found that these studies contained great inconsistencies in the methods. Furthermore, some important factors and details in the methods might influence outcomes, which are discussed respectively below.

**CA molecular structure**

CA was tested as a potential suture replacement because of its strong adhesiveness, which makes it possible to seal a technically flawed anastomosis, and even to create a sutureless anastomosis. Our previous ex-vivo study showed that adhesiveness is similar among different types of CA, but is much stronger than that adhesive strength in other categories of tissue adhesives (unpublished data). When choosing CA for specific surgical applications, it is therefore more important to take other factors into account, such as tissue toxicity [31, 32].

In general, shorter chain CA monomers (i.e. methyl-cyanoacrylate) create significant amounts of heat during polymerization, and are known to degrade into toxic end-products, resulting in severe tissue reaction and inflammation, while longer chain-length CA is associated with more hydrophobic and bacteriostatic properties and less tissue toxicity [2, 7]. However, in intestinal and colorectal anastomoses, data from the studies that compared different CA seem to prefer in NBCA to other shorter or longer monomers [21, 27, 33]. Our results in this review also agree with this, as most CA studies with MCA, isoBCA or OCA had negative outcomes, and more than half of the NBCA studies reported positive ones [8, 9, 14, 15, 20]. Nevertheless, one must note that, with the current limited data, it is still too early to conclude which CA is the best for intestinal and colorectal anastomoses. The biological properties of CA are influenced not only by its molecule structure, but also by the additional components added into the adhesives. Developments in biochemistry may bring further improvements in CA molecule structure for specific use as intestinal and colorectal anastomotic.

**CA dosage**

As well as the molecular structure, an important role in the tissue reaction of CA is also played by CA dosage. Unfortunately most studies did not provide details on this. One can imagine that an overdose of CA, comparable to a very high number of sutures or staples around the anastomosis, may lead to more side effects rather than a further increase in anastomotic strength. As CA is known to react exothermically during polymerization, CA overdose may cause direct tissue damage during polymerization, and increase adhesion formation, lengthening the long-term degradation time.

The currently available information is not enough to allow an analysis of the optimal amount of CA for intestinal and colorectal anastomosis in different animal models. According to the study of Paral et al., 1.0 mL of CA should be enough to construct a sutureless anastomosis in the porcine model [8]. Compared with the dosage for porcine anastomosis, 0.5 mL and 0.2 mL CA might be too much for rat anastomosis, as the rat colon is more than ten times smaller. Some clues on optimal CA dosage can be found from data in vascular surgery, where only 0.4 µl CA was enough to create vessel anastomosis in rats [34]. While the manufacturers’ original
Critical analysis of cyanoacrylate in intestinal and colorectal anastomosis

The applicator can be directly used in porcine or other big animal models, a small syringe with a blunt needle is recommended in rodent models to ensure accurate CA application.

Animal model
Not only due to the poor outcomes from the previous literatures, but also because of ethical concerns, canine models might not be suitable for future CA studies. This review shows that all previous studies using porcine models had positive results, implying that this might be the best large animal model for future CA studies regarding to intestinal and colorectal anastomosis. This is also supported by the previous systematic review, which also found the porcine model to be superior to those with other animal species, as the pig’s GI tract is much more similar to a human’s than a rodents’ [35]; this enables human-size surgical tools and human-dose CA to be used directly on porcine. However, the high costs of large animal models result in most animal studies on CA being performed on rat models. As stated earlier, most of the previous rat studies in this field were not a success. This is most probably due to the small size of the rat. Almost all techniques, and also the material size and dosage will thus need to be specifically adjusted for rats.

Anastomotic technique
Construction of a successful anastomosis is not simply connecting two endings together and reaching a mechanical strength as high as possible. A good and safe physiological wound healing without complications (i.e. anastomotic leakage, adhesion, stenosis) is more important from a clinical perspective [36]. For anastomosis of the digestive tract, the inverted-suture technique has been demonstrated to lead to a sufficient biomechanical strength as well as a better wound healing than the everted pattern; invaginated anastomosis is hardly used in clinic due to higher risks to develop stenosis and other complications [36-39]. Outcomes from CA research also confirm this, as all the studies using everted anastomosis had negative results, while more than half of those using inverted anastomosis had positive outcomes [8, 9, 15]. Comparing data from the included studies, we recommend that an inverted-suturing technique should also be used in future CA studies.

Overall, the use of CA in intestinal and colorectal anastomosis has two functions: to construct a sutureless anastomosis, or to reinforce a primary anastomosis as an anastomotic sealant. For sutureless anastomosis, various materials have been used to approximate the two bowel endings before CA application. Among these materials, the modified circular stapler (in which the staples are removed prior to use) in large animal models might be a good option because the CA can easily be applied on the inverted anastomosis [33]. As a small stapler for rodents is lacking, the use of holding sutures was described in most of the rat studies. However, it does not yet seem to be satisfactory according to our results. One possible reason is that the holding sutures are not able to guarantee the inverted connection, thus creating an everted anastomosis that may complicate wound healing if CA is polymerized between the two wound edges. Also, instructions for topical usage of CA in skin wound closure indicate that the application of CA between the wound edges should be prohibited [7]. To ensure an inverted anastomosis, a special stent might be a good replacement for holding sutures, but more work on this is still required.

For the use of CA as a sealant, the suture material and its size are also important factors for a good anastomosis. Our data shows that 3/0 sutures, often used in human intestinal and colorectal anastomosis, are suitable for large animal models; 5/0 sutures may be inappropriate.
for the rat intestinal and colorectal anastomosis, as no study reported positive outcomes with these. This may due to the large size of the 5/0 sutures (diameter of absorbable 5/0 suture: 0.15-0.199 mm [40]) relative to that of the rat colon (thickness of adult male rats: around 0.6 mm [41]). The 3/0 sutures (0.30-0.349 mm [40]) are much smaller and lighter compared to the human colon (thickness: 2.6 mm [42]) or porcine colon. For rat intestinal and colorectal anastomosis, smaller size sutures such as 7/0 (0.07-0.099 mm [40]) or 8/0 (0.05-0.069 mm [40]) seemed to be proper while more evidence is still required.

CONCLUSION
In view of the positive outcomes of the large animal experiments, the application of CA in intestinal and colorectal anastomosis seems promising. However, the great inconsistency and lack of detailed information in the previous literature made comparison of methodology difficult. To achieve a better consistency, studies should provide more details in the methods. If the dosage of CA, the choice of animal model, inverted anastomosis construction, and other method details also are improved, future studies will achieve better intestinal and colorectal anastomoses with CA.
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<td>Anastomosis</td>
<td>NS</td>
<td>2-4 min</td>
<td>Cuff</td>
<td>-</td>
<td>-</td>
<td>Invaginate</td>
<td>Intestine</td>
<td>+</td>
</tr>
</tbody>
</table>

* Shown on the picture
Abbreviation of different cyanoacrylate is specified on table 1. NS = not specified
REFERENCES


PART II

Mechanical strength of tissue adhesives
Mechanical strength and rheological properties of tissue adhesives with regard to colorectal anastomosis: an ex vivo study

Konstantinos Aristotelis Vakalopoulos, Zhouqiao Wu, Leonard Kroese, Gert-Jan Kleinrensink, Johannes Jeekel, Richard Vendamme, Dimitra Dodou, Johan Frederik Lange

ABSTRACT

**Objective:** To compare mechanical strength and rheology of existing tissue adhesives in a clinically relevant test setup with regard to colorectal anastomosis.

**Background:** Little is known on the mechanical strength of tissue adhesives directly after application. Furthermore, rheological profiling may be important in understanding mechanical performance and explaining differences between adhesives. This study provides new data on the mechanical strength and rheology of a comprehensive list of tissue adhesives with regard to colorectal adhesiveness.

**Methods:** Twelve surgical tissue adhesives were included: 4 cyanoacrylate adhesives (CA), 2 fibrin glues (FG), 3 polyethylene glycol (PEG) adhesives, and 3 albumin-based (AB) adhesives. Tubular rat colonic segments were glued together. Tensile (T), shear (S), and peel (P) strength were measured. Shear storage ($G'$) and shear loss ($G''$) moduli were also evaluated.

**Results:** CA adhesives were stronger than AB (T: $P = 0.017$; S: $P = 0.064$; P: $P < 0.001$), which, in turn, were stronger than PEG (T: $P < 0.001$; S: $P < 0.001$; P: $P = 0.018$). PEG were stronger than FG for shear ($P = 0.013$) and comparable for tensile and peel strength ($P > 0.05$). Within-group variation was smallest for CA. Mechanical strength correlated strongly between performed tests. Rheological properties ($G'$ and $G''$) correlated strongly with mechanical strength for all adhesives combined.

**Conclusions:** CA adhesives are the strongest and most homogenous group in terms of mechanical strength. Hydrogels (FG, AB) are heterogeneous, with lower mechanical strength than CA. FG are mechanically the weakest adhesives. Rheological profiles correlate to mechanical strength and may be useful for predicting mechanical performance.
INTRODUCTION

The field of tissue adhesives is gaining popularity in modern-day medicine. Tissue adhesives have become commonplace in several fields of medicine including dural repair, endoscopic fistula repair (cardio)vascular surgery, and mesh fixation. In the field of gastrointestinal surgery, recent research has reported using tissue adhesives to seal or create gastrointestinal anastomoses to decrease anastomotic leakage (AL) rates, which are known to be high in this field. These experiments, mostly on animal models, provide insight into the effectiveness of tissue adhesives on surgical complication rates, particularly AL.

Tissue adhesives work by forming a mechanical seal around an anastomosis, thus protecting it from leakage of intraluminal contents and ameliorating effects of AL. Before curing, all adhesives are low viscosity liquids that can efficiently flow into the pores of, in this case, biological tissue. After the polymerization phase, the cohesiveness of the adhesive increases, and the interface between the adhesive and the tissue is altered mechanically (ie, by interlocking of the adhesive with the porous tissue surface), physically, and/or chemically. Overall, the strength of the cured adhesive joint is the result of a balance between the cohesiveness of the adhesive and its adhesiveness to the tissue.

Tissue adhesives can be divided into categories on the basis of their composition. Cyanoacrylate adhesives (CA), also known as “superglues,” are synthetic adhesives, which contain cyanoacrylate monomers that polymerize after contact with water. Polymerization results in an exothermic reaction, the rate of which depends on the length of the cyanoacrylate monomers: the shorter the chain length, the more spontaneous the polymerization. CA are known to be strong but rigid and have been reported to induce tissue toxicity intracorporeally. Modern-day CA are becoming less histotoxic and more flexible. Another well-known group of tissue adhesives is fibrin glues (FG). These 2-component adhesives consist of concentrated fibrinogen and thrombin, simulating the final stage of the clotting cascade. FG form a flexible, mildly strong, adhesive bond. Some FG preparations use antifibrinolytics such as aprotinin to delay degradation time. FG are used as surgical hemostats, for the sealing of colostomies and in skin graft procedures. Polyethylene glycol (PEG) sealants are multicomponent preparations containing PEG combined with polymerization agents that form a hydrogel, resulting in a watertight tissue bond. PEG sealants have been approved for use in the sealing of spinal dura, with good clinical results. Furthermore, gelatin-formaldehyde-resorcinol (GRF) adhesives are 2-component synthetic adhesives containing a mixture of gelatin and resorcinol that is polymerized when a small amount of formaldehyde or glutaraldehyde is added. Despite concerns about tissue necrosis due to formaldehyde use, GRF is widely used for aortic dissection repair. In the same adhesive category and currently in use for the same clinical field, albumin-based adhesives are gaining popularity with good results, without concerns of formaldehyde-induced toxicity.

The mechanical strength of a tissue adhesive is an important parameter in its overall effectiveness as an anastomotic sealant. In in vivo studies, mechanical strength testing of the adhesive-tissue bond takes place directly after killing the animal. However useful as a quantitative measure of anastomotic strength, these methods do not provide information on mechanical strength directly after application, that is, before adhesive bond degradation and healing effects. This information is, in fact, important for the sealing of a bowel anastomosis, as its strength is lowest directly after creation, when wound-healing mechanisms have not yet
started to provide intrinsic anastomotic strength. Directly after construction, anastomotic strength thus relies entirely upon the used sutures or staples. Hence, one may postulate that the anastomosis is most prone to technical failure directly after its creation, and that this is when the added value of an anastomotic seal is most apparent. Therefore, the post-application adhesive strength of a tissue adhesive is an important parameter in the evaluation of a tissue adhesive as an anastomotic sealant.

Methodology is also a concern in the field of tissue adhesives. In in vivo studies, large differences exist in the choice of animal model, experimental endpoints, and adhesive strength testing methods (cf. anastomotic bursting pressure vs tensile strength tests). In ex vivo studies, various adhesive strength-testing methods exist, using various tissue substrates, tissue preparation methods, curing times, and testing protocols. Overall, these differences make the comparison of mechanical strength data between studies and a proper evaluation of the effectiveness of tested tissue adhesives problematic. This lack of consensus may also be a factor leading to the relatively low number of clinical studies in this research field.

Besides the mechanical strength of a tissue adhesive, it is also important to look into its rheological profile. The rheological profile of a viscoelastic material can be defined by dynamic mechanical analysis and can be described by 2 moduli: the shear storage modulus $G'$ and the shear loss modulus $G''$. These parameters provide information on the cohesion (strength of adhesive-adhesive bonds) and adhesion (strength of bonds between adhesive and tissue) and should ideally be balanced as not to create an adhesive, which is either too elastic or too brittle, which may result in suboptimal adhesive strength. Understanding the rheology of a tissue adhesive can provide insight into its cohesive response when under mechanical stress, which is important in understanding its clinical effectiveness, as recently shown by Serrero et al.¹⁴

In the current study, we have adapted existing guidelines of industrial adhesive testing for use with ex vivo rat colon to determine the mechanical adhesive strength of existing tissue adhesives, as a fundamental step in their evaluation as colorectal anastomotic sealants. Furthermore, rheological profiling of each tissue adhesive was undertaken, and the correlations between the rheological properties and the mechanical strength of the adhesives were calculated. All tissue adhesives were tested after the same testing protocol, ensuring fair comparison of results.

MATERIALS AND METHODS

Tissue adhesives
Twelve tissue adhesives were selected from each of the previously described tissue adhesive categories. A synopsis of the included adhesives can be found in Table 1. These adhesives were considered to be representative of the modern day commercially available tissue adhesives in surgical practice. Next to the 12 tissue adhesives, an industrial CA (Pattex Super Glue, Henkel, Germany) was used for comparative purposes. Tissue adhesives were purchased or provided for the purposes of this study. Companies providing the adhesives had no influence in the testing, results or conclusions of this study.
Table 1. Included tissue adhesives.

<table>
<thead>
<tr>
<th>Adhesive category</th>
<th>Commercial name</th>
<th>Company</th>
<th>Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyanoacrylates</td>
<td>Histoacryl Flex</td>
<td>B.Braun (Tüttlingen, Germany)</td>
<td>n-butyl-2-cyanoacrylate</td>
</tr>
<tr>
<td></td>
<td>Glubran 2</td>
<td>GEM Italia (Viareggio, Italy)</td>
<td>n-butyl-2-cyanoacrylate and methacryloyxysulfolane</td>
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<tr>
<td></td>
<td>Omnex</td>
<td>Ethicon (J&amp;J, Bridgewater, NJ, USA)</td>
<td>2-octyl-cyanoacrylate</td>
</tr>
<tr>
<td>Albumin based adhesives</td>
<td>Bioglu</td>
<td>Cryolife (Kennesaw, GA, USA)</td>
<td>Glutaraldehyde-albumin glue</td>
</tr>
<tr>
<td></td>
<td>Covabond</td>
<td>Covalent medical inc. (Ann Arbor, MI, USA)</td>
<td>Albumin, aldehyde cross linker</td>
</tr>
<tr>
<td></td>
<td>GRF</td>
<td>Cardial SA (St. Etienne, France)</td>
<td>Gelatin-resorcinol-formaldehyde glue</td>
</tr>
<tr>
<td>Polyethylene glycol adhesives</td>
<td>Duraseal Xact</td>
<td>Covidien (Mansfield, MA, USA)</td>
<td>Polyethylene glycol, trilisine amine, blue dye, N-hydroxy succinimide</td>
</tr>
<tr>
<td></td>
<td>Coseal</td>
<td>Baxter (Deerfield, IL, USA)</td>
<td>Polyethylene glycol, hydrogen chloride and sodium phosphate-sodium carbonate</td>
</tr>
<tr>
<td></td>
<td>Duraseal</td>
<td>Covidien (Mansfield, MA, USA)</td>
<td>Polyethylene glycol, trilisine amine and blue dye</td>
</tr>
<tr>
<td>Fibrin glues</td>
<td>Tissucol</td>
<td>Baxter (Deerfield, IL, USA)</td>
<td>Fibrin glue, with aprotinin</td>
</tr>
<tr>
<td></td>
<td>Evicel</td>
<td>Ethicon (J&amp;J, Bridgewater, NJ, USA)</td>
<td>Fibrin glue, without aprotinin</td>
</tr>
</tbody>
</table>

Adhesive substrate
Our objective was to develop a clinically relevant model for the testing of surgical tissue adhesives, in which the adhesive bond strength to colonic serosa could be tested without confounding factors such as suturing or anastomotic technique. We therefore chose to use intact tubular colonic segments to preserve the normal geometry and residual stresses of the colon. Colonic segments were obtained from male Wistar rats (250–350 g), which were killed for the purposes of other projects within our research group and in which the bowels were not disturbed. Approval for the study was received from the Erasmus University Medical Center (Rotterdam, The Netherlands), and guidelines for safe and hygienic tissue handling were followed. Directly after killing, the full colon of the rat was resected and the mesocolon removed. After the bowel contents were flushed using a syringe and tapwater, the colon was placed in Ringer’s lactate solution and cooled to 5 to 10°C pending mechanical testing. All tests were performed within 24 hours after resection.

Sample preparation
Directly before the experiments, the resected colon was cut into 2-cm long segments using surgical scissors. Per test, 2 segments were needed. A custom-made 4-mm wide U-shaped pin was inserted intraluminally into each colonic segment. Each colonic segment was ligated on both ends of the pin, outside of the gluing area, to prevent the colon from sliding during testing.
Tissue adhesive application

Adhesive application took place according to the manufacturers’ guidelines. Two of the above-mentioned pins (around which the colonic segments were placed) were each fixed onto a custom-made cylindrical holder with sunken screws and the colonic segments were glued while approximated, creating a tension-free adhesive bond. Curing time varied according to the manufacturer’s guidelines. The test setup is shown in Figure 1. To simulate intra-abdominal curing conditions, curing of the adhesive took place in an incubator that was kept at 37°C with a humidity level of greater than 95%. Two semicylindrical supports were used to lock the testing cylinders with the glued segments in position during curing and transportation from the incubator to the materials testing machine. These supports were removed as soon as the test setup was fixed to the testing machine, before mechanical testing.

Mechanical testing

To simulate the mechanical forces that a colonic tissue adhesive may encounter, we selected 3 mechanical tests: tensile, shear, and peel testing. Tensile and shear testing simulate contractile peristaltic waves, constricting the colon and pulling on the adhesive layer, and the effects of external viscera moving across the adhesive layer. Peel testing was considered to simulate the “weak point” of a tissue adhesive, when pull is exerted on the outer edge of the adhesive bond. These 3 tests also form the basis for the testing of tissue adhesives in the testing protocols of the American Society for Testing and Materials (ASTM) standards\textsuperscript{16–18}. For the purposes of our study, these ASTM standards were adapted for use with tubular colonic segments. Each test is illustrated in Figure 2. All tests were performed using an industrial static materials testing machine (Zwick, UK, type 1484/Testometric, UK, type AX M250-2.5 kN). Tests were performed with a 20-N load cell, at a testing speed of 10 mm per minute. Computer-based analysis software was used to record all tester data in real time. For each tissue, adhesive, tensile, shear and peeling strength were measured. Each test was performed 7 times.

Rheological testing

Rheological profiles were monitored at 37.5°C with an AR 2000 rheometer (TA Instruments, New Castle, DE) in parallel plate geometry. The liquid (uncured) adhesive samples were first
placed on the rheometer plate (8-mm diameter and 0.5-mm gap) and left to cure at 25°C until a stable value of $G'$ was reached. To prevent evaporation of water during the curing stage, silicon oil was applied around the sample (oil was removed before starting the frequency sweep to prevent influencing the measurement). Angular frequency sweep measurements were then performed in dynamic mode within the viscoelastic regime of the adhesives (ie, with $G'$ and $G''$ independent of strain) with a strain of 0.01 and frequencies ranging from 0.1 to 100 rad/s. All rheological tests were performed 3 times.

**Measure of solid content**
A given weight of liquid adhesive was left to cure at room temperature overnight. The cured amount was then placed in an oven at 70°C for 3 hours and the residual weight was measured. Solid content of the adhesive was obtained from the ratio of the residual weight divided by the initial sample weight.

**Data analysis**
A paired t-test was used to compare adhesive categories with each other with respect to their tensile, shear, and peel strength, and a 1-way analysis of variance with a post hoc Tukey-Kramer test was conducted to compare adhesives within categories. Pearson correlations were calculated between the tensile and shear, tensile and peel, and shear and peel data of all tested adhesives. Pearson correlations were also calculated between the rheological properties $G'$ and $G''$ versus each of the 3 mechanical strength tests. A $P$ value of 0.05 or less was chosen to define statistical significance. All data analyses were performed in MATLAB (Version R2010b; The MathWorks, Inc, Natick, MA).

**RESULTS**

**Mechanical testing**
First, mechanical strength between categories of adhesives was compared. CA showed the highest mechanical strength, stronger than the albumin-based (AB) adhesives in tensile ($t_{20} = 2.61, P = 0.017$) and peel ($t_{19} = 4.24, P < 0.001$) testing. CA also tended to be stronger than AB in shearing, although this result did not reach statistical significance ($t_{19} = 1.97, P = 0.064$). The AB group was significantly stronger than the PEG adhesive group in all 3 mechanical tests (T: $t_{17} = -4.01, P < 0.001$; S: $t_{15} = -6.13, P < 0.001$; P: $t_{18} = -2.60, P = 0.018$). Differences in mechanical strength between PEG and FG were small, and significant differences were only seen in the shear test, where PEG were superior to FG ($t_{11} = 2.95, P = 0.013$). An overview of these results is provided in Figure 2.
Second, mechanical strength within each adhesive category was analyzed for each mechanical
test (Fig. 3). Within CA, the largest variation in mechanical strength of different glues was found
for the tensile strength test, where Histoacryl Flex tended to be inferior to Omnex (P = 0.054).
However, the difference between the 4 CA did not reach significance (F_{3,24} = 2.60, P = 0.076).
Compared with tensile strength, shear and peel strength showed less variation between CA
adhesives (S: F_{2,23} = 1.22; P =0.325; P: F_{2,23} = 1.09; P = 0.372). AB were found to be rather
heterogeneous in terms of adhesive strength (F_{2,22} = 5.61, P = 0.011; P: F_{2,18} = 6.23, P = 0.009).
Specifically, GRF resulted in significantly lower tensile and peeling strength compared with
Covabond (P=0.010 and P=0.007, respectively). PEG showed the largest variation of all
categories in all 3 mechanical tests (T: F_{2,15} = 5.17; P = 0.020; S: F_{2,14} = 5.29, P = 0.020; P: F_{2,17} =
32.68, P < 0.001). DuraSeal yielded lower tensile strength than CoSeal (P = 0.019), whereas
DuraSeal Xact produced lower shear than CoSeal (P = 0.015) and lower peel from both DuraSeal
and CoSeal (both P < 0.001). Among FG, the only significant difference was found in the tensile
test results, for which Tissucol yielded higher strength than Evicel (t_{5} = -3.19, P =0.019). Finally,
we found that the results in all 3 tests correlated strongly with each other (T vs S: r = 0.504, P <
0.001; T vs P: r = 0.578, P < 0.001), as shown in Figure 4.
Mechanical strength and rheological properties of tissue adhesives
Chapter 4

(d)

<table>
<thead>
<tr>
<th>Adhesive categories</th>
<th>Tensile strength</th>
<th>Shear strength</th>
<th>Peel strength</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA</td>
<td>$F(3,24) = 2.60$ (p = .076)</td>
<td>$F(3,23) = 1.22$ (p = .325)</td>
<td>$F(3,23) = 1.09$ (p = .372)</td>
</tr>
<tr>
<td>AB</td>
<td>$F(2,22) = 5.61$ (p = .011)</td>
<td>$F(2,19) = 0.74$ (p = .491)</td>
<td>$F(2,18) = 6.23$ (p = .009)</td>
</tr>
<tr>
<td>PEG</td>
<td>$F(2,15) = 5.17$ (p = .020)</td>
<td>$F(2,14) = 5.29$ (p = .020)</td>
<td>$F(2,17) = 32.68$ (p = .001)</td>
</tr>
<tr>
<td>FG</td>
<td>$t(6) = -3.19$ (p = .019)</td>
<td>$t(6) = -0.303$ (p = .773)</td>
<td>$t(6) = -1.53$ (p = .176)</td>
</tr>
</tbody>
</table>

Figure 3. Overview of tensile, shear, and peel strength within each adhesive category: (a) Cyanoacrylate adhesives; (b) Albumin based adhesives; (c) Sealants; (d) FG. Results of statistical analysis are shown in the table. CA indicates Cyanoacrylate; AB, Albumin based adhesives; PEG, Polyethylene glycol adhesives; FG, Fibrin glues.

Pattex adhesive, used to compare tissue adhesives to industrial “super glue” yielded lower adhesive strength than the other tissue CA (T: mean = 1.57, standard deviation = 0.49, N = 7; S: mean = 1.68, standard deviation = 0.43, N = 7; P: mean = 0.31, standard deviation = 0.14, N = 7).

Figure 4. Correlation analyses. (a) Tensile strength test vs shear test; correlation coefficient $r = 0.504$ (P < 0.001). (b) Tensile strength vs peel test; $r = 0.578$ (P < 0.001).
Rheological testing

The highest values of \( G' \) and \( G'' \) over the entire frequency range were obtained for Pattex (industrial ethyl cyanoacrylate–based adhesive), indicating that this glue possesses the highest cohesiveness of all the CA specimens. Moreover, the high \( G' \) value (around \( 2 \times 10^8 \) Pa) and the low slope of the \( G' = f(\omega) \) curve both indicate that Pattex is a rigid material at 37.5°C. Among the tissue adhesives, the rheological profiles of the CA Histoacryl Flex and Dermabond, respectively, formulated from the monomers n-butyl cyanoacrylate and 2-octyl cyanoacrylate, are both characterized by lower values of \( G' \) and \( G'' \) and a higher slope for \( G' = f(\omega) \), indicating higher flexibility as compared with Pattex. Rheological profiles of CA are shown in Figure 5A.

The rheological behavior of PEG (Fig. 5B) is characterized by lower values of \( G' \) and \( G'' \) (from \( 1 \times 10^5 \) Pa to \( 1 \times 10^6 \) Pa) compared with CA, which is indicative of a low network concentration. Indeed, the solid content of DuraSeal was found to be 9.9%, which means FG Evicel was similar to the PEG-based DuraSeal (9.8% vs 9.9%), Evicel exhibited higher values of \( G' \) and \( G'' \), indicating that it is more cohesive than DuraSeal (Fig. 5B).

![Figure 5. Frequency (\( \omega \)) dependency of the real (\( G' \)) and imaginary (\( G'' \)) shear modulus components for: (a) Pattex, Histoacryl, Omnex, Glubran and Dermabond. (b) GRF, Covabond and Bioglue. (c) Coseal, Duraseal, Duraseal Xact, Evicel and Tissucol.](image)

The rheological behavior of the AB group (GRF, Bioglue, and Covabond) is shown in Figure 5C. The solid content of these adhesives was intermediate between those of CA and PEG, with values of 49.6, 40.1, and 38.3 for GRF, Covabond, and Bioglue, respectively. GRF exhibited intermediate \( G' \) between \( 1 \times 10^6 \) Pa to \( 1 \times 10^7 \) Pa. Bioglue and Covabond both displayed \( G' \) values in the same range as CA, which suggests that these adhesives are highly cohesive despite their moderate solid contents. At last, we also performed a correlation analysis between rheological profiles and mechanical tests of each tissue adhesive. Strong and significant correlations between both \( G' \) and \( G'' \) moduli and all 3 mechanical tests were found (Fig. 6; T: \( r_{G'} = 0.711; r_{G''} = 0.716 \); S: \( r_{G'} = 0.715; r_{G''} = 0.771 \); P: \( r_{G'} = 0.637; r_{G''} = 0.692 \)).
Figure 6. Correlation analysis between rheological results and tensile strength test. (a) Storage modulus vs tensile strength; correlation coefficient $r = 0.711$ ($P < 0.001$). (b) Loss modulus vs tensile strength; $r = 0.716$ ($P < 0.001$).

DISCUSSION

Tissue adhesives are gaining popularity in various fields of medicine. Except for their use as successful skin closure devices, tissue adhesives are also increasingly being used inside the human body for a number of indications\(^5,13\). Sealing of colonic anastomosis with tissue adhesives has been pointed out as a promising technique to prevent anastomotic leakage; however, in vivo studies have provided ambiguous results on its effectiveness\(^7\). This may be due to the interexperimental differences in animal models, testing protocol, and adhesive application. Ex vivo adhesive testing may provide a clear view of differences in the comparative mechanical performance between adhesives and may act as a platform for initial selection of tissue adhesives to be applied in subsequent in vivo testing. To date, data on ex vivo testing of tissue adhesives are scarce. Several authors report on the use of tissue adhesives in ex vivo models representing intracorporeal use. Shazly et al\(^20\) used rat duodenum for the testing of the adhesive strength of their PEG: Dextran glue. In their model, a full-thickness puncture wound was created using a needle and was then sealed off with the adhesive before burst pressure analysis was performed. In another study, Sidle et al\(^21\) evaluated the tensile strength of Bioglu in a model using peristomeum from human cadavers. Azadani et al\(^22\) compared the mechanical strength of several FG and Bioglu on human and porcine aortic grafts. In another study by Kull et al, the tensile, shear, and peel strengths of Glubran 2 and Tissucol were evaluated by using segments of fresh, shaven porcine skin as the biologic substrate and performing tests according to the ASTM guidelines for the testing of tissue adhesives\(^23\). To date, no experiments have reported using tubular colonic segments for the testing of tissue adhesives.

In our study, we evaluated the mechanical strength and rheological properties of a comprehensive list of surgical tissue adhesives from each tissue adhesive category, using the same experimental configuration and testing protocol, thereby overcoming the abovementioned limitation of heterogeneous testing protocols. Moreover, by using tubular colonic segments, we were able to test the adhesives in a clinically relevant setting by applying the adhesive only on the serosal surface of the bowel, the target site for its eventual clinical use, while leaving the mechanical properties of the colon intact.
Mechanical test setup
Peristalsis of the colon is a complex process consisting of various types of contractions. Individual phasic contractions occur spontaneously, and organized motor complexes assist in the propulsion of bowel contents. The effects of peristalsis consist of kneading of fecal material by circular muscle contraction and propulsion via longitudinal muscle activity. A bowel anastomosis is thus subjected to mechanical forces in various directions. Next to peristaltic forces, external forces may play a role such as in the case of adhesion formation to other viscera, and the direct adhesive effect of the tissue adhesive to other viscera. These forces can be simplified into 3 mechanical planes: forces acting to the plane of the anastomosis, forces parallel to the plane of the anastomosis, and peeling forces. To simulate these forces in our test setup, we therefore chose to test tensile strength, shear strength, and peel strength. To our knowledge, this is the first study in which fresh, circular bowel segments were used and in which an adhesive was applied only on the serosal surface of each segment. Our test setup can, therefore, enable surgical adhesive application in the same manner as it would be done perioperatively, while keeping the biomechanical characteristics of the colon intact.

Mechanical testing
In this study, CA was the strongest tissue-adhesive group in terms of adhesive strength. This group was also easy to use due to easy application procedures and quick curing time. Furthermore, when comparing the outcomes of the mechanical tests between CA, no significant differences were found. This points out that despite differences in composition and/or additives (Table 1), the group of CA was the most homogeneous group in terms of adhesive performance.

AB adhesives were characterized by diverse chemical compositions, resulting in larger differences in mechanical strength than in the case of CA. Significant differences were observed between AB for both tensile and peel tests. Of these, the AB adhesives Covabond and Biogluue exhibited similar mechanical strength, whereas the gelatin-based GRF resulted in lower adhesive strength for tensile and shear tests. In this group, it was found difficult to provide a precise adhesive application for GRF and the correct amount of formaldehyde hardener, as also previously acknowledged. To ensure reproducible and correct application, we used the application procedure described previously by Nishimori et al in which formaldehyde was applied using an insulin needle.

Adhesive strength testing yielded that PEG and FG are similar to each other. PEG adhesives differed significantly from each other in all mechanical tests. In this group, CoSeal resulted in the highest adhesive strength whereas DuraSeal and DuraSeal Xact yielded large differences between tests. DuraSeal Xact showed higher strength in the tensile strength test, but DuraSeal seemed to be stronger in shear and peel testing. The difference between DuraSeal and DuraSeal Xact is the additive N-hydroxy succinimide in DuraSeal Xact, used to prevent swelling in this adhesive. This additive may account for the differences in adhesive strength. Among FG, Tissucol and Evicel adhesives provided similar results for shear and peel strength, whereas Tissucol was stronger in terms of tensile strength. This may be due to the aprotinin additive in Tissucol, which is added to delay degradation time. In this study, we observed low mechanical strength of FG. Previous research wherein FG was used reported that FG created a very strong bond. A possible explanation for this finding is that the presence of blood or intraperitoneal fluid further strengthens the tissue adhesive bond, while being aided by the physiological action of fibrin.
We also observed that the 3 mechanical tests strongly correlated with each other. On the basis of this information, one may postulate that, if the purpose of an analysis is to compare ex vivo 2 or more adhesive formulations, using 1 of the 3 mechanical tests may suffice, thereby enabling considerable savings in material and time resources.

When comparing mechanical strength between adhesive groups (using the adhesive categories described previously), we observed that CA were the strongest tissue adhesives, followed by AB, PEG, and FG. Generally, the tensile and shear strength tests resulted in the highest adhesive forces and were mostly not significantly different to one another. Peel strength for all groups showed much lower mechanical strength in all adhesive samples, in line with previous research on tissue adhesives.²³

Rheological testing
Rheological testing of tissue adhesives is standard practice in the development phase of any industrial tissue adhesive. However, rheological data for commercialized tissue adhesives are not currently publicly available. Rheological analysis was performed to provide information on the degree of cohesiveness, and in turn, flexibility of the tested tissue adhesive. Higher values of $G'$ and $G''$ and a low slope of the $G' = f(\omega)$ curve are indicative of high cohesiveness and a rigid/brittle adhesive. When comparing the various categories of tissue adhesives, we observed that CA resulted in the highest cohesiveness and were therefore generally the least flexible tissue adhesives. AB were more flexible than CA, whereas the most flexible adhesives were found in the PEG and FG groups, which showed comparable rheological results.

Between CA, some differences were found in rheological profiles. Pattex, which was included for comparative purposes, representing non–tissue-oriented CA, was the most rigid adhesive. Within the tissue CA, Glubran 2 and Omnex provided the least flexible rheological profiles, whereas Histocryl Flex and Dermabond were the most flexible adhesives, yielding rheological profiles comparable to the AB. The increased flexibility of n-butyl cyanoacrylate–based (Histocryl) and 2-octyl cyanoacrylate–based (Dermabond) adhesives as compared with the industrial ethyl cyanoacrylate–based adhesive likely stems from the plasticizing effect of the alkyl side groups constituting the polymer backbone. This effect is especially pronounced for the longer octyl side groups, as indicated by the lowest values of $G'$ and $G''$ at high frequencies for the Dermabond adhesive. In the AB group, Covabond and Bioglu were relatively rigid, both displaying $G'$ values in the same range as CA, which suggest that these adhesives are highly cohesive despite their moderate solid contents. Moreover, these 2 adhesives had a very similar rheological profile, indicating that the albumin/aldehyde base, which both Covabond and Bioglu share, is a determinant factor of their rheological profile. In the same adhesive category, GRF showed low $G'$ and $G''$ indicating low cohesiveness and more flexibility. As stated previously, the lowest $G'$ and $G''$ were observed for PEG and FG. Although these categories differed significantly in mechanical strength, they share very similar rheological profiles and are very flexible adhesives. In this group, it was noteworthy that DuraSeal Xact was the most flexible adhesive sample, whereas its composition is similar to the DuraSeal adhesive. Interestingly, although the solid content of the FG Evecel was almost similar to the PEG-based DuraSeal (9.8% compared with 9.9%), Evecel seemed to be much more cohesive. This observation suggests that the fibre-like supramolecular architecture of FG creates a stiffer structure as does the more flexible network of interconnected PEG chains in PEG adhesive. Rheological results are interesting due to the implications for their target use. Keeping the rheological profiles in mind,
one may predict which tissue adhesive is the best choice for the desired use. This information may aid a surgeon to decide which adhesive is most suitable for the targeted indication.

When used in our mechanical test setup, CA polymerized within seconds after coming into contact with fluid, but polymerization between the 2 plates of the rheometer took considerably longer. This was true for all the CA except for Omnex, which integrates a polymerization catalyst in the applicator and cures within a few minutes even in a “dry” environment. Furthermore, it should be noted that the rheological profiling of the PEG group was the most difficult in our experimental setup, because of the very low grip of the hydrogels on the plates, the low modulus of the cured gels, and the fast evaporation of water. Nevertheless, satisfactory results could be obtained in each case.

**Rheology and mechanical testing**
Both storage and loss modulus (the 2 moduli defining the rheological profile of adhesives) were significantly correlated with each of the 3 mechanical tests. This finding indicates that the rheological characteristics of an adhesive can, in turn, predict its mechanical strength. As rheological tests are easily performed only requiring several drops of an adhesive, this technique may be promising in the future evaluation of tissue adhesives. Another interesting finding comes from the rheological profile of the Pattex adhesive. Despite the highest values of $G'$ and $G''$, Pattex provided relatively low results in mechanical strength. This indicates that, in general, a tissue adhesive’s mechanical strength may rely upon an “optimum range” of $G'$ and $G''$, which may not necessarily be the highest value of $G'/G''$, in line with previous research on tissue adhesive rheology\textsuperscript{14}.

**Study limitations**
In this study, we attempted to create intra-abdominal circumstances as closely as possible, simulating a physiologic environment for adhesive application. An ex vivo approach was chosen to be able to systematically test each tissue adhesive in a reproducible fashion and enable comparisons without confounding factors resulting from surgical intervention or wound healing. Naturally, ex vivo testing is clinically less relevant than in vivo testing, as the structural integrity of the bowel wall starts to degrade directly after resection. This problem was partly overcome by cooling the tissue in a preservation solution. Rat colon has been previously used by many researchers in the testing of tissue adhesives and was therefore chosen as the substrate in this study. Another practical problem we encountered was that the application procedure was difficult as most applicators are non-interchangeable and meant for use in human colon, which, of course, is larger than the rat colon. At last, in this study, we only observed the mechanical strength and rheology of ex vivo colonic segments, which does not provide information on the effects of the body’s healing process on the adhesive, and also the effects of the adhesive on the tissue. This aspect should be examined in future studies.

**CONCLUSION**
In this study, we have provided information on the adhesive strength and rheological characteristics of a comprehensive list of tissue adhesives spanning across all present-day adhesive categories. Modern-day cyanoacrylates are the strongest in terms of mechanical strength and form a homogeneous group based on rheological endpoints. Of the AB adhesives, Covabond and Bioglue adhesives were also strong and showed rheological profiles similar to that of cyanoacrylates. From the PEG group, DuraSeal Xact and CoSeal seemed to be promising
in terms of mechanical strength. FG showed the lowest adhesive strength, with Tissucol providing slightly better results. The mechanical test results correlated to each other, implying that the choice of 1 single test contains sufficient information to evaluate the mechanical strength of a tissue adhesive. Importantly, in this study, a standardized testing protocol was used enabling us to compare results between tissue adhesives in a methodologically appropriate manner. Rheological profiling of tissue adhesives aided in explaining differences in mechanical strength and in understanding the behavior of tissue adhesives. Furthermore, the rheological profiles of the tissue adhesives were significantly correlated to their mechanical strength, making it possible to predict mechanical strength by examining rheological endpoints. It could be recommended that the combination of mechanical and rheological data should become part of a standard testing protocol in future studies with tissue adhesives.

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Reducing anastomotic leakage by reinforcement of colorectal anastomosis with cyanoacrylate glue

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Chapter 5

ABSTRACT

**Introduction:** Technical failure of sutured or stapled anastomoses may lead to anastomotic leakage (AL), which is one of the most important complications after colorectal surgery. Cyanoacrylate glue (CA) provides strong mechanical attachment, making it a good candidate for suture reinforcement. This study aimed to demonstrate that CA is the most important factor in the strength of a sealed colorectal anastomosis, in both normal and insufficient anastomoses.

**Methods:** *ex-vivo* porcine colorectal segments were resected. A one-layer continuous anastomosis or an insufficient 6 interrupted-suture anastomosis was created, and the baseline anastomotic bursting pressure (ABP) was measured. The primary anastomosis was then reinforced either by CA or with 4 additional interrupted sutures, further inverting the anastomosis. After reinforcement a second ABP test was performed.

**Results:** Thirty-two segments were used. Reinforcing the anastomosis by CA significantly increased ABP in both normal and insufficient anastomosis when compared to the primary anastomosis (p < 0.05 for all groups); no significant difference in ABP was found between normal and insufficient anastomosis groups after CA reinforcement. Anastomotic reinforcement with CA was not inferior to the reinforcement with sutures in both normal and insufficient anastomoses, and had significant less ABP variances in normal anastomosis groups (p = 0.042).

**Conclusion:** Reinforcing a colorectal anastomosis with CA increases its mechanical strength in both normal and technically insufficient situations, which may contribute to the reduction of anastomotic leakage. CA is promising for anastomotic reinforcement based on mechanical improvement of the anastomosis and *in-vivo* studies are needed to evaluate its biological effects.
Reducing anastomotic leakage by reinforcement of colorectal anastomosis with cyanoacrylate glue

INTRODUCTION

Anastomotic leakage (AL) is one of the most important complications in gastrointestinal (GI) surgery. Especially in ultra-low colorectal anastomosis, a higher rate of leakage has been observed, which varies from 8% to 20% [1] and leads to a mortality as high as 33% [2]. The etiology of AL is still not yet fully understood. Several mechanisms and risk factors are known to contribute to its occurrence [3-5]. Of these, the most important are considered to be technical failure and ischemia [6].

In order to reduce the incidence of AL, substantial surgical techniques have been tested [7, 8]. One recent research from Gadiot et al. indicated that supportive sutures of laparoscopic left-sided anastomosis might significantly reduced AL rate [9]. In this technique, three interrupted supportive sutures were circumferentially placed parallelly to the primary anastomosis. They offered sufficient additional strength to protect the primary anastomosis from mechanical stress, thus preventing the occurrence of AL. However, hand-sewn suturing for low-level colorectal surgery is technically challenging and hence requires a longer learning curve, which may slow its further implementation in clinical practice.

Cyanoacrylate glue is now becoming increasingly popular in surgery for different indications [10]. It has been shown to provide strong mechanical attachment and polymerize quickly within 30 to 60 seconds after application [11]. Clinical practice showed promising results on the use of CA as a sealant after primary anastomosis in pancreaticoduodenectomy [12]; experimental studies also used CA to create sutureless colorectal anastomoses [13]. These results suggested that CA seemed to be a promising alternative to supportive sutures in low colorectal anastomosis.

In this study, we used CA to reinforce normal and insufficient colorectal anastomoses in an ex vivo porcine model. We aimed to demonstrate that CA is the most important factor and is responsible for the anastomotic strength of an invertedly reinforced anastomosis, regardless of the primary anastomosis being normal or insufficient.

METHODS

Animals

Distal colon segments of 10-15 cm in length were resected from male Yorkshire pigs (6 months old, 90-100 kg) from a local slaughterhouse, directly after euthanasia by CO2 overdose. The specimens were flushed using tap water and preserved in a phosphate-buffer solution, and then immediately transported to the laboratory in the medical center. All experiments were then performed within 6 hours after euthanasia.

Primary anastomosis

The experimental methodology is depicted in figure 1. During the first part of the experiment a primary anastomosis was constructed in an end-to-end, inverting way using synthetic suture (3/0 Vicryl, Ethicon, J&J, USA). In the normal anastomosis group (NG), a continuous one-layer anastomosis was created. Bite size was 5 mm from the colonic edge and subsequent sutures were placed at 5 mm intervals. In the insufficient anastomosis group (IG), six equidistant interrupted sutures were placed equally around the circumference with 5 mm bite size. After that, the first anastomotic bursting pressure (ABP) test was performed.
**Anastomotic reinforcement**

The anastomosis was subsequently reinforced with either CA or sutures. In the suture-reinforced groups, the anastomosis was reinforced with four supportive sutures (3/0 Vicryl, Ethicon, J&J, USA). These supportive sutures were placed equidistantly around the anastomosis, further inverting the primary anastomosis. Sutures were placed submucosally at 5 mm of either side of the primary anastomosis, and the bite size was also 8 to 10 mm.

In the CA-reinforced groups, 2-octyl-cyanoacrylate glue (Dermabond, Ethicon, J&J, USA) was used to reinforce the anastomosis. One capsule (0.5 mL) of Dermabond was used to form a continuous glue bond around each primary anastomosis. The CA was applied on one side of the primary anastomosis, 10 to 15 mm in length along the anastomosis and 5 mm in width; then forceps were used to approximate two serosal edges, which further inverted the anastomosis for 5 mm (the width of CA). This procedure was repeated several times until the whole primary anastomosis was reinforced. The schematic overview is depicted in figure 2. After CA application, a waiting time of five minutes was set to ensure full polymerization.

After reinforcement with either CA or sutures, the second ABP test was performed again following the below described protocol.

**Anastomotic bursting pressure test**

An air-infusing probe was introduced into the proximal end of the colonic segment. Both bowel ends were then ligated to ensure an airtight compartment. The specimen was submerged in a tank filled with tap water and air was infused through the probe at a rate of 99 ml/hour via an automatic syringe pump (Perfusor Secura, B. Braun, DL). The setup was connected to a digital pressure indicator (DPI 101, Druck, Leicester, UK). Two researchers observed the anastomosis for signs of anastomotic leakage (i.e. air bubbles from the anastomosis). The pressure at the time of the first leakage of air was defined as the anastomotic bursting pressure (ABP) and noted.
Reducing anastomotic leakage by reinforcement of colorectal anastomosis with cyanoacrylate glue

Figure 2. Cross-sectional view of anastomoses with reinforcement (Schematic). Primary anastomoses: (a) Normal one layer continuous sutures, (b) Insufficient 6 sutures; Reinforcement: (c) CA reinforcement (colored in yellow); (d) Suture reinforcement.

Statistical analysis
Data were shown in the form of mean ± standard deviation (S.D.). Statistical analysis was performed using SPSS 17.0 (SPSS, Chicago, IL, USA). Data were analyzed using Wilcoxon Signed ranks, Mann-Whitney, and nonparametric Levene’s tests according to proper indications. A p-value of 0.05 or less was chosen to define statistical significance.

RESULTS

Overall, 32 colon specimens were used. Sixteen of them were used for the creation of a one layer continuous anastomosis: the normal anastomosis group (NG). The NG was divided into two groups of eight specimens; in one group the anastomoses were reinforced with cyanoacrylate glue (NGG), and in the other group the anastomoses were reinforced with four supportive sutures (NGS). The other 16 specimens were used to create an insufficient six-suture
anastomosis: the insufficient anastomosis group (IG). Again, two groups of eight specimens were made, one with reinforcement of CA (IGG) and the other with sutures (IGS).

All the anastomoses burst at the anastomotic site during the ABP test. As is shown in figure 3, the mean ABP of the primary anastomosis was significantly higher in NG (32.5 ± 14.7 mmHg) than in IG (12.5 ± 5.0 mmHg, p = 0.000, Mann-Whitney test); the respective ABP in NGG, NGS, IGG and IGS groups were 48.2 ± 13.0 mmHg, 65.1 ± 27.6 mmHg, 39.0 ± 9.9 mmHg and 57.6 ± 18.8 mmHg. Reinforcing the primary anastomosis with CA significantly increased ABP in both normal and insufficient anastomosis groups (NGG / NG, p = 0.012; IGG / IG, p = 0.012, Wilcoxon Signed Ranks Test); no significant difference in ABP was found between normal and insufficient anastomosis groups after CA reinforcement (NGG / IGG, p = 0.161, Mann-Whitney test). Similar results were also found in suture-reinforced groups.

The CA-reinforced anastomoses had similar ABP values to the suture-reinforced anastomoses in normal anastomosis groups (NGG / NGS, p = 0.195, Mann-Whitney test), while the ABP variances were significantly less in CA-reinforced group (NGG / NGS, p = 0.042, nonparametric Levene’s test). Similar ABP between CA- and suture-reinforced anastomoses also was found in the insufficient anastomosis groups, including similar ABP variances (IGG / IGS, p = 0.065, Mann-Whitney test; p = 0.248, nonparametric Levene’s test).

The ABP of the insufficient anastomoses reinforced with CA was higher (but not significant) than that of the normal anastomosis group (IGG / NG, p = 0.136, Mann-Whitney test).

![Figure 3a NG vs. NGG](image1)
![Figure 3b NG vs. NGS](image2)
![Figure 3c IG vs. IGG](image3)
![Figure 3d IG vs. IGS](image4)

Figure 3. ABP (mmHg) before and after reinforcement. (a) Normal anastomosis before and after CA reinforcement (27.9 ± 10.8 mmHg vs. 48.2 ± 13.0 mmHg, p = 0.012); (b) Normal anastomosis before and after suture reinforcement (37.2 ± 17.3 mmHg vs. 65.1 ± 27.6 mmHg, p = 0.025); (c) Insufficient anastomosis before and after CA reinforcement (12.3 ± 5.3 mmHg vs. 39.0 ± 9.9 mmHg, p = 0.012); (d) Insufficient anastomosis before and after suture reinforcement (12.7 ± 5.0 mmHg vs. 57.6 ± 18.8 mmHg, p = 0.012). *p < 0.05 (Wilcoxon Signed Ranks Test).
DISCUSSION

Despite many years of research and countless technical alterations, leakage remains a major problem after creation of a colorectal anastomosis. Previous studies revealed that the strength of skin closure with CA approximates that of closure with 5/0 sutures [11]. In concordance with this finding, we found that with a limited amount (0.5 mL), CA reinforcement significantly increased the anastomotic bursting pressure in both normal and insufficient colorectal anastomoses with less variance, and it was not inferior to the suture reinforcement. Even in the insufficient anastomoses, CA reinforcement still provided good anastomotic strength, which was slightly higher than the standard one-layer sutures, although this difference was non-significant. These results indicate that strong anastomotic strength could be guaranteed after CA application regardless of the strength of the primary anastomosis. Furthermore, this strength comes into effect immediately after its application around the anastomosis, which may contribute to the prevention of AL caused by technical failure.

Previous clinical work introduced the concept of inverted suture reinforcement in colorectal anastomosis to prevent the occurrence of AL [9]. This concept was quite promising because of the significant reduction of AL rate, yet suture reinforcement does have several shortcomings in practice. Firstly, the technique of sub-mucosal suturing may be difficult to perform, especially in ultra-low anastomoses. Its strength relies on the skill of the surgeon, which may vary according to experience, and has been known to affect the incidence of leakage [14]. Secondly, due to individual variations between patients, it may also be challenging to decide how many sutures are needed and how to space them equidistantly, especially if an extra suture is needed to ensure the full inversion. Lastly, creating a second layer of reinforcement sutures is a time consuming process, which was already addressed previously in the comparison of single- and double-layer colorectal anastomosis [15]. In our current experimental setup, we found these problems might be overcome with the use of CA. Firstly, CA reinforcement significantly reduced ABP variances when compared with the suture reinforcement, indicating that CA application was more standardized. Also, different from interrupted sutures, CA provided an equally distributed seal around the whole circumference of the anastomosis. Lastly, CA application was easier and faster than hand-suture technique, and it might be further improved if a specific applicator would be developed in the future.

Cyanoacrylate use in gastrointestinal surgery is still limited. Although its application in sealing vascular [16] and pancreaticoduodenal [12] anastomosis showed promising results, CA reinforcement of a primary anastomosis has not been widely accepted in intestinal or colorectal surgery. Most comments on CA reinforcement focus on its negative biological effects such as an increased tissue reaction, which may counteract its mechanical effects [17, 18]. Certainly, CA’s biological characteristics determine the late outcomes such as tissue reaction and wound healing after its application; however technical aspects during application also influence these outcomes.

When comparing experimental methods among different studies, some technical differences, which might partly explain the inconsistent results between those studies, were noticed. For instance, the amount of CA used in our porcine model was 0.5 mL per anastomosis, while in other rat studies 0.2 mL or 0.5 mL of CA was applied [18, 19]. Furthermore, most other studies did not specify the CA amount they used. Comparing the size of a porcine colon with a rat colon, one can imagine that the required CA amount is much smaller in the latter. Only 0.4 μL (0.0004
mL) CA was enough to create a vessel anastomosis in a previous rat model [20]. In our previous work, we found that 0.02 mL CA was already enough to seal a primary anastomosis in a rat model, leading to a sufficient clinical and histological healing (unpublished data from our group). Conversely, overdose of CA may lead to more side effects, such as increased inflammatory response and adhesion formation [21], rather than a further increase in bursting pressure. Besides CA dosage, inversion of a gastrointestinal anastomosis has been considered as a key factor in anastomotic healing [22], and was also essential to the reinforcement technique discussed in this study. Some previous studies reported a leakage rate of 34% in evertting CA sealed anastomoses [23, 24]; while in another study, no leakage and good wound healing was found when a modified circular stapler was used to create inverted anastomoses sealed with CA [13]. These aspects and other technical details of CA’s application should also be taken into consideration for AL studies. For that, ex-vivo tests prior to in-vivo models might help to determine the least possible CA amount, the best suturing technique, and other details to achieve a strong and immediate anastomotic strength. Afterwards, the biological effects can be evaluated in in-vivo models.

Due to our current experimental test setup, there are still some limitations in our study. First, as mentioned above, the method of CA application in this study, though easy, is still not yet practical for widespread clinical implementation; instruments for CA’s application, especially in laparoscopy, are needed. Also, surgical staplers are widely used in GI anastomosis now. Inclusion of CA reinforcement after stapling would further improve the integrity and clinical relevance of this experiment. Lastly, we used ex-vivo porcine segments because they were easily available and inexpensive, but the non-vital period up to 6 hours, though relatively short, might still influence the colon’s biomechanical properties. In-vivo tests will be necessary to minimize those effects and, furthermore, to determine the biological effects of CA reinforcement.

In conclusion, Cyanoacrylate reinforcement increased the bursting pressure of colorectal anastomoses in both normal and technically insufficient anastomoses; as hypothesized, a reinforced anastomosis derived most of its biomechanical strength from CA reinforcement, regardless of the primary anastomotic configuration. The use of CA as a supportive agent may contribute to the reduction of AL and is promising for suture replacement, while in-vivo studies are needed for further implementation.

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PART III

Can tissue adhesives be safely used on living tissue?
Prevention of leakage by sealing colon anastomosis: experimental study in a mouse model

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ABSTRACT

**Introduction:** In colorectal surgery, anastomotic leakage (AL) is the most important complication. Sealants, applied around the colon anastomosis, may help prevent AL by giving the anastomosis time to heal by mechanically supporting the anastomosis and preventing bacteria leaking into the peritoneal cavity. The aim of this study is to compare commercially available sealants on their efficacy of preventing leakage in a validated mouse model for AL.

**Methods:** Six sealants (Evicel, Omnex, VasculSeal, PleuraSeal, BioGlue, Colle Chirurgicale Cardial) were applied around an anastomosis constructed with five interrupted sutures in mice, and compared to a control group without sealant. Outcome measures were AL, anastomotic bursting pressure (ABP), and death.

**Results:** In the control group there was a 40% death rate with a 50% rate of AL. None of the sealants were able to diminish the rate of AL. Furthermore, use of the majority of sealants resulted in failure to thrive, increased rates of ileus, and higher mortality rates.

**Conclusion:** If sealing of a colorectal anastomosis could achieve a reduction of incidence of clinical AL this would be a promising tool of prevention of leakage in colorectal surgery. In this study we found no evidence that sealants reduce leakage rates in a mouse model for AL. However, the negative results of this study make us emphasize the need of systemic research, investigating histologic tissue reaction of the bowel to different sealants, the capacity of sealants to form a watertight barrier, their time of degradation, and finally their results in large animal models for AL.
INTRODUCTION

In the field of colorectal surgery, anastomotic leakage (AL) is the most important complication. Incidence of AL has gone unchanged for many years, with high mortality (10 to 20%), and often need of second surgery, increased rate of subsequent complications, and prolonged hospitalization. A major problem of AL is timing of the diagnosis due to lack of sensitive and specific diagnostic tools. This increases the morbidity of AL, due to the delay in diagnosis and subsequent therapy in symptomatic, ill patients. In some patient categories, alertness for AL is raised, thanks to known risk factors for AL such as low anastomosis, male gender, smoking and corticosteroid use. Despite higher vigilance in patients with risk factors for AL, and the use of deviating stomas in low anastomoses, a tool for primary prevention of leakage of a colorectal anastomosis is not yet clinically available.

Different approaches to AL prevention have been investigated, such as omental wrapping, staple line reinforcement, intraluminal devices, and sealing. None have yet shown convincing results or achieved broad implementation. Sealants, applied around the colon anastomosis, may help prevent AL by giving the anastomosis time to heal by mechanically supporting the anastomosis and preventing bacteria leaking into the peritoneal cavity. Some sealants have been tested in an experimental setting, however with different models and outcome measures, making it difficult to evaluate their safety and benefits in colorectal surgery, and their use in a clinical setting remains rare.

Prevention of AL should be a priority in colorectal surgery considering the potential benefits in morbidity and mortality. Sealing with tissue adhesives may be an effective technique in this field. The aim of this study is to compare a number of available sealants on their efficacy of preventing leakage in a validated mouse model for colorectal AL.

METHODS

Leakage model

Within this research group an animal model was developed for AL. In this model, described by Komen et al., approximately 40% of the animals will develop AL in seven days based on technical failure by creating an anastomosis with five full-thickness interrupted sutures, compared to a standard, non-leaky, anastomosis constructed with 12 full-thickness interrupted sutures. C57BL6-mice were used since they are considered to be more sensitive to infections than rats. Outcome measures are AL, anastomotic bursting pressure (ABP), and death.

Anesthesia and operation

Before and after the intervention standard mouse chow and water were supplied ad libitum. The intervention consisted of anesthetizing the mouse (nose mask, FiO2 60%, isoflurane 2%), shaving, and disinfecting the abdomen. The abdomen was entered through a 3-cm midline incision. On the ascending colon (one centimeter aborally to the cecum), the bowel was transected without damaging the vessels. An end-to-end anastomosis was constructed with 5 interrupted full-thickness sutures (Dafilon 8-0; B. Braun Melsungen, Germany). In the study groups a sealant was applied around the anastomosis (extraluminally), using the minimum quantity necessary to cover it circularly. The colon was repositioned and the abdominal wall and skin are both closed with a continuous suture (Safil 5-0 B. Braun). All intra-abdominal manipulations were done with microscope to ensure optimal vision. After the operation, the
mice were examined daily for signs of AL, and their weight noted. When mice died before day 7, necropsy was performed aiming to determine the cause of death.

**Measurements at day 7**

After seven days the experiment was ended. The mice were anesthetized and re-laparotomy performed. The abdominal cavity was examined for abscesses or signs of fecal peritonitis, regarded as a manifestation of AL. Then the anastomotic bursting pressure (ABP) was determined: a canula was inserted proximal of the anastomotic site; the bowel was ligated distally to the anastomosis and proximally over the canula. Air was injected into the isolated anastomotic segment and the pressure monitored. The pressure at the point of rupture of the anastomosis represented ABP. After completing the ABP test the animal was sacrificed. In case of death before day 7 a necropsy was performed. In case of unethical sickness relaparotomy was performed before day 7.

**Study groups**

Six different sealants were tested, from each of the existing sealant categories: fibrin sealants, cyanoacrylate sealants, polyethylene glycol sealants, and albumin-based sealants. Their properties are described beneath. All groups contained eight mice, with a control group containing 16 mice. The Evicel experiment was performed separately, and contained 14 mice with 14 controls.

1. **Evicel** (Ethicon Inc, Johnson & Johnson, Sommerville, NJ) is a human fibrin sealant indicated as an adjunct to hemostasis. It initiates the last phase of physiological blood coagulation: thrombin activates the conversion of fibrinogen into fibrin. Factor XIIIa, which is activated form factor XIII by thrombin, crosslinks fibrin. Fibrin sealants are metabolized in the same way as endogenous fibrin, by fibrinolysis and phagocytosis.

2. **Omnex** (Ethicon, USA) is a (synthetic) cyanoacrylate sealants consisting of a blend of two monomers, 2-octyl cyanoacrylate and butyl lactoyl cyanoacrylate. The sealant polymerizes to form a flexible, strong film adherent to the tissue. Omnex biodegrades in approximately 36 months.

3. **VascuSeal** (Covidien, Mansfield, MA) is a 100% synthetic polyethylene glycol based hydrogel sealant. When mixed together, the precursors cross link to form the surgical sealant, visible through a blue coloration. The polymer hydrolyzes over seven days liberating polyethylene glycol molecules, which are cleared by the kidneys.

4. **PleuraSeal** (Covidien, USA) is also a synthetic, polyethylene glycol based hydrogel sealant. It is similar to VascuSeal, except for later absorption in approximately four to eight weeks. It has been withdrawn from commerce since this experiment, due to a higher than anticipated persistent air leak rate and inconsistent efficacy at interim results of a clinical study.

5. **BioGlue** (CryoLife Inc, Kennesaw, GA) is an albumin-based surgical adhesive. It is composed of purified bovine serum albumin (BSA) and glutaraldehyde. BioGlue makes a mechanical seal after crosslinking when being mixed, bonding tissues and sealing defects.

6. **Colle chirurgicale Cardial** (Cardial-Bard, St Etienne France) is an albumin-based sealant, consisting of resorcin-gelatin glue with formaldehyde-glutaraldehyde polymerizing agent. The polymer is applied on the surface to be glued; the polymerizing agent is added afterwards with a syringe.
RESULTS

Application was only possible when the polymerizing or crosslinking agent was mixed in the syringe or at the tip, making a semi-liquid gel-like substance when applied on the bowel, which stayed in place. The only sealant in which we could not achieve this was Cardial Colle Chirurgicale, being two completely fluid components that had to be applied on the bowel one after the other. This technique does not make a ‘sticky’ solution and therefore could not be applied at the correct position. For this reason the experiment with Cardial Colle Chirurgicale was aborted after surgery of four mice out of ethical considerations. The easiest application of sealants was through the help of an air-assisted applicator (Evicel, VascuSeal, PleuraSeal).

In the control group there was a 40% death rate with a 50% rate of AL (10% abscess, 40% peritonitis). In this group there was no observation of mechanical ileus, there was a weight gain after three days, and the mean ABP was 134.6 mmHg (SEM 12.45). None of the sealants were able to diminish the rate of AL. Evicel showed similar results as the control group: weight gain after 4 days, 43% AL, ABP was 118 mmHg (SEM 16.8). However 1 mouse developed ileus (7%). Omnex, VascuSeal and Cardial Colle Chirurgicale showed similar results considering the rate of AL and death (0% AL in the uncomplete Cardial-group with 4 mice), but significantly more ileus (figure 2) and higher mortality (figure 3). In two mice of the Cardial-group macroscopic hepatic tissue damage was visible. PleuraSeal and BioGlue had a 100% death rate. During necropsy, in some mice origin of death could not be determined anymore.

Rates of AL, ileus, death, and results of ABP for each group are shown in figure 1 to 4. In conclusion, the sealants tested in this study seem to worsen morbidity and mortality in a mouse-model for colonic anastomotic leakage, with the exception of Evicel fibrin sealant which gave similar results as the control group.

![Figure 1. Percentage of AL.](image-url)
Figure 2. Percentage of ileus.

Figure 3. Mortality rate.

Figure 4. Anastomotic bursting pressure (ABP, in mmHg). No significant differences between groups (Mann Whitney U test).
DISCUSSION

Prevention of complications is something every surgeon aims for. Disinfection, preoperative prophylactic antibiotics, and choice of suture material aim to prevent wound infection; rapid mobilization and analgesia aim to prevent postoperative ileus; subcutaneous heparin aims to prevent thrombosis and emboli. Anastomotic leakage (AL) is the most feared after colorectal surgery due to its increased rate of subsequent complications, need of re-intervention, prolonged hospitalization, and high mortality (10-20%)\(^{3-6}\). A tool of prevention of leakage of bowel content into the abdominal cavity, therefore significantly lowering the morbidity of AL, would be a reassurance for surgeon and patient, significantly lowering hospital costs and mortality.

Several techniques have been tested to date. Merad et al. performed a randomized controlled trial in 1998 on omentoplasty to prevent AL. No difference between groups was found\(^{17}\). Several intraluminal devices have been tested, such as the Coloshield or C-Seal (Polyganics, Groningen, the Netherlands)\(^{28,29}\). There are only few clinical studies with these devices, some with promising results; however they are small and have not brought the convincing results needed for broad implementation\(^{19}\). A temporary protective stoma has been shown to reduce clinical AL after low anterior resection\(^{16}\). However, a temporary protective stoma is also related to complications and associated morbidity, requires re-intervention for its closure, or is not closed at all due to complications and increased morbidity encountered during the postoperative phase\(^{30-32}\). Therefore it is only indicated in situations of high risk of AL.

Sealants, applied around the colon anastomosis, may help prevent AL by giving the anastomosis time to heal by mechanically supporting the anastomosis and preventing any apparent leaks. In other fields of surgery sealants have been tested clinically, such as for (cardio)vascular anastomoses with positive results\(^{33-35}\), in pancreatic surgery without any benefit on pancreatic leaks\(^{36,37}\), in thoracic surgery with inconsistent results regarding diminishing air leaks after pulmonary resection\(^{38-42}\), and one randomized study in bariatric surgery where fibrin sealing of staple lines has not shown superiority\(^{43}\).

The optimal sealant should break down so that no foreign body remains, not hindering the natural healing process. Four large categories of sealants exist\(^{26}\): fibrin sealants, cyanoacrylate sealants, polyethylene glycol sealants, and albumin-based sealants. The majority of clinical experience exists with fibrin sealants: they do not impair wound healing and degrade quickly, however do not have the properties for a watertight seal that can last for at least one week. Cyanoacrylate makes a stronger, watertight seal. However it takes a long time to degrade and most cyanoacrylate sealants are intended only for extracorporeal use in skin approximation. Polyethylene glycol is a non-toxic, biocompatible hydrogel that mechanically bonds to the tissue forming a flexible bond, staying intact from a few days to several weeks. Albumin-based sealants contain formaldehyde (toxic) or glutaraldehyde (non-toxic) polymerizing agents. They are very solid, but increase the risk of causing a strong inflammation reaction, especially when based on formaldehyde. They are mostly used in arterial wall repair.

In this experiment we used sealants out of each of these groups. There is no tissue adhesive that has been developed for sealing of gastro-intestinal anastomoses; the existing sealants we used are intended for vascular sealing, pleural sealing, or arterial wall repair. We believe surgical sealants should be tested in a situation where the risk of an inadequate anastomosis
exists. The perfect anastomosis that will heal without (micro)leaks is not a problem in colorectal surgery, and does not need sealing. Therefore, the tested hypothesis is that if a (micro)defect exists or occurs within the first days, the healing-process can still continue with help of the watertight seal around it. In the model we used we know approximately 60-70% of anastomoses will heal properly, leaving 30-40% of the anastomoses with abscesses or leakage within 7 days. In our opinion this is a correct model for sealant testing: sealants should not make any difference in the anastomoses that heal properly (or potentially make them a bit stronger). However they should prove their use in insufficient anastomoses, for this is the indication for which they are studied.

The fibrin glue used in this study indeed showed no complications related to toxicity, however it did not diminish the risk of AL. Omnex (cyanoacrylate) showed similar incidences of AL and mortality as the control group; however there was also a 40% ileus-rate, showing the glue generates a reaction at the site of the bowel resulting in obstruction. VascuSeal (polyethylene glycol, absorption within 1 week) showed an even higher risk for AL, and also a 40% rate of ileus. PleuraSeal (polyethylene glycol, absorption 4-8 weeks, withdrawn from market) resulted in a 100% death rate, with at least 40% AL and 40% ileus, considering the difficulty of determining cause of death in necropsies. This also applied for BioGlue (albumin-based), showing both AL and ileus, but most of all a 100% death rate. Cardial Colle chirurgicale (albumin-based) is composed of the polymerizing agent formaldehyde. This liquid must be dripped on the polymer without applying it on living tissue due to its reported toxicity\textsuperscript{44}. Two or three drops being sufficient for one cm\textsuperscript{2}, one can imagine that its application is almost impossible in a mouse model. Considering this the experiment with Cardial Colle Chirurgicale was ended after difficult application in the first four mice, out of ethical considerations. Liver damage was seen in two out or four mice on postoperative day 7, and an 80% ileus rate.

In conclusion, the results for each of the included sealants used in this experiment are grim. Until now, most experiments on the sealing of colon anastomosis have large variations in methodology and outcome measures. Some studies have used a normally healing anastomosis, others a model with insufficient anastomosis (less sutures, chemo/radiotherapy, ischemia, peritonitis etc). Majority of studies with a fibrin sealant on a sufficient anastomosis found a higher ABP in the fibrin sealant group\textsuperscript{45-49}. In studies with insufficient anastomoses and fibrin sealant, three studies from Kanellos et al. found less AL and higher ABP with a fibrin sealant\textsuperscript{50-52}, however three studies by van der Ham found no AL in all groups, and no improvement of ABP after fibrin sealing\textsuperscript{49,53,54}. One study with a cyanoacrylate sealing on a sufficient anastomosis found no AL, but more strictures, less ABP, and more inflammation in the cyanoacrylate group\textsuperscript{55}. Three studies with cyanoacrylate and insufficient anastomoses found no differences in AL, ABP was inferior or equal, and there was more inflammation in the cyanoacrylate sealant group\textsuperscript{22,55,56}. One study with a polyethylene glycol sealant and one study with an albumin-based sealant on a sufficient anastomosis found no differences in AL or ABP\textsuperscript{21,57}.

Almost all studies mentioned above are rat studies. Pommergaard et al. showed, in a recent review on experimental anastomotic leakage models, that mouse or pig models seem superior to the rat, since the rat seems less sensitive to infection, therefore creating a leaking anastomosis with clinical consequences is difficult to achieve in the rat\textsuperscript{25}. However, the use of sealants in mice with devices that are made for humans is also considered not ideal. Application
and amount of sealant applied is difficult; therefore rat or pig studies may be superior for this type of research.

This study shows that sealants are unsuccessful in lowering the incidence of colon AL in mice, and that the majority of sealants increase morbidity and mortality, with progressive weight loss of mice and ileus. The exact bowel tissue reaction to different sealants should be investigated with histologic evaluation, to determine which sealants do not impair wound healing and do not cause strictures, thus identifying potential sealants for colorectal surgery for additional research.

In conclusion, if sealing of a colorectal anastomosis could achieve a reduction of incidence of clinical AL this would be a tremendous tool of prevention of leakage in colorectal surgery. In this study we found no evidence that sealants reduce leakage rates in a mouse model for colon AL. However, the negative results of this study make us emphasize the need of systemic research, investigating histologic tissue reaction of the bowel to different sealants, the capacity of sealants to form a watertight barrier, their time of degradation, and finally their results in large animal models for AL.
REFERENCES

Clinical, mechanical and immunohistopathological effects of tissue adhesives on the colon: an in-vivo study


ABSTRACT

Background: Tissue adhesives may be useful for sealing bowel anastomoses by preventing anastomotic leakage. Prior to clinical implementation, an in-depth analysis of the clinical and immunohistopathological effects of tissue adhesives on the target tissue and of the mechanical strength of the adhesive bond in an in vivo model is needed.

Materials and methods: In 84 rats, two bowel segments were glued using one of the following tissue adhesive: Bioglu, Gelatin-resorcinol-formaldehyde (GRF), Glubran 2, Histoacryl Flex, Omnex, Duraseal Xact, or Tissucol. Rats were followed for 7 or 28 days. Endpoints were clinical complication rate, mechanical strength, and immunohistopathological reactions.

Results: Of the seven tissue adhesives, GRF and Bioglu showed the highest rates of bowel wall destruction and ileus and the most severe immunohistopathological tissue reactions at 7 and 28 days. Cyanoacrylates (Histoacryl Flex, Omnex, Glubran 2) showed high mechanical strength and mild immunohistopathological reactions at 7 and 28 days. Duraseal Xact and Tissucol were the most inert tissue adhesives, but exhibited low mechanical strength. At 28 days, mechanical strength was significantly correlated to CD8, CD68, and Ki67 cell counts.

Conclusion: Based on the clinical and immunohistopathological outcomes, GRF and Bioglu were found to be the least suitable tissue adhesives for colonic use. Duraseal Xact and Tissucol were inert but also showed low mechanical strength. Cyanoacrylates exhibited mild clinical and immunohistopathological effects while maintaining high strength, which makes them promising as colonic sealants.
INTRODUCTION

In the field of gastrointestinal surgery, anastomotic leakage rates remain unacceptably high. This is especially true for the colorectal anastomosis, where leakage rates between 5% and 15% are still being reported, with subsequent mortality rates as high as 20%\(^1\)\(^-\)\(^4\). The sealing of an anastomosis (i.e., the surgical connection of two bowel endings by staples or sutures) with a tissue adhesive (TA) has been a focus of surgical research during the past years\(^5\)\(^-\)\(^7\).

Present-day tissue adhesives (TAs) can be divided into four categories based on their chemical composition\(^8\). Cyanoacrylates (CA), the largest TA category, are known to form a rigid and watertight bond and have been recently proposed to be potential candidates for the sealing of bowel anastomoses\(^5\)\(^,\)\(^9\). Fibrin glues (FGs) act as hemostats by enhancing the final stage of the clotting cascade and form a network of fibrin molecules with the adhesive substrate, and have been useful in the sealing of experimental colorectal anastomoses\(^10\)\(^-\)\(^13\). Polyethylene glycol adhesives (PEGs) are flexible hydrogels, primarily used in neurosurgery for sealing the Dura mater\(^14\)\(^,\)\(^15\). Albumin-based (AB) adhesives, including gelatin–resorcinol–formaldehyde (GRF) adhesives, form a strong and flexible adhesive bond with the tissue and are used for the sealing of vascular anastomoses and in aortic surgery\(^16\)\(^-\)\(^18\). A disadvantage of GRF adhesive is that it contains formaldehyde, which has been linked to toxic effects on tissue\(^6\)\(^,\)\(^11\)\(^,\)\(^19\).

In gastrointestinal surgery, FGs are used for staple line sealing after gastric bypass in bariatric surgery\(^20\). Furthermore, recent research reported promising results for the sealing of the esophageal and the pancreatoco-duodenal anastomosis with various TAs, including FG, CA, and PEG adhesives\(^21\)\(^-\)\(^26\). Despite extensive experimental research in the field of colorectal surgery, anastomotic sealing with TAs has not yet been implemented into regular clinical practice. As previously described in the literature, this may be (at least partially) attributed to a lack of methodological consensus between experimental studies, inhibiting the comparison of available experimental data\(^5\)\(^,\)\(^27\)\(^,\)\(^28\).

An in vitro study on the mechanical strength and the rheological properties of 12 clinically relevant TAs was recently performed by the authors\(^8\). The results showed that large differences exist between TAs in terms of mechanical strength, with CAs being the strongest TAs, followed by AB and PEG adhesives. FGs were mechanically the weakest among the tested TAs. Besides mechanical testing, evaluating the clinical and immunohistopathological effects of TAs on the target tissue is imperative prior to clinical implementation. Information on the immunohistopathological effects of TAs on bowel tissue remains scarce. In this study, a set of TAs from all four abovementioned adhesive categories were selected. Short- and long-term clinical effects, immunohistopathological effects, and the mechanical strength of the TA bond on colonic tissue were examined.

METHODS

This study was approved by the ethical committee on animal experimentation, under supervision of the Erasmus University Rotterdam (permit number 105–12-08). Eighty-four specified-pathogen-free male Wistar rats weighing 250–300 g were obtained from a licensed breeder (Charles River Laboratories, MA, USA). 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. Rats were 
scored daily using a validated wellness score to assess the onset of peritonitis.\(^{29}\)

**Tissue adhesives**

Seven TAs were evaluated, as listed in Table 1. These TAs were chosen based on their 
mechanical and rheological profiles as derived from previous in vitro research from the authors, 
and had to be in use clinically.\(^{5}\) In total, 12 rats were included per TA: 6 for short-term (7 days) 
and 6 for long-term (28 days) follow-up. Rat allocation was performed in a randomized manner 
by an independent researcher not otherwise involved in the experiment.

Table 1. Included tissue adhesives.

<table>
<thead>
<tr>
<th>Group</th>
<th>Tissue adhesive</th>
<th>Composition</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Bioglace</td>
<td>Glutaraldehyde-albumin</td>
<td>Cryolife (Kennesaw, GA, USA)</td>
</tr>
<tr>
<td>2</td>
<td>GRF</td>
<td>Gelatin-resorcinol-formaldehyde</td>
<td>Microval (St. Juste Malmont, FR)</td>
</tr>
<tr>
<td>3</td>
<td>Histoacryl Flex</td>
<td>n-butyl-2-cyanoacrylate</td>
<td>B. Braun (Tuttlingen, GER)</td>
</tr>
<tr>
<td>4</td>
<td>Omnex</td>
<td>2-octyl-cyanoacrylate and butyl lactoyl acrylate</td>
<td>Ethicon (J&amp;J, Sommerville, NJ, USA)</td>
</tr>
<tr>
<td>5</td>
<td>Glubran 2</td>
<td>n-butyl-2-cyanoacrylate and methacryloylsulfolane</td>
<td>GEM S.r.l. (Viarregio, IT)</td>
</tr>
<tr>
<td>6</td>
<td>Duraseal Xact</td>
<td>Polyethylene Glycol, trilysine amine, N-hydroxy succinimide, blue dye</td>
<td>Covidien (Mansfield, MA, USA)</td>
</tr>
<tr>
<td>7</td>
<td>Tissucol</td>
<td>Fibrin glue with aprotinin</td>
<td>Baxter (Deerfield, IL, USA)</td>
</tr>
</tbody>
</table>

**Surgical technique**

Rats received analgesia (Rimadylo; 5 mg/kg subcutaneously) preoperatively and were 
anesthetized by isoflurane/oxygen 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, a 1 cm antimesenteric segment of the 
proximal colon was mobilized and placed in direct contact with the serosal surface of the cecum 
and then fixed with two single serosal sutures (Dafilon 8–0, Ethicon, USA), one on each edge of 
the segment. In this manner, a 1 cm tension-free seroso-serosal bowel approximation, that is, 
the “proximal TA bond,” was created, on which the TA was applied. The surgical model is 
illustrated in Figure 1. Next, a distal segment of the ascending colon was mobilized and sutured 
to the descending colon in a tension-free manner following the abovementioned protocol, 
creating a second seroso-serosal approximation: the “distal TA bond.” For each rat, 0.25 mL of 
TA was used per TA. Care was taken to prevent spillage of glue into the abdomen. Sufficient 
curing time was allowed, based on the manufacturers’ guidelines of each TA. The abdominal 
wall was closed in two layers using a continuous suture technique (Safil, 5–0. B. Braun, GER). A 
second dose of Rimadylo was administered 24 h postoperatively.

**Clinical endpoints**

At the end of the follow-up period, rats were anaesthetized, and the abdomen was opened 
using a U-shaped incision. The abdomen was macroscopically inspected for signs of bowel wall 
destruction, that is, the presence of abscess or fecal matter, ileus and adhesion formation. The 
tenacity of the adhesions was graded using the four-degree Zühlke classification, a universally 
accepted classification of adhesions based on surgical adhesiolysis (grade 0: no adhesions; 
grade 1: filmy adhesions, easily separated by blunt dissection; grade 2: stronger adhesions, 
separated by combination of blunt and sharp dissection; grade 3: strong adhesions, sharp
dissection necessary; grade 4: very strong adhesions with organ attachment, sharp dissection with high risk of organ damage\textsuperscript{30}.

![Figure 1. Surgical model. (1) Liver. (2) Cecum. (3) Proximal tissue adhesive bond. (4) Ileum (cut for the sake of clarity). (5) Colon. (6) Distal tissue-adhesive bond.](image)

**Immunohistopathological analysis**

After assessing the clinical endpoints and prior to euthanization by cardiac puncture, the proximal TA site was resected and used for immunohistopathological analysis. All samples were fixed overnight in 4% paraformaldehyde, dehydrated using a graded ethanol series and xylene and subsequently embedded in paraffin, after which 5-mm-thick tissue sections from the paraffin blocks were cut. Automated hematoxylin and eosin (H&E) staining of the slides was performed using the MICROM slide stainer HMS 70 (MICROM International GmbH). Representative slides from each rat were used for immunohistochemical staining for CD4, CD8, CD20, CD68, and Ki67. Table 2 summarizes antibodies, manufacturers, and dilutions.

<table>
<thead>
<tr>
<th>Marker</th>
<th>Meaning of marker</th>
<th>Company</th>
<th>Dilution</th>
<th>Secondary antibody</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4</td>
<td>Specific immuno response, expressed on T-helper lymphocytes</td>
<td>Emelca Bioscience, Breda, Netherlands</td>
<td>1:100</td>
<td>rabbit-anti-rabbit</td>
</tr>
<tr>
<td>CD8</td>
<td>Specific immuno response, expressed on cytotoxic ('Killer') T-lymphocytes</td>
<td>AbD Serotec, Kidlington, United Kingdom</td>
<td>1:200</td>
<td>rabbit-anti-mouse</td>
</tr>
<tr>
<td>CD20</td>
<td>Specific immuno response of B-cells, involved in antibody production</td>
<td>Emelca Bioscience, Breda, Netherlands</td>
<td>1:100</td>
<td>rabbit-anti-rabbit</td>
</tr>
<tr>
<td>CD68</td>
<td>Innate immuno response, expressed on macrophages</td>
<td>AbD Serotec, Kidlington, United Kingdom</td>
<td>1:1000</td>
<td>rabbit-anti-mouse</td>
</tr>
<tr>
<td>Ki-67</td>
<td>Cell-proliferation marker</td>
<td>Monosan, Uden, Netherlands</td>
<td>1:4000</td>
<td>rabbit-anti-rabbit</td>
</tr>
</tbody>
</table>

**Scoring of Hematoxylin and eosin (H&E) and Ki-67**
Upon staining, H&E slides were scored on inflammatory cell infiltration, fibroblast activity, neoangiogenesis, and collagen deposition using the Modified Phillips Scale. In this scale, each of the histological parameters is scored from 0 to 4 as follows: 0 = no evidence; 1 = occasional evidence; 2 = light scattering; 3 = abundant evidence; and 4 = confluent cells or fibers. Furthermore, a general descriptive histological analysis was made per TA. For Ki-67, a cellular marker of proliferation, per rat the image field (10X enlarged) containing the highest concentration of cells was chosen, in which 10 fields were randomly chosen for scoring. Tissue at the TA/tissue interface was scored based on the amount of stained cells as part of the total cell population as: 1 = <5%, 2 = 5-25%, or 3 = >25%. H&E and Ki-67 scoring was performed during a single session in which four of the authors, including an experienced pathologist, evaluated each slide and provided their scores independently while blinded to the type of adhesive used. In case of discrepancies in scoring, slides were re-examined and discussed until consensus was reached.

**Counting of the cells involved in the inflammatory response**

After staining, slides were scanned with a slide scanner (Hamamatsu, Hamamatsu City, Japan). The TA–tissue interface was located on the computer screen and was enlarged 10 times (screen size 1024 X 768 pixels), after which five fields were randomly chosen for cell counting. The average cell count of the five fields was calculated for CD4, CD8, CD20, and CD68 using Image J software (National Institutes of Health, Bethesda, MD, USA).

**Mechanical strength testing**

The distal TA site was resected and, the two approximation sutures were cut without disturbing the TA bond. A custom made 4-mm-wide U-shaped pin was inserted intraluminally into each colonic segment, and then fixed in a tensile strength tester (Testometric, Rochdale, UK, type AX M250-2.5 kN). Tests were performed with a 20 N load cell, at a testing speed of 10 mm/min. No preload was applied. Computer-based analysis software was used to record force data as a function of time and the maximum tensile force was extracted from these data. All mechanical tests were performed directly after resection.

**Statistical analysis**

A Shapiro–Wilks test for normal distribution was performed prior to statistical analysis. Tensile strength data were normally distributed and compared between TA groups using one-way ANOVA and post-hoc Bonferroni multiple comparisons testing. Immunohistological data were non normally distributed and were compared between TA groups using the Kruskal–Wallis one-way analysis-of-variance test, followed by post-hoc Dunn’s multiple comparisons test. A p value of 0.05 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 clinical outcomes is provided in Table 3. At 7 days, macroscopic signs of fecal peritonitis, subsequent to bowel wall destruction at the distal TA site, were seen in two of the 42 rats, both in the GRF group. Furthermore, two cases of mechanical ileus were identified, both in the Bioglue group. GRF showed the largest number of adhesions as compared to the other six TAs. Histoacryl Flex also showed a large number of adhesions, mostly at the distal TA site and between other visceral structures. GRF and Omnex yielded the highest maximum
Zühlke scores (i.e., tenacity of adhesions) for visceral and distal adhesions (GRF) and for adhesions to the proximal glue site (Omnex) when compared to the other TAs.

At 28 days, GRF had the highest complication rate, including one mortality at day 6, bowel wall destruction in two rats, and mechanical ileus in four rats. One rat in the Bioglu group was also found to have bowel wall destruction. All TAs showed numerous adhesions except for Duraseal Xact, which did not lead to any adhesions. As in the short-term group, GRF yielded the highest amount of adhesions and Zühlke scores.

Table 3. Synopsis of clinical outcomes.

<table>
<thead>
<tr>
<th>Tissue adhesive</th>
<th>Number of rats</th>
<th>Fecal peritonitis*</th>
<th>Mechanical ileus*</th>
<th>Adhesions, Proximal**</th>
<th>Adhesions, Distal**</th>
<th>Adhesions to other viscera**</th>
<th>Max. Zühlke score</th>
<th>Mean tensile strength (N (SD))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bioglu</td>
<td>5***</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>4</td>
<td>2</td>
<td>3</td>
<td>1.05 (0.47)</td>
</tr>
<tr>
<td>Histoacryl Flex</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>11</td>
<td>9</td>
<td>2</td>
<td>1.48 (0.46)</td>
</tr>
<tr>
<td>GRF</td>
<td>6</td>
<td>2</td>
<td>0</td>
<td>4</td>
<td>16</td>
<td>13</td>
<td>4</td>
<td>0.38 (0.33)</td>
</tr>
<tr>
<td>Duraseal Xact</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.39 (0.20)</td>
</tr>
<tr>
<td>Glubran 2</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.64 (0.39)</td>
</tr>
<tr>
<td>Tissucol</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.06 (0.07)</td>
</tr>
<tr>
<td>Omnex</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>3</td>
<td>0.58 (0.24)</td>
</tr>
<tr>
<td>Bioglu</td>
<td>6</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>3</td>
<td>2.26 (1.12)</td>
</tr>
<tr>
<td>Histoacryl Flex</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>7</td>
<td>0</td>
<td>2</td>
<td>1.83 (0.46)</td>
</tr>
<tr>
<td>GRF</td>
<td>6</td>
<td>2</td>
<td>4</td>
<td>13</td>
<td>16</td>
<td>13</td>
<td>4</td>
<td>4.25 (1.29)</td>
</tr>
<tr>
<td>Duraseal Xact</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>0.00 (0.00)</td>
</tr>
<tr>
<td>Glubran 2</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>4</td>
<td>4</td>
<td>2</td>
<td>2.56 (0.24)</td>
</tr>
<tr>
<td>Tissucol</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>0.07 (0.16)</td>
</tr>
<tr>
<td>Omnex</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>5</td>
<td>0</td>
<td>1</td>
<td>1.80 (0.31)</td>
</tr>
</tbody>
</table>

*Number of affected rats.
**Amount of adhesions.
***One rat in this group died perioperatively following anaesthesia-related complications.

Mechanical strength

Results of the tensile strength tests are summarized in Table 3. Shapiro–Wilk testing for normal distribution found that mechanical strength data was normally distributed (p = 0.07). One-way ANOVA testing showed significant differences in tensile strength between TAs at both 7 (F(6,33) = 11.5, p < 0.001) and 28 days (F(6,32) = 28.1, p < 0.001). At day 7, Histoacryl Flex was stronger than all other TAs (all p values < 0.003) except Bioglu (p = 0.855), whereas Tissucol exhibited the lowest tensile strength, significantly lower than Bioglu (p = 0.001) and Histoacryl Flex (p < 0.001).

At day 28, the strongest TA was GRF, which was statistically higher than all other TAs (p < 0.01), whereas the weakest TAs were Duraseal Xact and Tissucol, which both were significantly weaker than all other TAs (p < 0.01). There was no significant correlation between the tensile
strength of the adhesives at day 7 and day 28. When excluding the tensile strength data of the sealant category, which were in an outlying lower range when compared to the other adhesives, a significant negative significant correlation between the tensile strength at day 7 and day 28 ($r = 0.2044$, $p = 0.023$) was found.

**Histological and Immunohistochemical analysis**

A descriptive summary of the histological results of each TA is provided in Table 4. An overview of the cell counts per immunological marker is provided in Figure 2(a,b) (at 7 and 28 days, respectively). At day 7, Ki-67, a marker of cell proliferation, was highest in Tissucol and lowest in the CA s Histacryl Flex and Omnex, though no significant differences between TAs were found between groups ($F(6,33) = 11.1$, $p = 0.087$). At 28 days, GRF showed the highest Ki-67 count and Tissucol, Duraseal Xact and Omnex the lowest. At 28 days, significant differences were found between the seven TAs ($F(6,32) = 14.9$, $p = 0.021006$); however, no significant differences remained after post-hoc testing.

Kruskal–Wallis one-way analysis of variance testing revealed significant differences for all the remaining histopathological analyses at day 7 and day 28 ($p < 0.001$ in all cases). Results of post-hoc analyses by Dunn’s multiple comparisons test are shown below. For CD4, a marker of T-helper cells, Bioglue and Histacryl Flex showed the highest cell counts when compared to Tissucol at day 7 ($p = 0.004$ and $p = 0.037$, respectively). CD4 reaction at day 28 was highest in Omnex, significantly higher than in Tissucol ($p = 0.001$). CD8, a marker of cytotoxic T-cells, was found to be the highest for GRF at day 7, significantly higher than Duraseal Xact ($p = 0.004$), Glubran 2 ($p = 0.003$), and Tissucol ($p = 0.002$). At 28 days, GRF maintained the highest CD8 count, significantly higher than Tissucol ($p = 0.001$). For CD20, a marker of B-cell response, Bioglue and GRF showed the highest cell counts, both significantly higher than Omnex at 7 days ($p = 0.001$ and $p = 0.003$, respectively) and 28 days ($p = 0.01$ and $p = 0.004$, respectively). At 28 days, Glubran 2 also showed high CD20 counts, significantly higher than Omnex ($p = 0.023$). Lastly, CD68 score, a marker of macrophage response, was found to be the highest in Bioglue at day 7, significantly higher than Duraseal Xact ($p = 0.006$) and Glubran 2 ($p = 0.007$). At 28 days, it was GRF that showed the highest CD68 count, significantly higher than Duraseal Xact ($p = 0.015$) and Tissucol ($p < 0.001$). Glubran 2 and Bioglue were, in turn, both significantly higher than Tissucol ($p = 0.041$ and $p = 0.031$, respectively).
Table 4. Descriptive histopathological analysis. Illustrated per follow-up time point and amount of magnification. T: Tissue adhesive (*: Remnant) I: Inflammatory response. C: Collagen formation. **: Visible at higher magnification.

<table>
<thead>
<tr>
<th>TA</th>
<th>7d. 1.25x</th>
<th>7d. 5x/10x</th>
<th>28d. 1.25x</th>
<th>28d. 5x/10x</th>
<th>Report</th>
</tr>
</thead>
</table>
| Biogluce | ![Image](image1.png) | ![Image](image2.png) | ![Image](image3.png) | ![Image](image4.png) | 7d: Acute inflammatory reaction to TA with peritonitis and muscle lysis, extending into the submucosal layer. Some giant cells are present**.  
28d: Deep bowel wall necrosis and leakage of bowel contents, causing a chronic inflammatory reaction. |
| Duraseal | ![Image](image5.png) | ![Image](image6.png) | ![Image](image7.png) | ![Image](image8.png) | 7d: Local inflammatory reaction to TA remnants. Presence of plasma cells and neutrophils**. Degradation of TA evokes second stage inflammatory response. Inert TA.  
28d: Local inflammatory reaction, no signs of ongoing response. Limited macrophage/giant cell mediated foreign body reaction**. |
| Glubran 2| ![Image](image9.png) | ![Image](image10.png) | ![Image](image11.png) | ![Image](image12.png) | 7d: Extended inflammatory response and local bowel wall necrosis, until the submucosal layer.  
28d: Extended inflammatory response and local bowel wall necrosis, until the submucosal layer. |
| GRF      | ![Image](image13.png) | ![Image](image14.png) | ![Image](image15.png) | ![Image](image16.png) | 7d: Acute inflammatory response with complete bowel wall necrosis. TA is scattered around the examined area.  
28d: Neutrophilic granulocyte acute response**. Large amounts of toxicity and macrophagic phagocytosis. Bowel wall necrosis until the lamina propria. |
| Histoacryl Flex | ![Image](image17.png) | ![Image](image18.png) | ![Image](image19.png) | ![Image](image20.png) | 7d: Local inflammatory reaction to TA, no muscle lysis  
28d: No inflammation or reaction to TA. Neangiogenesis present, indicating ongoing healing. Inert TA. |
| Omnex    | ![Image](image21.png) | ![Image](image22.png) | ![Image](image23.png) | ![Image](image24.png) | 7d: Limited local inflammatory reaction. A few inflammatory cells at TA. Inert TA.  
28d: Limited local inflammatory reaction. Few inflammatory cells at TA. Inert TA. |
| Tissucol | ![Image](image25.png) | ![Image](image26.png) | ![Image](image27.png) | ![Image](image28.png) | 7d: Tendency of cell encapsulation into TA and subsequent scattering of TA. Very inert TA.  
28d: TA is completely dissolved. No ongoing reaction. Very inert TA. |
Figure 2. Immunohistochemical analysis at (a) 7 and (b) 28 days.

Correlation analysis between tensile strength and immunohistochemistry
At 7 days, tensile strength correlated significantly only with CD4 ($r = 0.50, N = 7, p = 0.001$) and CD8 ($r = 0.39, N = 7, p = 0.014$) counts. At 28 days, tensile strength was significantly correlated to CD8 ($r = 0.48, N = 7, p = 0.003$), CD68 ($r = 0.68, N = 7, p = 0.001$), and Ki67 (Ranked transformed Spearman correlation; $r = 0.50, N = 7, p = 0.001$), indicating that high tensile strength was associated with a more severe response of CD4 and partly CD8 positive T-cells to the tissue adhesive. Correlation analysis between the short-term tensile strength and the long-term immunohistopathological outcomes of each tissue adhesive yielded no significant outcomes.

DISCUSSION
Sealing of colonic anastomoses with tissue adhesives (TAs) has been proposed as a promising new technique for preventing leakage of intraluminal contents through a (technically)
insufficient anastomosis into the abdominal cavity. In this study, a comparative analysis of clinical, mechanical, and immunohistopathological endpoints of seven commercially available TA was performed, in a new experimental model that enables gluing two separate bowel segments per rat, while maintaining anatomical configuration and functionality during the follow-up period. By applying the TAs between two serosal surfaces without the presence of a bowel anastomosis, it was possible to observe the effects of the TA without confounding factors such as operative technique and anastomotic complications, thus providing a clear picture of the direct effects of the use of TA on the colon, information crucial to the understanding of the effectiveness and future clinical use of TAs in visceral surgery.

**Clinical effects of TAs**

Bioglue was associated with a higher rate of mechanical ileus (MI) compared to the other TAs, a finding which has previously been reported after use on mouse colonic anastomoses. GRF use was associated with the only cases of bowel wall destruction and, subsequently, fecal peritonitis at day 7. Furthermore, at day 28, most complications were attributed to GRF use, which led to a higher incidence of mechanical ileus and bowel wall destruction than with the other TAs. These findings are in line with previous research on GRF, which reported toxicity after application of GRF on the bowel. Of the seven TAs evaluated in this study, GRF showed the highest amount of adhesion formation. This finding may be explained by the severity of bowel wall destruction, mostly leading to fecal peritonitis and a subsequent strong inflammatory response, as seen in the immunohistochemical results. Histoacryl Flex showed more adhesion formation and higher tensile strength when compared to the other CAs at 7 days, while no differences remained at 28 days. This may indicate that the early adhesion formation seen in Histoacryl Flex depends on its strong adhesive bond to the surrounding tissue rather than collagen formation by the host, as was seen in the other TAs. The sealants Tissucol and Duraseal Xact showed the smallest amount of adhesions and the lowest complication rates at both 7 and 28 days, indicating safe use on the bowel surface. These findings are in line with previous research on the use of PEG adhesive.

**Tensile strength**

At both 7 and 28 days, CAs generally showed high tensile strength with a small standard deviation. The tissue sealants Duraseal Xact and Tissucol exhibited the lowest mechanical strength in this study. Duraseal Xact was completely dissolved at 28 days in all rats, resulting in a tensile strength of nil. FG also showed low tensile strength in this study, a finding that contradicts previous research in which sealing of rat colonic anastomosis with FG yielded high anastomotic bursting pressure, possibly due to the lack of a bowel defect and thus a TA bond directly on the serosal surface of the colon. In general, mechanical strength was higher at 28 days than at 7 days, indicating that the strength of a TA bond grows after initial application. GRF showed high tensile strength at 28 days, much higher than any other TA. This finding may be explained by extensive adhesion formation, as indicated by the high Zühlke scores that were observed with GRF. When comparing the in vivo results in this article to the in vitro results previously published by the authors, it was found that in vitro mechanical strength was higher than the 7 days tensile strength measurements for all TAs and lower than the mechanical strength at 28 days. This finding infers that adhesive degradation may start sooner than expected after intracorporeal application.
\textit{Histopathology}

Previous studies have primarily focused on the histopathology of FG and CA. Several authors reported promising effects of FG sealing of colonic rat anastomoses in which FG use led to only mild inflammatory reaction and no tissue toxicity\textsuperscript{10,12}. Regarding CA, early studies pointed out that long-chain CA formulations elicited an exothermic reaction leading to a severe inflammatory tissue response\textsuperscript{34}. However, present-day short-chain CA formulations have become more inert, indicating safe use intracorporeally\textsuperscript{35,36}. Information on the histological effects of the other TA categories on colonic tissue is scarce. A study by Yol et al. reported a higher infiltration of inflammatory cells, collagen, and fibroblasts for Biogluce applied on a rat colonic anastomosis compared to the use of platelet-rich plasma, a hydrogel which is thought to promote tissue healing\textsuperscript{37}.

In this study, Biogluce and GRF induced the most severe inflammatory reaction of all tested TAs. Of the CAs, Glubran 2 induced an extended inflammatory response with mild local muscle lysis as deep as the submucosal colonic layer. This finding was unexpected, as the chemical composition of Glubran 2 (n-butyl-2-cyanoacrylate/methacryloxyasulfolane) does not differ considerably from either Histoacryl Flex (nbutyl- 2-cyanoacrylate) or Omnex (n-octyl-cyanoacrylate/butyl lactoyl acrylate), which were both histologically inert. The mild toxic effects of Glubran 2 may possibly be attributed to methacryloxyasulfolane, an additive in Glubran 2 that increases flexibility; this finding remains, however, unclear. Histoacryl Flex induced a limited local host reaction without tissue necrosis, with subsequent neoangiogenesis seen at day 28, indicating that this CA is relatively inert, without toxic effects on the bowel. The same can be stated for Omnex, which elicited a local and mild inflammatory response. The sealants, Duraseal Xact and Tissucol, were the most inert adhesives. Duraseal Xact caused a second inflammatory response after the initial degradation of the adhesive, which became apparent at day 28. This finding has not been observed in earlier research on PEG\textsuperscript{38}. This indicates that byproducts created through degradation of this TA elicit a more intensive tissue reaction than the response to the initial adhesive layer. This effect was, however, not clinically relevant as can be seen from the clinical and pathological evaluation. Tissucol showed an inflammatory response that was different to the other adhesives, with the body’s host reaction encapsulating parts of the adhesive and cleaning these up rapidly before day 28, at which time Tissucol was almost completely dissolved. Note that in Tissucol, aprotinin is added to increase degradation time, meaning that in other FGs without aprotinin, the adhesive layer may dissolve even faster, possibly resulting in lower tensile strength.

\textit{Immunohistochemistry}

This is the first study on TAs that implements the use of immunohistochemistry to aid in the understanding of the clinical and histological effects of TAs. CD4, CD8, and CD20 counts were evaluated, which indicate presence of T-helper cells, T-cytotoxic lymphocytes, and B-cells in the inflammatory response infiltrate, respectively. These cells play an important role in the response of the adaptive immune system of the host, and, more importantly in this study, in the regulation of the inflammatory response, which, in turn, affects the degradation of the TA. CD68 stains the macrophages, which have the double role of regulating the intensity of the inflammatory response as well as contributing in the healing process with the formation of collagen, an important contributor to long-term tensile strength. Lastly, Ki-67 indicates the rate of cell proliferation, which in this study is most likely linked to the intensity of wound healing or ongoing inflammatory reaction. When taking all parameters into account, it was found that
the most severe inflammatory reaction was seen with Biogluce and GRF. At day 7, Biogluce showed the highest scores for CD4, CD20, and CD68, indicating that this TA was associated with the most intense short-term inflammatory response. This may be due to a direct toxic effect on the bowel surface, or to the initial degradation of the adhesive into toxic by-products. The large amount of CD4-positive T cells and CD68-positive macrophages indicate that there was a more intense inflammatory response directed to this TA. The macrophages can, in turn, stimulate collagen formation, aiding in the high tensile strength found in Biogluce.

Interestingly, the number of CD20-positive B-cells was also found to be high in both Biogluce and GRF at day 7. These B-cells possibly also play a regulatory role in the inflammatory response. Moreover, there may be a relationship between allergic reactions of the host and these TAs involving the adaptive immune system, but this remains outside the scope of this article. The cell proliferation marker Ki67 was found to be the highest in Tissucol at 7 days. Taken together with its inert tissue reaction, this may indicate that physiological tissue healing may take place early on in the presence of this TA. At 28 days, GRF showed an ongoing (chronic) inflammatory response. This is confirmed by a high amount of CD4, CD8, CD20, and CD68. Also Ki67 was highest in this TA, and points toward high cell proliferation as the result of the chronic inflammatory response. Omnex induced an isolated CD4 response higher than the other adhesives, also higher than that of the other CAs, a finding that remains unclear.

**Study limitations and implications for future research**
This study evaluated the effect of TA on intact bowel, without the presence of a defect, as would be the case after the creation of a bowel anastomosis. This aspect should be examined in future research. Moreover, the combination of TA use in the presence of a stapled colon anastomosis remains an interesting aspect for future research.

**CONCLUSION**

In this study, the use of a new experimental rat model was implemented for the comparative analysis of clinical effects, mechanical strength, and inflammatory response of a clinically relevant set of surgical tissue adhesives. Clinical complications were found only for GRF and Biogluce at both short- and long-term endpoints. Tensile strength analysis showed that the CA Histoacryl Flex was the strongest TA at 7 days, while GRF was the strongest at 28 days. Histopathological evaluation was in line with the clinical findings, with Biogluce and GRF eliciting the most severe inflammatory response and inducing bowel wall necrosis. Glubran 2 showed mild local muscle lysis in some cases while the other CAs and sealants were inert. The immunohistochemical findings correlated with TA tensile strength at 28 days. From this study, it seems that an optimal TA should elicit a minimal to moderate immune response to initiate high tensile strength without presence of an ongoing inflammatory response and subsequent clinical complications. These parameters were found in the included CAs, in particular in Histoacryl Flex and Omnex.
REFERENCES


Clinical, mechanical and immunohistopathological effects of tissue adhesives on the colon

PART IV

Prevention of colorectal anastomotic leakage with tissue adhesives
Sutureless closure of colonic defects with tissue adhesives: an in-vivo study in the rat


Chapter 8

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 0.5-cm incisions were created, one in the proximal and another in the distal colon. Incisions were sealed with a TA: Histoacryl Flex, Biogluve, 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, Biogluve, 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.
INTRODUCTION

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 four categories based on their chemical composition: cyanacrylates (CAs), fibrin glues (FGs), polyethylene glycol (PEG) adhesives and, lastly, 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 pancreatico-duodenal anastomosis with PEG adhesives and FGs may decrease anastomotic leakage 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. Rheological 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 out of the 11 TAs were used to glue the serosal surface of two intact (e.g., 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)histopathological analysis pointed towards large differences between TAs, with the biological TAs (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)histopathological 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 seven 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 two
months old weighing 250–300 grams were obtained from a licensed breeder (Charles River Laboratories, MA, USA). 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 one week was observed prior to the start of the experiment. Rats were scored daily using an adapted wellness score to assess the onset of peritonitis. 

We evaluated seven 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 mmHg (6) in bursting pressure between the different experimental groups at day 3. With a standard deviation of 20 mmHg and an alpha of 0.05, for a power of 80%, 10 rats were needed per group. All TAs except GRF and Glubran 2 were approved by the U.S. food and drug administration at the time of the study and were used in an off-label manner for the purposes of the current 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. 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, anastomotic leakage, especially when due to technical factors and to differences in colonic perfusion would have played a confounding role in the evaluation of TAs.

<table>
<thead>
<tr>
<th>Group</th>
<th>Tissue adhesive</th>
<th>TA Category</th>
<th>Composition</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>None</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1</td>
<td>Biogluce</td>
<td>BT</td>
<td>Glutaraldehyde-albumin</td>
<td>Cryolife (Kennesaw, GA, USA)</td>
</tr>
<tr>
<td>2</td>
<td>GRF glue</td>
<td>CA</td>
<td>Gelatin-resorcinol-formaldehyde</td>
<td>Microval (St. Juste Malmont, FR)</td>
</tr>
<tr>
<td>3</td>
<td>Histoacryl Flex</td>
<td>CA</td>
<td>n-butyl-2-cyanoacrylate</td>
<td>B. Braun (Tuttingen, GER)</td>
</tr>
<tr>
<td>4</td>
<td>Omnex</td>
<td>CA</td>
<td>2-octyl-cyanoacrylate/ butyl lactoyl cyanoacrylate</td>
<td>Ethicon (J&amp;J, Sommerville, NJ, USA)</td>
</tr>
<tr>
<td>5</td>
<td>Glubran 2</td>
<td>CA</td>
<td>n-butyl-2-cyanoacrylate and methacryloxy sulfolane</td>
<td>GEM S.r.l. (Viarregio, IT)</td>
</tr>
<tr>
<td>6</td>
<td>Duraseal Xact</td>
<td>PEG</td>
<td>Polyethylene Glycol with N-hydroxy succinimide</td>
<td>Covidien (Mansfield, MA, USA)</td>
</tr>
<tr>
<td>7</td>
<td>Tissucol</td>
<td>FG</td>
<td>Fibrin glue with aprotinin</td>
<td>Baxter (Deerfield, IL, USA)</td>
</tr>
</tbody>
</table>
Surgical technique
Figure 1 depicts the surgical model. Rats received analgesia (Temgesic; 0.05 mg/kg subcutaneously) preoperatively and were anaesthetized by isoflurane/O₂ 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 0.5-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 histopathological testing, and the distal segment for bursting pressure testing. Afterwards, the wound edges of each incision were approximated, enabling TA application over the full length of the defect. For each rat, 0.05 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 two layers using a continuous suture technique (Safil, 5-0. B. Braun, GER). 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.](image)

Clinical outcomes
At the end of the follow-up time of 3 or 10 days, or upon the onset of clinical signs of peritonitis based on the abovementioned 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 intra-peritoneal abscess or fecal matter and ileus formation. The Zühlke score, which depicts the tenacity of intra-abdominal adhesions was also determined. Each animal was euthanized by cardiac puncture upon completion of the experimental protocol.
**Bursting pressure 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 bursting pressure was recorded for each rat.

**Histopathological analysis**
Prior to sacrifice, the proximal incision site was resected and used for histopathological analysis. All samples were processed with standard histopathological techniques resulting in 5-μm hematoxylin and eosin (HE)-stained sections for evaluation. Upon staining, HE slides were scored for inflammatory cell infiltration, fibroblast activity, neoangiogenesis and collagen deposition using the Modified Phillips Scale \(^21\). In this scale each of the histological parameters is scored from 0 to 4 (0= no evidence; 1= occasional evidence; 2= light scattering; 3= abundant evidence and 4= confluent cells or fibres). H&E scoring was performed during a single session using a multiple-head microscope, in which three of the authors (KV, ZW, KL), including an experienced pathologist (KL), 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 re-examined and discussed until consensus was reached.

**Statistical analysis**
For the clinical and histological 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 0.05 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 (\(p < 0.001\) \(\chi^2 (7, 71) = 45.45\); post-hoc analysis: all \(p\)-values between the control group and the TA groups < 0.001) and did not differ significantly between the TA groups. Also fecal peritonitis rate at day 3 was higher for the control group (\(p < 0.001, \chi^2 (7, 70) = 35.74\); post-hoc analysis: all \(p\)-values between the control and the TA groups ranging between 0.0001 and 0.031, expect for GRF: \(p = 0.101\)). 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 (\(p = 0.002\)).
Table 2. Synopsis of clinical outcomes. Numbers depict total amount of rats per specified outcome. GRF = gelatin-resorcinol-formaldehyde.

<table>
<thead>
<tr>
<th>Follow-up</th>
<th>Study group</th>
<th>N</th>
<th>Premature mortality**</th>
<th>Leakage (Fecal peritonitis, Abscess)</th>
<th>Mechanical ileus</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Short term (3d)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>10</td>
<td>7</td>
<td>9 (8,1)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Bioglace</td>
<td>10</td>
<td>0</td>
<td>10 (0,10)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Histoacryl Flex</td>
<td>10</td>
<td>0</td>
<td>7 (0,7)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Omnex</td>
<td>9*</td>
<td>0</td>
<td>6 (1,5)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Glubran 2</td>
<td>10</td>
<td>0</td>
<td>4 (0,4)</td>
<td>0</td>
<td></td>
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<tr>
<td>Duraseal Xact</td>
<td>10</td>
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<td>5 (2,3)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>GRF</td>
<td>10</td>
<td>0</td>
<td>7 (3,4)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Tissucoll</td>
<td>10</td>
<td>0</td>
<td>7 (0,7)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td><strong>Long term (10d)</strong></td>
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<td></td>
<td></td>
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<tr>
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<td>4</td>
<td>7 (4,3)</td>
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<td></td>
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<tr>
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<td>10</td>
<td>2</td>
<td>5 (2,3)</td>
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<tr>
<td>Omnex</td>
<td>9*</td>
<td>0</td>
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<tr>
<td>Glubran 2</td>
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<td>0</td>
<td>2 (0,2)</td>
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<tr>
<td>Duraseal Xact</td>
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<td>3 (3,0)</td>
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<td>10</td>
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<td>10</td>
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<td>0</td>
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<td></td>
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</tbody>
</table>

* Died perioperatively due to anesthetic complications  
** Mortality occurring prior to the completion of the follow-up time (either 3d or 10d)

At day 10, no significant differences were found in mortality rates between groups (p = 0.07, χ² (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 = 0.018) and Tissucol (p = 0.025). Post-hoc analysis showed no further differences between groups for either fecal peritonitis or rate of abscess formation.

Figure 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 = 0.010, χ²(7,63) = 18.39) and the Zühlke score of proximal (p = 0.001, χ²(7,63) = 25.90) and distal adhesions (p = 0.010, χ²(7,63) = 18.41), but not for the number of distal adhesions (p = 0.524, χ²(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 = 0.004), GRF (p = 0.009), Bioglace (p = 0.013), and Histoacryl Flex (p = 0.046), and the higher Zühlke score of distal adhesions for Bioglace as compared to Glubran 2 (p = 0.031).
At 10 days significant differences were found between groups for the number of proximal \( (p < 0.001, \chi^2(7,60) = 32.53) \) and distal adhesions \( (p = 0.007, \chi^2(7,60) = 19.28) \), but not for the corresponding Zühlke scores (proximal: \( p = 0.100, \chi^2(7,60) = 12.02 \); distal site: \( p = 0.037, \chi^2(7,60) = 14.91 \)). The most prominent result was that Duraseal Xact exhibited a higher number of proximal adhesions as compared to Glubran 2 \( (p < 0.001) \), Omnex \( (p = 0.004) \), and Histoacryl Flex \( (p = 0.007) \).

**Bursting pressure (BP)**

BP of the distal incision site is illustrated in Figure 3. At 3 days, no significant differences were observed between groups \( (p = 0.153, F(7,57) = 1.62) \). At 10 days, significant differences were observed \( (p < 0.001, F(7,57) = 9.42) \), with Histoacryl Flex, Glubran 2 and Tissucol being the three strongest TAs. Specifically, Tissucol was stronger than GRF \( (p = 0.004) \), Biogluce \( (p = 0.007) \), and Duraseal Xact \( (p = 0.010) \) and Glubran2 was stronger than GRF \( (p = 0.029) \) and Biogluce \( (p = 0.047) \). Histoacryl Flex was stronger than GRF \( (p = 0.039) \).

**Histopathology**

Analysis of histopathological data is illustrated in Figure 4. At day 3, no significant differences were observed between groups for any of the analysed parameters, except that Biogluce exhibited a lower inflammation rate than Tissucol \( (p = 0.030) \). At day 10, Histoacryl Flex exhibited a higher amount of collagen formation than Duraseal Xact \( (p = 0.002) \), Biogluce \( (p = 0.034) \) and Tissucol \( (p = 0.040) \).
Sutureless closure of colonic defects with tissue adhesives

Figure 3. Mean BP at day 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.

COMMENTS

The sealing of colonic anastomoses with tissue adhesives (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 study we included seven 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 histological 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\textsuperscript{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 focussing 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\textsuperscript{22,25}. At day 10, the best performing TAs in the current 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. Importantly, the control group and the TA groups were associated with different presentation of bowel leakage. In the control group, as expected, the majority of 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\textsuperscript{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\textsuperscript{27,28}. Based on the results of the current study as well as previous literature, Duraseal Xact does not seem suitable as a colonic sealant\textsuperscript{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\textsuperscript{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\textsuperscript{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.

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

**Histopathology**

Naturally, the histopathological data in this study were influenced by a combination of the foreign body reaction of each TA after tissue application, as well as the inflammation brought on by the leakage of bowel content through the defect. It is worth noting that Bioglu, 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 Bioglu 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 days follow up. 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 following a bowel defect. It should be noted that histopathological 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.

Lastly, 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 \textsuperscript{32-34}.

CONCLUSION

Prior to the clinical use of tissue adhesive (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. Cyanoacrylate TAs generally seem to prevent the onset of fecal peritonitis by stopping bowel leakage from spreading into the abdominal cavity. Biological (Biogluce, GRF) and PEG adhesives (Duraseal Xact) were associated with the most leakage-related complications and low mechanical strength, making these tissue adhesives unsuitable for use as colonic sealants. In this study, the cyanoacrylates Histoacryl Flex, Glubran 2 and Omnex, as well as the Fibrin glue Tissucol showed the most promising results, combining fewer leakage-related complications compared to the other TAs, while maintaining high mechanical strength and an inert histological 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 prior to clinical implementation.
REFERENCES

The prevention of colorectal anastomotic leakage with tissue adhesives in a contaminated environment is associated with the presence of anti-inflammatory macrophages

Zhouqiao Wu, Konstantinos A Vakalopoulos, Geesien SA Boersema, Leonard F Kroese, King H Lam, Paul H van der Horst, Irene M Mulder, Yvonne M Bastiaansen-Jenniskens, Gert-Jan Kleinrensink, Johannes Jeekel, Johan F Lange

ABSTRACT

**Background:** Colorectal anastomoses created in a contaminated environment result in a high leakage rate. This study investigated whether using anastomotic sealants (TissuCol®, Histoacryl® Flex, and Duraseal®) prevents leakage in a rat peritonitis model.

**Study Design:** Sixty-seven Wistar rats were divided into control and experimental groups (TissuCol-, Histoacryl-, and Duraseal-group). Peritonitis was induced one day before surgery with the caecal ligation puncture model. On day 0, colonic anastomosis was constructed with sutures and then sealed with no adhesive (control group) or one select adhesive (experimental groups). Bursting pressure, abscess formation and adhesion severity were evaluated on day 3 or day 14. Hematoxylin and eosin staining and immunohistochemical staining for CD4, CD8, CD206 and iNOS were performed.

**Results:** On day 3 bursting pressures of the TissuCol-group (120.1±25.3 mmHg), Histoacryl-group (117.3±20.2 mmHg), and Duraseal-group (123.6±35.4 mmHg) were significantly higher than the control-group (24.4±31.7 mmHg, p<0.001). Abscesses around the anastomosis were found in the control-group (6/7) and Duraseal-group (2/9), but not in the TissuCol-group or Histoacryl-group. A higher number of CD206+ cells (M2-macrophages), a lower number of iNOS+ cells (M1-macrophages), a higher M2/M1 index, and a higher CD4+/CD8+ index were seen at the anastomotic site in all experimental groups compared with the control group on day 3. On day 14 abscesses were only found in the control group. Adhesion severity in the Duraseal-group was significantly lower than that in the control group (p=0.001).

**Conclusions:** Anastomotic sealing using TissuCol®, Histoacryl® Flex, or Duraseal® seems to be an effective and safe option to prevent leakage in contaminated colorectal surgery. The presence of large numbers of anti-inflammatory macrophages seems to be involved in preventing the leakage.
INTRODUCTION

Under certain conditions such as abdominal trauma or perforation in diverticulitis and colorectal carcinoma, emergency surgery is initiated in order to repair bowel defects in a contaminated environment. Instead of a Hartmann’s procedure for perforated diverticulitis, primary anastomosis has become a well-accepted intervention in selected patients [1-4], resulting in similar or even better clinical outcomes regarding postoperative mortality and complication rates than Hartmann’s procedure [1, 5, 6]. However, performing primary anastomosis in a contaminated environment is challenging and still causes substantial leakage [7-9], especially in urgent situations. Patients with perforated diverticulitis who underwent primary anastomosis alone suffered a leakage rate of 19.3% [10], which is much higher than the leakage rate of approximately 9% following a low anterior resection for rectal cancer [11].

Intra-abdominal sepsis induces nitric oxide production at the anastomotic site, which activates substantial inflammatory responses and subsequently impairs the collagen synthesis thereby delaying anastomotic healing [12, 13]. Macrophages are one of the main factors in the inflammatory response, and based on their behavior this response is either pro-inflammatory (M1) impairing wound healing or anti-inflammatory (M2) promoting wound healing; other immune cells such as T lymphocytes, though not fully understood, were also reported to be involved in the response [14]. Interestingly, the deleterious influence of inflammation is localized on the anastomosis and does not affect new collagen synthesis in the uninjured colon, where the biological barrier is intact [12]. This suggests that using a tissue adhesive or sealant as an artificial barrier to obstruct contact between intra-abdominal pathogens and anastomosis may reduce the deleterious effects of inflammation, thus preventing anastomotic leakage. Among different tissue adhesive compounds, fibrin glue and cyanoacrylate glue have been substantially investigated in both experimental and clinical studies with promising results [15-17]. Other sealants, such as polyethylene glycol glue, also had satisfactory results as an adhesion barrier system [18]. These tissue adhesives have been tested in different environments [17, 19, 20], however knowledge regarding their influence on anastomotic healing in a contaminated environment is still limited. We therefore conducted an experiment under conditions of peritonitis induced by the rat caecal ligation puncture (CLP) model that has been used in previous studies [21, 22]. Colonic anastomoses were constructed and sealed with select tissue adhesives. This study aimed to investigate the influence of the select tissue adhesives on anastomotic healing, and to determine whether they are safe and effective solutions to prevent anastomotic leakage under contaminated conditions.

METHODS

Animals
Male Wistar rats, weighing 250-350 grams, were purchased from a licensed breeder (Harlan Laboratories, Boxmeer, the Netherlands). All rats were bred under specific pathogen-free conditions, and kept under standard laboratory conditions. Standard rat chow and water were supplied ad libitum. The research protocols of all experiments were approved by the Ethical Committee on Animal Experimentation of Erasmus University Rotterdam.
Peritonitis model
To induce peritonitis, the rat caecal ligation puncture (CLP) model was used [21, 22]. In brief, rats were anaesthetized with isoflurane/oxygen inhalation, and the abdomen was opened through a midline incision. Then, the caecum was ligated distally to the ileocecal valve with a non-absorbable nylon suture (Ethilon 4-0, Ethicon, Somerville, USA), maintaining the continuity of the bowel. The distal caecum was punctured once with an 18-gauge needle, and was gently compressed until feces were extruded. The abdomen was closed with two layers of running sutures (Safil 5-0, B Braun, Melsungen, Germany). Wellness of all rats was evaluated during the follow-up, and animal with severely compromised wellness (i.e. ceased food intake, circulatory or respiratory difficulty, severe weight loss, severely abnormal locomotion) would be euthanized and examined prematurely for humane endpoint.

Surgical technique and follow up
Twenty-four hours later, the rat was anesthetized again. The abdomen was reopened, and a culture swab was taken to confirm peritonitis. After that, 6 mg/kg of gentamicin (Centrafarm, Etten-Leur, The Netherlands) was injected intramuscularly. The ligated caecum was resected, the abdominal cavity was rinsed with at least 20 mL phosphate buffered saline (PBS, 37°C), and colorectal anastomosis was performed afterwards. A colon segment of 1 cm in length was resected approximately 3 cm proximally to the peritoneal reflection. An end-to-end one-layer continuous anastomosis was constructed in an inverted fashion with Dafilon 8-0 (B. Braun, Melsungen, Germany). One researcher (ZW) performed all anastomoses under microscopic vision enhancement. Following that, one tissue adhesive was selected and applied at two parts: at the descending colon around the anastomosis as a sealant (distal segment), and 1 cm in length at the beginning of the ascending colon (proximal segment). The tissue adhesives were prepared according to the instruction manuals. Because all tissue adhesive were designed for human patient with much larger amount of adhesive than the amount we applied on rats, one tissue adhesive was randomly chosen on the operation day and reused within the manual-instructed time period until reaching the planned group size. If necessary, a blunt needle was used to guide an accurate adhesive application around the anastomosis. The average amount of applied tissue adhesive is listed in Table 1. According to the applied tissue adhesive, rats were divided into the control group, TissuCol-group, Histoacryl-group, and Duraseal-group. To ensure full polymerization of tissue adhesives after application, we allowed the adhesives to set for five minutes before closing the abdomen with a running suture (Safil 5-0, B Braun, Melsungen, Germany).

On postoperative day (POD) 3 or POD14, rats were anesthetized again and re-laparotomy with a U-shape incision was performed. The abdomen was examined for manifestations of abscess formation, anastomotic dehiscence, and adhesions. Adhesion severity was recorded using the Zühlke score [23]. Bursting pressure was determined afterwards, and the bursting location was noted. The samples from the distal and proximal segments were harvested for histological examination, then the rat was euthanized.

Histology and immunohistochemistry
All the harvested segments were fixed overnight in 4% buffered formaldehyde and embedded in paraffin. The distal samples were cut longitudinally and the proximal samples were cut transversely, both in a depth of 5 micrometers. Hematoxylin and eosin (HE) staining was performed in all samples.
Three parameters including inflammatory cell infiltration, fibroblast activity, and collagen deposition at the anastomotic site were evaluated for all the HE stained distal samples. For each parameter a ranking was made for all the slides with the following strategy: first, one researcher (ZW) and one pathologist (KL) performed a blind evaluation of each slide under a microscope using the Ehrlich and Hunt numerical scale as modified by Phillips et al. [24]. After scoring, a ranking was made within the slides having the same score by cross-comparing the slides. Then the slides on the margin of each score were compared and adjusted again (e.g. compare highest ranked ones with score 1 with lowest ranked ones with score 2). Finally all the ranked slides were consecutively rearranged (sorted from the lowest to highest rank) and minor modifications were made if necessary.

Immunohistochemical staining for CD4 (T helper cells), CD8 (T suppressor cells), CD20 (B lymphocytes), iNOS (M1-macrophages), and CD206 (M2-macrophages) was performed on distal samples. The slides were first deparaffinized and boiled in Tris/EDTA pH 9.0 for 15 min. Endogenous peroxidase activity was blocked with 1.5% H2O2 in PBS for 10 min. Slides were blocked for 30 min with a 5% non-fat dry milk in PBS solution. The primary antibody of CD4 (1:100, Emelca Bioscience, Breda, Netherlands), CD8 (1:200, AbD Serotec, Kidlington, UK), CD20 (1:100, Emelca Bioscience, Breda, Netherlands), CD206 (1:400, Abcam plc, Cambridge, UK), or iNOS (1:1600, Abcam plc, Cambridge, UK) was applied respectively, and the slides were incubated overnight at 4°C. On the second day the slides were washed with PBS, and subsequently incubated with Envision secondary rabbit-anti-mouse or rabbit-anti-rabbit antibody (DAKO, Glostrup, Denmark) for 30 min. Diaminobenzidine (DAKO, Glostrup, Denmark) was used for visualization of antigen-antibody reactivity. Finally all slides were counterstained with hematoxylin, dehydrated and mounted.

A blinded investigator using a microscope under 40x10 magnification counted the positive cell amount of each staining. For the anastomotic site, three fields were selected: one on each cutting edge, the other one on the interface. For the serosa-glue site, three fields were also chosen at the interface of the tissue adhesive and adjacent tissue; in the control group, three fields were chosen at the interface of adhesion and adjacent tissue. The number of positive cells for each staining was counted, and the average of the three fields was used for analysis. An M2/M1 index was calculated with the equitation blew. A natural logarithm was used to adjust the data from exponential distribution into linear distribution. A similar equitation was also used for the CD4/CD8 index.

\[
M2/M1 \text{ index} = \ln \frac{\text{Number of CD206 + cells}}{\text{Number of iNOS + cells}}
\]

Statistical analysis
Statistical analysis was performed with SPSS 21.0 (IBM software, USA). Data were presented as mean (S.D.) or percentage. The one-way analysis of variance was performed with the Kruskal Wallis Test or Chi-square Test, and a p-value < 0.05 was considered to indicate statistical significance. In multiple comparisons, α was corrected with the number of comparisons with the following formula: \( \alpha' = \alpha / N \) (\( \alpha = 0.05, N = \text{number of comparisons} \)). When a significant difference was reached in the Kruskal Wallis Tests, multiple comparisons were made between the control group and each corresponding tissue adhesive the Mann-Whitney U Tests or Chi-square Tests in this study, so \( N = 3 \) was chosen for correction. Only a \( p' \)-value < 0.017 (0.05/3)
was considered to indicate statistical significance in the multiple comparisons. All reported p-values were two-sided.

RESULTS

A total number of 67 rats were used in the experiment and seven rats (10.4%) died during follow-up. Six deaths occurred on the day of the operation due to septicemia, but no anastomotic related complications were observed during autopsy. One rat had bloody stools after its operation and died on POD3, with its autopsy showing abscess formation on the anastomosis. There was no statistical difference in mortality rate between groups (Table 1).

<table>
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<tr>
<td>TissuCol®</td>
</tr>
<tr>
<td>Histoacryl® Flex</td>
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<tr>
<td>Duraseal®</td>
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<td>Total</td>
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*Note: Overall mortality 7 / 67 = 10.4%.*

After induction of fecal peritonitis with CLP model, all rats manifested septic symptoms such as compromised activities, nasal/ocular exudates, fluffy hair, diarrhea and weight loss, but no rat was prematurely euthanized for humane endpoint. On the operation day, weight loss were seen in all rats with an average of 10 to 12 grams in each group; no significant difference was found between groups. Abdominal fecal peritonitis were observed in all rats, manifesting as existence of ascites with fecal content. Abdominal culture tests of the ascites further confirmed bacterial contamination with Gram-positive (*e.g.* Enterococcus, Staphylococcus) and Gram-negative (*e.g.* Escherichia coli, Proteus) flora.

*Intra-abdominal observations*

On POD3, anastomotic dehiscence occurred in 28.6% (2/7) of rats in the control group, but not in other groups. Abscesses on the anastomosis was found in 85.7% (6/7) of rats in the control group, 22.2% (2/9) of rats in the Duraseal-group, but not in the TissuCol-group or Histoacryl-group, and it was significantly different between groups (*p* < 0.0001). On POD14, abscess formation on the anastomosis were found in one rat from the control group whereas none was found in the other groups.

The bursting pressure in the control group was 24.4 ± 31.7 mmHg. This was significantly lower than that in the TissuCol-group (120.1 ± 25.3 mmHg, *p* = 0.001), Histoacryl-group (117.3 ± 20.2
mmHg, \( p = 0.001 \), and Duraseal-group (123.6 ± 35.4 mmHg, \( p = 0.001 \), Figure 1). In the control group, 85.7% of the segments burst at the anastomotic line during the test, while the rates in the other groups differed between 28.6% (Histoacryl-group) and 50% (Tissucol-group). On POD14, most anastomotic segments did not burst at the site of the anastomosis in the ABP tests.

![Figure 1. Comparison of anastomotic bursting pressures (ABP) on postoperative day (POD) 3 and 14. Values are mean (S.E.M.). The On POD3, overall comparison, \( p = 0.001 \), Kruskal-Wallis Test. On POD14, overall comparison, \( p = 0.039 \), Kruskal-Wallis Test. The \( p \) values of Mann-Whitney pairwise comparisons are illustrated in the figure when relevant.](image)

Anastomotic adhesion formation was found in all rats. On POD3, the number of adhesions was significantly different between groups (\( p = 0.012 \)). An average adhesion number of 3.7 was found in the control group, which was significantly higher than the Tissucol-group (average: 1.8; \( p = 0.004 \)) and Histoacryl-group (average: 1.9; \( p = 0.015 \)), but not significantly higher than the Duraseal-group (average: 2.8). Most rats were scored 2 in the Zülke score (blunt dissection possible but partly sharp dissection possible; beginning of vascularization), and no difference was found between groups. In contrast, on POD14, the number of adhesions was similar between groups (average varied between 1.4 and 1.7), while their severity significantly differed (\( p = 0.004 \)). The lowest adhesion score was found in the Duraseal-group (average of 1.3; firm adhesion, easy to separate by blunt dissection; no vascularization). It was significantly lower than the control group (\( p = 0.001 \)), which had an average severity of 3.1 (lysis possible but sharp dissection only, clear vascularization).

**Histology and immunohistochemistry**

**Anastomotic site:**
All the distal-segment slides showed an acute inflammatory response at the anastomotic site on POD3. The inflammatory cell infiltration was highest in the control group, which was significantly higher than that in the Histoacryl-group (\( p = 0.009 \)). Lower fibroblast activity and collagen deposition was seen in the control group compared with the experimental groups, but their differences were not statistically significant (Figure 2). A lower number of CD206+ cells (M2) and a higher number of iNOS+ cells (M1) were seen in the control group, and the
M2/M1 index for the control group was also statistically significantly lower than all the tissue adhesive groups respectively (p = 0.002; Figure 3). A significant correlation was found between the ABP value and the M2/M1 index (R = 0.682; p < 0.0001). Similar changes were also seen in the CD4+ cells (T helper cells), CD8+ cells (T suppressor cells), and CD4+/CD8+ index (Figure 4), although only the difference between the control group and the TissuCol-group regarding CD8+ cells was statistically significant.

On POD14 most slides demonstrated sufficient wound healing on the anastomosis, showing as re-continuity of mucosal and muscle layers, less inflammatory cell infiltration and higher collagen deposition. There was no significant difference in fibroblast activity and collagen deposition between the control group and the adhesive groups. A higher number of iNOS+ cells (M1) and a lower M2/M1 index was seen in the control group compared with the tissue adhesive groups (Figure 3).

**Serosa-glue interface:**
On POD3, similar numbers of Inos+ cells and CD206+ cells were seen at the serosa-glue interface. The M2/M1 index, though higher in the tissue adhesive groups, was not significantly different between groups. On POD14, a significant higher M2/M1 index was observed in the Histoacryl-group compared with the control group at the serosa-glue interface (Figure 5).

**Proximal samples:**
In proximal samples, except for a minimal number of inflammatory cells, evidence of an inflammatory response was not seen in the control group. Similar to the distal samples, a moderate reaction was seen at the serosa-glue interface in the TissuCol-group, Histoacryl-group and Duraseal-group on POD3, which manifested as macrophage infiltration around the tissue adhesive, without interrupting the continuity of the mucosal, sub-mucosal and muscle layers of the colon. On POD14, the foreign body reaction in the TissuCol-group was observed to have significantly reduced, and the glue was not observed (neither macroscopically nor microscopically) in 75% (6/8) of the rats. The reaction in the Histoacryl-group and Duraseal-group was still moderate.
Figure 2. Comparison of histological parameters on postoperative day (POD) 3 and 14. Values are mean ranking (S.E.M.). On POD3, overall comparison of inflammatory cell infiltration ranking (2.A) yielded a p = 0.003 with the Kruskal-Wallis Test. Overall comparisons resulted a p > 0.05 in the other parameters, Kruskal-Wallis Test. The p values of Mann-Whitney pairwise comparisons are illustrated in the figure when relevant.
Figure 3. Comparison of macrophage subtype (i.e. M2 and M1) amount at anastomotic site on postoperative day (POD) 3 and 14. Values are mean of cell count (S.E.M.). On POD3, overall comparison of M2 (3.A), p = 0.003; M1 (3.B), p = 0.011; M2/M1, (3.C) p = 0.002, Kruskal-Wallis Test. On POD14, overall comparison of M2 (3.A), p > 0.05; M1 (3.B), p = 0.016; M2/M1, (3.C) p = 0.010, Kruskal-Wallis Test. The p values of Mann-Whitney pairwise comparisons are illustrated in the figure when relevant.
Figure 4. Comparison of T lymphocyte amount at anastomotic site on postoperative day (POD) 3 and 14. Values are mean of cell count (S.E.M.). On POD3, overall comparison of CD4+ (4.A), p = 0.042; CD8+ (4.B), p = 0.027; CD4+/CD8+, p > 0.05 (4.C), Kruskal-Wallis Test. Overall comparisons on POD14 all resulted a p > 0.05, Kruskal-Wallis Test. The p values of Mann-Whitney pairwise comparisons are illustrated in the figure.
Figure 5. Comparison of macrophage subtype (i.e. M2 and M1) amount at serosa-adhesive site on postoperative day (POD) 3 and 14. Values are mean of cell count (S.E.M.). Despite overall comparison of M2/M1 on POD14 (5.C) yielded a $p = 0.036$, all the other overall comparisons on POD3 or 14 resulted a $p > 0.05$, Kruskal-Wallis Test. The $p$ values of Mann-Whitney pairwise comparisons are illustrated in the figure.
DISCUSSION

Performing anastomosis in a contaminated environment results in a high leakage rate of colorectal anastomosis and poor clinical outcomes, threatening patient’s safety. To investigate whether using tissue-adhesive sealants prevents leakage in a contaminated environment, we constructed colorectal anastomoses under fecal contamination using the rat CLP model. All three chosen tissue adhesives increased the biomechanical strength of anastomosis in the short term, without increasing the risk on adhesion formation in the long term. The increased presence of anti-inflammatory macrophages (M2) and the decrease of the presence of pro-inflammatory (M1) macrophages is associated with increased biomechanical strength and most likely contributed to the positive effect of the tissue adhesives. These results support the further application of these tissue adhesives in a contaminated environment.

In this study, bacterial peritonitis induced with the CLP model caused high mortality, intra-abdominal abscess formation, anastomotic dehiscence, and low bursting pressures in the control group rats on POD3. These results are in line with previous studies [22, 25, 26]. In comparison, we found that under sterile conditions colonic anastomotic bursting pressure approximated an average of 80-90 mmHg (unpublished data). Lipopolysaccharide (LPS) from intra-abdominal bacteria triggers the classical activation of macrophages (M1), secreting nitric oxide, proinflammatory cytokines (i.e. tumor necrosis factor (TNF)-α, interleukin (IL)-12), subsequently enhancing cell-mediated immunity [27]. Although the exact mechanisms of nitric oxide in anastomotic healing is not yet determined, previous studies found that sepsis-induced nitric oxide production subsequently impaired collagen synthesis and thus delayed colonic anastomotic healing in the early phases [12, 13]. Accumulation of nitric oxide also causes apoptosis of neutrophils [28], manifesting as abscess formation. These histological changes were also observed in the control group, and more importantly correlation between the M2/M1 index and the bursting pressure was also found on POD3. These data demonstrate that bacterial peritonitis impaired anastomotic healing in the short term in our CLP model and that macrophages play a role in this process.

We also observed that tissue adhesives reduced abscess formation and increased the bursting pressure in the short term. These positive influences on wound healing are unlikely to be completely determined by the sealant adhesiveness. We previously evaluated the adhesiveness of twelve commercially available tissue adhesives in an ex-vivo rat colon model [29]. According to that study, only cyanoacrylate (including Histoacryl® Flex) had a strong adhesiveness that may instantly increase anastomotic strength. In contrast, all tested fibrin and PEG glues (including TissuCol® and Duraseal®) had very limited adhesiveness, which might hardly give additional strength to the anastomosis [29, 30]. However, as is shown in our results, both TissuCol® and Duraseal® significantly increased the bursting pressure on POD3, indicating involvement of other mechanisms.

Activation of macrophages is critical in the acute phase of wound healing. In the early phase, the wound strength mainly comes from the sutures and type III collagen produced by fibroblasts [31]. The fibrogenesis by fibroblasts is enhanced by alternatively activated macrophages (M2), while the classical activation of macrophages (M1) has negative influence on collagen deposition [27, 32]. In addition to the actual cell count, the M2/M1 index is also a representative parameter for macrophage function in tissue reaction [33, 34]. Our data
showed a lower number of M1, a higher number of M2, and a higher M2/M1 index in the tissue adhesive groups in the short term. The data suggest an alteration in the macrophage activation. We hypothesized that the tissue adhesive might isolate the contact between the anastomosis and intra-abdominal bacteria, and thus prevented the endotoxin-induced proinflammatory responses. This was further supported by the results of the Duraseal-group. In that group, a similar amount of iNOS+ cells as in the control group was found at the serosa-glue site (macroscopically presenting as abscess formation), but the anastomotic site was protected from this deleterious environment by the adhesive sealant, and far fewer iNOS+ cells were seen around the anastomosis, probably contributing to the high bursting pressure on POD3.

T lymphocytes also play an important role in the wound healing process. Previous studies reported that accumulation of CD8+ cells had a negative influence on collagen deposition and thus impairing early phase wound healing [35], which was also seen in our data accordingly. After exposure to LPS, type I interferon (IFN-α, β) produced by antigen-presenting cells activates CD8+ cells (T suppressor lymphocytes) [36], which trigger the apoptosis process of the infected somatic cells via the caspase cascade. The involvement of CD4+ cells (T helper cells) in wound healing is complicated. T helper 1 cells are involved in cellular immunity, and enhance iNOS production in macrophages [37]. T helper 2 lymphocytes and mast cells, however, produce IL-4 and other cytokines, which stimulates fibroblasts to produce extracellular matrix proteins, fibronectin, and collagen [38]. Although we did not further differentiate between the subpopulations of T helper cells, in a previous study on thermal injury, a decrease in CD4+/CD8+ ratio could only be found in infected wounds but not in the wounds without infection [14, 39]. A similar phenomenon was only seen in our control group which had a contaminated anastomotic wound. These data are in line with the observation in macrophage activation, and they further elucidate that applying the tissue adhesives prevents localized infection on the anastomosis and thus activates an alternative inflammatory response.

Previous studies found that the protective effect of tissue adhesive on the anastomosis was temporary, mainly in the short term [20, 40, 41]. De Hingh et al. reported that biomechanical strength of anastomoses is most vulnerable on POD3, and then gradually recovers in the CLP model [26]. Other studies showed that the strength at the anastomosis was higher than the intact colon in the long term, when the bursting location was not at the anastomotic site [25, 42]. Similar changes in bursting pressure and inflammatory cells were also seen in our data. Such phenomena are consistent with clinical observations, as most clinical anastomotic leakages occur within the first seven postoperative days, especially after contaminated procedures [25, 43]. In this regard tissue adhesives are required to provide effective protection during the first critical days after surgery prior to a long-lasting protection. Our results showed application of select tissue adhesives assists wound healing during the crucial period.

Increasing the anastomotic strength in the short term, it is also important that the applied tissue adhesives do not cause other adverse events in the long term. Among those events, adhesion formation and foreign body reaction are the most concerning ones for intra-abdominal application of biomaterials [19, 20]. Our data showed that the tissue adhesives used did not increase adhesion formation. The foreign body reaction after adhesive application were
The prevention of colorectal anastomotic leakage with tissue adhesives

moderate in all tissue adhesive groups, which were also shown in the M2/M1 index in the long term.

Among the select tissue adhesives, fibrin glue has been known as inert [41, 44, 45], and polyethylene glycol glue has been used as an adhesion barrier [18]. A moderate foreign body reaction and adhesion formation after application of the cyanoacrylate glue, however, was not expected because it was reported to increase inflammatory reactions, necrosis and adhesion formation in normal or high-risk conditions [46-48]. The inconsistency between previous cyanoacrylate studies and our data can be explained by several reasons. First, our amount of cyanoacrylate, 0.02 mL, was much smaller than that in the previous studies [20, 46], and thus fewer adverse events could be expected. In addition, most cyanoacrylate studies with positive results used n-butyl-cyanoacrylate, which causes a less inflammatory response and tissue toxicity than other cyanoacrylate molecules [20]. Therefore Histoacryl® Flex was also used in this study which is made of n-butyl-cyanoacrylate. These differences, though small, might significantly influence outcomes.

In conclusion, the application of the select tissue adhesives (i.e. TissuCol®, Histoacryl® Flex, and Duraseal®) increased anastomotic strength in the short term without increasing the long-term risk for adhesion formation. The alternative activation of macrophages and T cells most likely mediated these positive effects. Our results support the further application of anastomotic sealants in contaminated colorectal surgery.

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REFERENCES

Sealing insufficient colonic anastomoses with cyanoacrylate tissue adhesives: an in vivo study

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Submitted.
ABSTRACT

Background: Tissue adhesives (TA) may be useful to strengthen colorectal anastomoses, thereby preventing anastomotic leakage (AL). Previous studies have identified cyanoacrylate (CA) TAs as the most promising colonic anastomotic sealants. This study investigates the protective effects of sealing colonic anastomoses with various CAs.

Materials and methods: 55 Wistar rats underwent laparotomy and transection of the proximal colon. An anastomosis was created with 4 interrupted sutures followed by either application of Histoacryl Flexible, Omnex, Glubran 2, or no TA seal. An additional control group was included with a 12 suture anastomosis and no TA seal. After 7 days the rats were sacrificed and scored for presence of AL as the main outcome. Secondary outcomes were occurrence of bowel obstruction, adhesions and anastomotic bursting pressure. Histological evaluation was performed.

Results: The highest AL rate was found in the Glubran 2 group (7/11), followed by the 4-sutures group without TA (5/11), and the Omnex group (5/11). Histoacryl Flexible showed the lowest AL rate (2/11). In the control group only 1 rat showed signs of AL. Histologically, the highest influx of inflammatory cells was found in the 4-suture group without TA and for Omnex and Glubran 2. Histoacryl Flexible caused more mature collagen deposition when compared to the other TA groups.

Conclusions: Histoacryl Flexible showed the lowest leakage rate compared to the other TA groups and to the 4-suture control group. Glubran 2 showed the highest AL rate and a high inflammatory response. Histoacryl Flexible was associated with the presence of more mature collagen, and seems to promote anastomotic healing.
INTRODUCTION

Anastomotic leakage (AL) in colorectal surgery remains a major problem. Despite years of extensive research, AL is still commonplace, occurring in 5-15% of colorectal anastomoses. Over the years, various approaches have been proposed to prevent the onset of colorectal AL. Mechanical intraluminal devices creating a magnetic anastomosis, intraluminal sheets of plastic covering the stapled anastomosis, and exotic techniques such as tissue welding have all been proposed, but most were quickly abandoned due to inefficacy or high complication rate.

Recently, the idea of sealing an anastomosis externally with a tissue adhesive is gaining popularity, and has been linked to promising results. The benefit of such a technique is that a surgeon can create an anastomosis in a conventional manner using sutures or staples and perform an intra-operative leak test or other anastomotic test, before applying an extra layer of protection on the serosal surface of the anastomosed colon. Of the various available tissue adhesives (TAs), a special interest has arisen for cyanoacrylate (CA) TAs. CA is a type of chemical polymer, also known as ‘superglue’. In the early years of CAs, its use was linked to the impairment of tissue healing by exothermic polymerization and tissue toxicity. Newer CA formulations have eliminated this highly exothermic curing process by adjusting the length of the polymer-chains and adding various additives, also increasing the flexibility of the CA. Recent studies show that these new CAs are inert when used on the colon, not causing toxic reactions, while maintaining enough elasticity to cope with peristaltic movement and intraluminal forces.

Several experimental studies have been performed using CA glues to prevent AL, yielding ambiguous results. This may be partly due to a large spectrum of experimental methodology in the various studies, in which large differences exist in the used animal models, TA dosage and experimental end-points. This is a well-recognized problem in the field of experimental research on colorectal anastomoses. Previous research on the use of TAs for colonic sealing has proposed a stepwise approach, evaluating a large number of TAs following a similar experimental protocol, yielding promising results for CA-based TAs.

In the current study we use a rat model to simulate high rates of AL, based on the creation of a mechanically insufficient colonic anastomosis, which is sealed by a protective barrier of one of three different CA glues. The aim of this study is to identify promising CAs for the prevention of AL, which may prevent the intra-peritoneal leakage of bowel contents.

METHODS

Study design

Three clinically available CAs were included in this study. These TAs were chosen based on their mechanical and rheological profiles as derived from previous in vitro research from our group. TA composition and manufacturer details are listed in Table 1. A positive control group, in which a 12-suture colonic anastomosis was used as well as a negative, 4-suture control group, without a TA seal. The included study groups are listed in Table 1. Rat allocation was performed in a randomized manner using a lottery system. Data are reported according to the ARRIVE guidelines. A power analysis was calculated based on a reduction of 20% (6) in inflammation,
as scored on histological data, between the different experimental groups with a variance of ±16% (σ). The number of animals per group was calculated as follows: \( n = \frac{2(\alpha/2 - z)\sigma}{\sigma/\delta} = \frac{n = 2(1.96 + 0.84)^2(16/20)^2}{15.7*0.64 = 10.03 \approx 11.}

Table 1. Study groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Name</th>
<th>Anastomotic technique</th>
<th>Tissue adhesive (TA)</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>12 sutures</td>
<td>-</td>
<td>N/A</td>
</tr>
<tr>
<td>2</td>
<td>No TA (negative control)</td>
<td>4 sutures</td>
<td>-</td>
<td>N/A</td>
</tr>
<tr>
<td>3</td>
<td>Histoacryl Flexible</td>
<td>4 sutures</td>
<td>n-butyl-2-cyanoacrylate and a softener</td>
<td>B. Braun (Tuttingen, GER)</td>
</tr>
<tr>
<td>4</td>
<td>Omnex</td>
<td>4 sutures</td>
<td>2-octyl-cyanoacrylate/ butyl lactoyl cyanoacrylate</td>
<td>Ethicon (J&amp;J, Sommerville, NJ, USA)</td>
</tr>
<tr>
<td>5</td>
<td>Glubran 2</td>
<td>4 sutures</td>
<td>n-butyl-2-cyanoacrylate and methacryloxy sulfonole</td>
<td>GEM S.r.l. (Viarregio, IT)</td>
</tr>
</tbody>
</table>

**Animals**

Male specified-pathogen-free Wistar rats (250-350g) were housed at the Central Animal Facility of the Maastricht University Medical Center, the Netherlands. Rats were housed according to standard laboratory conditions, including individually ventilated cages with unrestricted access to standard rat chow and water. The experimental protocol complied with the Dutch Animal Experimentation Act and was approved by the local Animal Experimental Committee.

**Surgical procedure**

Experienced researchers certified for animal research performed all surgical procedures. Rats received buprenorphine 0.1mg/kg (Temgesic, Schering-Plough, USA) pre-operatively for analgesia, which was repeated at 24 hours post-operatively. Anesthesia was induced by inhalation of isoflurane 5.0 vol% (Forene, Abbott Laboratories, USA), followed by a maintenance dose of 2.5 vol%. The abdominal skin was shaved, disinfected with iodine 1% and covered with sterile drapes. A 5-cm midline incision was made, the cecum was identified and exteriorized onto moist sterile gauzes. The ascending colon was transected 2-cm distally to the cecum, without damaging the mesenteric vessels. An insufficient end-to-end colo-colonic anastomosis was created using 4 evenly distributed polypropylene 6/0 sutures (Prolene, Ethicon, Johnson & Johnson, USA).

After construction of the anastomosis, in the TA groups, 0.025 mL of TA was applied evenly to the anastomotic site using the provided applicators. Care was taken to avoid spillage into the abdomen and, if necessary, a blunt needle was used to accurately guide the TA around the anastomosis. Curing time varied based on the manufacturer’s guidelines. In the control group, 12 sutures were used instead of 4, obtaining a sufficient anastomosis. Postoperative hydration was provided by a bolus of 5 mL sterile saline solution (37°C), injected into the abdominal cavity after repositioning of the abdominal contents. The abdominal wall was closed with a running suture of polyglactin 4/0 (Vicryl, Ethicon, Johnson & Johnson, USA). Skin closure was performed
Sealing insufficient colonic anastomoses with cyanoacrylate tissue adhesives

with an intracutaneous running suture of poliglecaprone 4/0 (Monocryl, Ethicon, Johnson & Johnson, USA). Postoperatively, daily evaluation of all animals was carried out. Animal weight and signs of distress were noted. In case of severe distress/illness (humane endpoints), animals were sacrificed prior to the completion of the follow-up time.

**Outcome measures**
The main outcome of the study was anastomotic leakage (AL), including macroscopic anastomotic dehiscence, fecal peritonitis or large anastomotic abscesses. After 7 days or when humane-endpoints were reached, rats were sacrificed using an overdose of carbon dioxide. The abdomen was re-opened and macroscopically inspected for signs of leakage or TA-related complications, that is, presence of intraperitoneal abscesses or fecal matter and mechanical ileus. Abscess formation was scored using the following scoring method: 1) one or several perianastomotic millimetric abscesses; 2) abscess covering up to ¼ of anastomotic circumference; 3) Large abscess; more than 1/4 of anastomotic circumference; 4) intra-abdominal abscess formation. Based on our previous research, in which we found that an abscess score of 1 was not associated with any clinical complications and was therefore not clinically significant, we defined AL in this study as the presence of fecal peritonitis or an abscess score of >2\textsuperscript{12,20}. The Zühlke score, which depicts the tenacity of intra-abdominal adhesions, was also determined\textsuperscript{14}.

**Anastomotic bursting pressure**
To measure anastomotic bursting pressure (ABP), a plastic tube was inserted into the colon proximally to the anastomotic site, and ligated with a single polyglactin 4/0 suture. The distal colonic segment was clamped to ensure an airtight compartment. Pressure was gradually increased in the anastomotic compartment using an automatic pressure pump (IDEE, Maastricht, the Netherlands). ABP was monitored and recorded using a digital manometer until air bubbles appeared. The maximum bursting pressure was recorded for each rat.

**Histological evaluation**
After ABP testing, the anastomotic segment was subsequently resected and prepared for histological evaluation. Tissue samples were embedded in paraffin and cut in 4µm sections. To evaluate the morphology of cells, standard hematoxylin-eosin (H&E) staining was performed. Specimens were scored based on inflammation, fibroblast activity, collagen deposition and neo-angiogenesis according to the Ehrlich and Hunt numerical scale as modified by Phillips et al, which: 0: No evidence, 1: occasional evidence, 2: Light scattering, 3: abundant evidence, 4: confluent cells or fibers\textsuperscript{15}. All slides were evaluated by an experienced pathologist (MG) who was blinded for the experimental groups.

**Evaluation of collagen formation**
Tissue sections were stained for collagen using Picro Sirius red, as previously described\textsuperscript{16}. It was chosen not to include collagen staining in the 12-suture control group due to ethical reasons, as these findings are well-known and have been reported in numerous recent studies\textsuperscript{17-19}. In short, sections were exposed to a 0.1% solution of Sirius red in saturated aqueous picric acid for 90 minutes, followed by 2 min of washing in 0.01N HCl, dehydration and mounted with Entellan. Images of the anastomotic region were taken (200x magnification) using cross polarization light microscopy (Leica DM5000B, Leica Microsystems, Switzerland). Collagen percentage of anastomotic tissue was calculated. Maturity level of collagen was
estimated by calculating the red (mature fibers, collagen type I) versus green (immature fibers, collagen type III) area ratio using the Qwin morphometry-system (Leica QWin V3.5.1, Leica Microsystems).

Statistics
One-way ANOVA was used in case of continuous variables, with a Bonferroni post-hoc test. A \( \chi^2 \)-test or Fisher’s exact was used in case of categorical variables. A p-value \( \leq 0.05 \) was considered statistically significant. All analyses were performed using IBM SPSS Statistics, version 21.0 for Mac (IBM SPSS, USA), while graphs were composed using GraphPad Prism, version 5.0a for Mac (GraphPad Software, USA).

RESULTS

Anastomotic Leakage
Both in the 4-suture non-TA group and in the Glubran 2 group, one rat died prior to completion of the follow-up period due to fecal peritonitis caused by AL. Except for these two rats, AL only consisted of the presence of anastomotic abscesses. In the 12-suture control group AL occurred in one rat, associated with an abscess score of 1. In the 4-suture non-TA group, 4 rats showed signs of AL in the form of abscess formation, with an abscess score of 2 in 3 rats and an abscess score of 4 in 1 rat. In the TA groups there was a large difference in AL-rate. Glubran 2 had the highest AL rate, consisting of 1 total anastomotic dehiscence and subsequent fecal peritonitis, and 6 cases of abscess formation. Abscess scores in this TA group ranged from 1 to 4. 5 rats in the Omnex group and 2 rats in the Histoacryl Flexible group showed signs of AL in the form of abscess formation, with maximum abscess scores of 4 and 2, respectively. Statistical analysis shows that Glubran 2 had a significantly higher amount of abscesses when compared to the positive control group (\( p = 0.013 \)) and Histoacryl Flexible (\( p = 0.049 \)). A synopsis of AL-rates is provided in figure 1A.

Clinical outcomes
Discomfot in the various groups is reflected by changes in weight loss throughout the follow-up period (Fig 1B). The negative control group, in which no TA was used, showed the highest rate of weight loss. For the TA groups, the Glubran 2 group showed the most weight loss on postoperative day 7, significantly higher than in the control group (\( p < 0.01 \)) and the Histoacryl Flexible group (\( p < 0.01 \)). Mechanical ileus rate varied significantly between the TA groups, with Glubran 2 showing the highest ileus rate, significantly higher than the negative control group (\( p = 0.01 \)). The number and Zühlke score of adhesions did not differ significantly between the experimental groups. A synopsis of the clinical outcomes is presented in Table 2.
Sealing insufficient colonic anastomoses with cyanoacrylate tissue adhesives

Figure 1. A) Histoacryl Flexible had the lowest amount of anastomotic leakage (2/11) compared to the other intervention groups Omnex 5/11 and Glubran2 7/11 and the no TA group 5/11. The control group with 12 sutures only showed one case of AL (X²=9.43, p=0.05). B) Weight loss was monitored as a measure of discomfort and the percentage weight loss was highest in the no TA group, followed by the Glubran 2 group with was significantly higher than the Histoacryl Flexible group and the control group. C) No significant differences were found between the experimental groups regarding anastomotic bursting pressure. Control group (12 sutures) and the group without TA showed significantly different ABP (p=0.004).

Table 2. Synopsis of clinical outcomes

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of rats</th>
<th>Fecal peritonitis*</th>
<th>Abscess score (&gt; 2)*</th>
<th>Mechanical ileus**</th>
<th>Adhesions, total (mean)***</th>
<th>Median Zuhlke score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) 12 sutures</td>
<td>11</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>4 (4)</td>
<td>3</td>
</tr>
<tr>
<td>2) 4 sutures</td>
<td>11</td>
<td>1</td>
<td>5</td>
<td>0</td>
<td>5 (5)</td>
<td>3</td>
</tr>
<tr>
<td>3) Histoacryl Flexible</td>
<td>11</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>5 (5)</td>
<td>3</td>
</tr>
<tr>
<td>4) Omnex</td>
<td>11</td>
<td>0</td>
<td>5</td>
<td>1</td>
<td>5 (7)</td>
<td>3</td>
</tr>
<tr>
<td>5) Glubran 2</td>
<td>11</td>
<td>1</td>
<td>7</td>
<td>5</td>
<td>5 (8)</td>
<td>3</td>
</tr>
</tbody>
</table>

* Number of rats in which a clinically relevant abscess score (i.e. >2) was found.
** Number of affected rats.
*** Data are presented as the total amount of adhesions found, with the mean amount of adhesions between parentheses.
Anastomotic Bursting Pressure (ABP)
The highest ABP, as depicted in Fig. 1C, was found in the 12-suture control group (272 mmHg ±7) and differed significantly from the 4-suture no-TA group (147 mmHg ±37, p<0.01). The use of TA resulted in an increase in ABP in all 3 TA groups, however no statistically significant differences were found. The highest increase in ABP was found in the Histoacryl Flexible group (217 mmHg ± 53), followed by the Glubran 2 group (205 mmHg ± 67). Omnex showed the lowest ABP of the TA groups (173 mmHg ± 69).

Histological evaluation
The use of a 4-suture anastomosis, with or without the presence of a TA, led to more inflammation when compared to the 12-suture control group (Fig 2A). A significantly higher inflammation score was found in the Omnex and Glubran 2 groups when compared to the 12−suture control group (p < 0.01). Significantly more collagen deposition was found in the Histoacryl Flexible group and the Omnex group in comparison with the 12-suture control group (fig 2B; p < 0.01). The number of fibroblasts and level of neo-angiogenesis did not differ between the experimental groups (fig 2C & 2D).

Picro Sirius red staining, which depicts level of collagen maturity, of the anastomotic region showed comparable percentages of collagen for all groups (Fig. 3A, p = 0.214). When focusing on collagen maturity, a significant difference was found in red/green ratios between the Histoacryl Flexible and Glubran 2 group (Fig. 3B, p< 0.05), illustrating higher levels of mature collagen in the Histoacryl Flexible group.

DISCUSSION

The sealing of colonic anastomoses with tissue adhesives (TAs) has been proposed as a promising method for the prevention of anastomotic leakage (AL) by forming a mechanical barrier that can protect from leakage of intraluminal contents into the abdominal cavity[20]. Of the large amount of available TAs, recent research has provided evidence that cyanoacrylate (CA) TAs may be useful in the prevention of AL[6]. In the current study, we selected three clinically available CAs to seal insufficient, 4-suture, colonic anastomoses in a rat model. We also included a positive control group, in which a 12-suture colonic anastomosis was used as well as a negative, 4-suture control group, without a TA seal. We evaluated the effectiveness of each TA in the prevention of AL, also taking into account mechanical strength and histological profile.

Overall, there were large differences between AL rates of the various groups. AL mostly presented as peri-anastomotic abscess formation. The described abscess score was used to score the severity and amount of abscesses; in this study, an abscess score of <2 was not associated with any clinical symptoms, and therefore not considered clinically relevant. Histoacryl Flexible, a combination of n-butyl-2-cyanoacrylate and a softener, showed the lowest rate of AL of the TA groups, occurring in two rats. Furthermore, the maximum abscess score in Histoacryl Flexible was lower than in the other TAs, and consisted only of punctiform abscesses around the anastomosis, which did not have any clinical consequences. The histological evaluation showed that this TA resulted in the least inflammation and the highest level of collagen formation and healing of the TA groups. Overall, Histoacryl Flexible showed promising results, with an AL rate comparable to the 12-suture control group, with positive
Sealing insufficient colonic anastomoses with cyanoacrylate tissue adhesives | clinical outcomes and improved histological assessment. This TA seems to be a safe and effective colonic sealant.

Figure 2. A) significantly more inflammation occurred in the Omnex and Glubran 2 group compared to the control group. B) No differences were found between groups regarding fibroblast activity. C) More collagen deposition was found in the Histoacryl group and the Omnex group compared to the control group. Neoangiogenesis (D) did not differ between the experimental groups.

Figure 3. A) No differences were found between groups in the relative collagen area (quantified as the percentage of total tissue surface). B) Maturity of collagen was estimated by calculating the red/green ratio, which was significantly higher in the Histoacryl Flexible group compared to the Glubran 2 group, indicating more mature collagen.
Glubran 2, based on an n-butyl-2-cyanoacrylate and methacryloxy sulfolane mixture, showed the poorest results in our study. In terms of AL, the use of this TA resulted in one case of premature death due to fecal peritonitis, as well as the highest rates of abscess formation and abscess severity. Furthermore, its use was associated with a higher incidence of mechanical ileus, occurring in five rats, significantly higher than in the 4-suture control group. Rats in this group showed the most weight loss of all study groups. Histological analysis associated Glubran 2 use with the highest degree of inflammation, and a significantly more premature collagen ratio, indicating less healing capability. Glubran 2 induced an extended inflammatory response with mild local muscle lysis as deep as the submucosal colonic layer. This finding was also reported in a previous study by Kayaoglu\textsuperscript{21}. Omnex, a 2-octyl-cyanoacrylate / butyl-lactoyl-cyanoacrylate mixture, showed similar results to the negative control group in terms of AL rate, clinical effects, mechanical strength and histological analysis. Presence of this TA thus did not improve outcomes nor lead to any complications when used on the colon.

Results of this study are in line with previous research on the use of CA in experimental AL models. In a previous study from our group the same set of CAs were applied on rat colon without the presence of a colonic defect and followed for 1 or 4 weeks\textsuperscript{12}. That study showed that Histoacryl Flexible retained the lowest complication rate and a relatively inert histological profile, with a limited local host reaction and an increase in inflammatory markers at seven days, a finding which did not persist at 28 days when no ongoing inflammatory reaction was found. In the present study, Histoacryl Flexible was associated with a higher inflammatory reaction than the 12-suture control group at seven days, comparable to the 4-suture no-TA group. The long-term inflammatory reaction of this TA in an anastomosis model may be an interesting subject for further research.

All three TAs included are modern CAs, in use clinically. When examining the chemical compositions of each CA (as depicted in table 1), one may note that the main ingredients of the included CAs are similar to one another. Glubran 2 (n-butyl-2-cyanoacrylate/methacryloxy sulfolane) does not differ considerably from either Histoacryl Flexible (nbutyl-2-cyanoacrylate) or Omnex (n-octyl-cyanoacrylate/butyl lactoyl acrylate), while differences exist in the various additives and softeners. As reported in previous research, Glubran 2 use elicits a significantly higher inflammatory response that the other included CAs, and may possibly be attributed to methacryloxy sulfolane, an additive in Glubran 2 that increases flexibility\textsuperscript{6}. This finding should be addressed in future studies.

Our study has several limitations. Firstly, we opted for a follow-up time of 7 days to evaluate short-term effectiveness after creation of an anastomosis. Therefore, we cannot comment on the long-term safety of CA use on colonic anastomosis. This may be in part extrapolated from our previous studies, however, a future objective would be to use several follow-up time-points. By doing this, one could also evaluate the clinical relevance of the (sub)clinical local abscesses we encountered, which, in fact, may reflect perioperative spillage of bowel contents instead of AL. Secondly, as a large number of colorectal anastomoses are stapled nowadays, a study on the interaction between CA and a stapled anastomosis may an interesting step for further research.

In conclusion, this study shows that the use of Glubran 2 was directly associated with poor outcomes in this study and does not seem to be a suitable TA for the sealing of the colonic
anastomosis. Furthermore, we found limited evidence of a protective effect of the use of Histoacryl Flexible as an anastomotic sealant. The use of this CA showed a trend for the decrease of AL, the increase in mechanical strength and stimulation of wound healing when compared to the other CAs and to a negative control group. It was associated with an incidence of AL comparable to a standard, 12 suture anastomosis and should be further evaluated in future research.
REFERENCES


Summary, General Discussion and Future Perspectives
Anastomotic leakage (AL) is one of the most feared complications in colorectal surgery. Despite years of research the overall AL-rate remains unacceptably high: between 5 and 25%\textsuperscript{1-3}. In this thesis, our research on the role of tissue adhesives (TAs) as colorectal anastomotic sealants was described.

**The importance of methodology in experimental research: a stepwise approach**

In the presented research a novel stepwise approach to tissue adhesive testing was proposed, focused on the standardization and communication of methodology. The aim of this approach was to validate TAs for the sealing of the colorectal anastomosis by minimizing confounding factors and enabling a sound comparison between studies.

It was decided to define the efficacy of a TA by three pillars: its mechanical strength (i.e. mechanical strength of TA bond), its clinical and histopathological effects on living tissue and its capability to prevent AL. This thesis was structured according to those pillars. Firstly mechanical strength was investigated, focusing on the direct post-application strength of the TA bond, in two ex-vivo models (chapters 3 and 4)\textsuperscript{4,5}. Secondly the best performing TAs in terms of mechanical strength were chosen for in vivo testing and used to attach the serosal surface of two intact (e.g. without any defect) colonic segments to each other in a sutureless manner. This study was described in Chapter 6, and provides information on the inertness of each TA when used on the colon\textsuperscript{6}. In that study mechanical strength was evaluated at short- and long-term follow-up, after the onset of tissue healing and TA degradation. By using the same mechanical testing protocol as in Chapter 3, it was possible to observe the differences in mechanical strength over time. Furthermore, Chapter 5 presents our experience with a mouse colon anastomosis model that proved to be suboptimal for the testing of TAs as the surgical technique and TA application were not easily standardizable due to the small size of the animal model, leading to a high incidence of clinical complications\textsuperscript{7}. In Chapter 7, TA use was evaluated in a novel in vivo model in which iatrogenic colonic defects were sealed using the same set of seven TAs as in Chapter 3\textsuperscript{8}. That study investigated the protective effect of a TA barrier in terms of intraperitoneal leakage of bowel contents as well as effects on tissue healing, when used to seal a colonic defect in a sutureless manner, thus without the eventual variability introduced by an additional suture or staple closure. In Chapter 8, standard colonic anastomoses were sealed with TAs in the cecal ligation and puncture model. With this model, the effect of ongoing peritonitis on AL rate, clinical and (immuno-) histopathological endpoints could be evaluated\textsuperscript{9}. Lastly in Chapter 9 the three most promising TAs were selected based on our previous results and used to seal insufficient colonic anastomoses in a rat model\textsuperscript{10}. AL rate, clinical complication rate, anastomotic bursting pressure (ABP) and histological endpoints were evaluated. An overview of the research approach implemented in this thesis is provided in Fig. 1.
Part I: Where are we now?

Chapter 1
In Chapter 1 a systematic review on TAs for the sealing of gastrointestinal anastomoses was presented\textsuperscript{11}. Experimental and clinical studies on esophageal, gastric, pancreatic, ileal and colorectal anastomosis were included. Of the twenty studies identified in the field of colorectal surgery only one study was on humans, far less than for the other anastomotic locations\textsuperscript{12}. Several studies using the same animal models and tissue adhesives reported contradicting results. A closer look into the methodology of each respective study revealed a large amount of heterogeneity with regard to the included studies. The largest differences were found in the type of anastomosis and experimental endpoints. Mostly, authors employed rat models in which a colonic anastomosis was made. However, there was no consistency between the amount of sutures used, the location of the anastomosis and whether a colonic resection was performed. Regarding mechanical strength endpoints, most authors used the ABP-test. Although this is a useful test, large variations in each testing protocol were observed. Lastly, information on the tissue adhesive application protocol, such as the exact amount of TA and curing time, which is imperative for replication studies, was mostly not reported, as was the case for information on animal type, species, age and perioperative care. Overall, this lack of
consensus in methods (and subsequently in results), together with incomplete reporting of data, might be partially responsible for the relatively small number of clinical studies conducted in the field of TAs in colorectal surgery.

Lastly it was found that TA research concentrated almost exclusively on two types of TA: fibrin glue (FG) and cyanoacrylate (CA). Studies using FG for colorectal anastomotic sealing showed promising results. However, CA use in colorectal surgery showed ambiguous results without clear conclusions to be drawn. As shown in that chapter more proximally located anastomoses, i.e. esophageal and small intestinal anastomoses, in which CA was tested, showed positive outcomes.

Chapter 2
Based on the findings reported in Chapter 1 regarding the ambiguous results found for the CA adhesives, it was decided to perform a systematic review, focusing only on this TA group. Methodological details of each study were examined and compared to experimental outcomes. When examining the progression of CAs throughout the years, an evolution was noted in their chemical characteristics with newer CA formulations containing longer chain CA monomers than older formulations. Although longer chain CA monomers are generally associated with more hydrophobic and bacteriostatic properties and less tissue toxicity, it was found that n-butyl cyanoacrylate (NBCA), a CA with intermediate chain length, showed the best results in the included studies: better than CAs with shorter (i.e. methyl-cyanoacrylate and ethyl-cyanoacrylate) and longer (i.e. octyl-cyanoacrylate) chain lengths. Furthermore, in line with Chapter 1 this review identified large inconsistencies in experimental methodology, especially with regard to the presentation of chosen animal model, type of anastomosis and anastomotic technique and of CA dosage. More detailed reporting of these parameters might facilitate comparison between studies and should be stimulated in future research.

Part II: Mechanical strength of tissue adhesives

Chapter 3
Research until now has mostly focused on in vivo models, in which the mechanical strength of a TA was evaluated at the time of sacrifice, thus after a variable period of time. These studies provide information on the mechanical strength of a TA bond after a given time of interaction with the host, at which time the effects of healing and TA degradation have already affected the TA bond. As the kinematics of each TA differ, one cannot extrapolate these results towards the direct post-application strength of the TA. Understanding the strength of a TA right after its application is important because the anastomosis is most prone to failure directly after its creation, when it relies solely on the closure technique and the value of a TA seal is most apparent. In Chapter 3 the testing protocol used by the American Society for Testing and Materials (ASTM) was adapted for the testing of tissue adhesives, to evaluate the mechanical strength of a large number of commercially available TAs from all known TA categories (cyanoacrylates (CA), fibrin glues (FG), polyethylene glycol, albumin-based adhesives). An overview of TA categories and included TAs per chapter is provided in Table 1. Three mechanical tests were used to simulate the mechanical forces that a colonic tissue adhesive may encounter: tensile, shear, and peel testing. This study showed that the new generation CAs (Omnex, Glubran 2, Histoacryl Flexible, Dermabond) were the strongest TAs, as well as the most homogeneous in terms of experimental outcomes. Interestingly FGs were
the weakest TAs, which contradicted previous in vivo studies that reported high mechanical strength for FG\textsuperscript{18-20}. This might be due to the lack of blood or intraperitoneal fluid in our ex vivo setting; it was possible that the presence of blood strengthened the tissue-adhesive bond by the physiological action of fibrin\textsuperscript{21}.

Serrero et al. found that the rheological behavior of various TA formulations was correlated to their mechanical strength and that an optimal rheological profile existed\textsuperscript{22}. In Chapter 3 rheological testing of each included TA was performed to gain information on cohesiveness and in turn: flexibility. It was found that the rheological characteristics of the TAs were correlated to their mechanical strength and thus might have a predictive function. As rheological tests are easily performed, only requiring few drops of the TA, rheological profiling may be a promising future endpoint in TA research. Based on this study a standardized protocol for the testing of TAs was proposed, combining ex vivo mechanical strength testing and rheological profiling.

Table 1. Overview of TA categories and included TAs per chapter.

<table>
<thead>
<tr>
<th>Tissue adhesive category</th>
<th>Commercial name</th>
<th>Composition</th>
<th>Chapters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyanoacrylates (CA)</td>
<td>Histoacryl Flexible (B.Braun, DE)</td>
<td>n-butyl-2-cyanoacrylate</td>
<td>X X X X X</td>
</tr>
<tr>
<td></td>
<td>Glubran 2 (GEM Italia, IT)</td>
<td>n-butyl-2-cyanoacrylate and methacryloxyxulfolane</td>
<td>X X X X</td>
</tr>
<tr>
<td></td>
<td>Omnex (Ethicon, USA)</td>
<td>2-octyl-cyanoacrylate</td>
<td>X X X X X</td>
</tr>
<tr>
<td>Dermabond (Ethicon, USA)</td>
<td>2-octyl-cyanoacrylate</td>
<td>X X</td>
<td></td>
</tr>
<tr>
<td>Albumin based adhesives (AB)</td>
<td>Bioglu (Cryolife, USA)</td>
<td>Glutaraldehyde-albumin glue</td>
<td>X X X X</td>
</tr>
<tr>
<td></td>
<td>Covabond (Covalent Inc., USA)</td>
<td>Albumin, aldehyde cross linker</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>Colle chirurgicale (Cardial SA, FR)</td>
<td>Gelatin-resorcinol-formaldehyde glue (GRF)</td>
<td>X X X X</td>
</tr>
<tr>
<td>Polyethylene glycol adhesives (PEG)</td>
<td>Duraseal Xact (Covidien, USA)</td>
<td>Polyethylene glycol, trilisine amine, blue dye, N-hydroxy succinimide</td>
<td>X X</td>
</tr>
<tr>
<td></td>
<td>Coseal (Baxter, USA)</td>
<td>Polyethylene glycol, hydrogen chloride and sodium phosphate-sodium carbonate</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>Duraseal (Covidien, USA)</td>
<td>Polyethylene glycol, trilisine amine and blue dye</td>
<td>X X</td>
</tr>
<tr>
<td></td>
<td>PleuraSeal (Covidien, USA)</td>
<td>Polyethylene glycol</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>VascuSeal (Covidien, USA)</td>
<td>Polyethylene glycol</td>
<td>X</td>
</tr>
<tr>
<td>Fibrin glues (FG)</td>
<td>Tissucol (Baxter, USA)</td>
<td>Fibrin glue, with aprotinin</td>
<td>X X X X</td>
</tr>
<tr>
<td></td>
<td>Evicel (Ethicon, USA)</td>
<td>Fibrin glue, without aprotinin</td>
<td>X X</td>
</tr>
</tbody>
</table>
Chapter 4
In Chapter 4 ex vivo mechanical strength testing of TAs was continued in a porcine colon model. ABP was compared between insufficient anastomoses protected by CA and standard, sutured, anastomoses. It was found that insufficient anastomoses with a protective CA layer were comparable to standard, one-layer sutured anastomoses.

Following these initial studies on the mechanical strength of a TA directly after application, in part II of this thesis research was presented on the post-application evolution of TA mechanical strength. Clinical studies had shown that AL might occur up to one month after the creation of an anastomosis, marking the importance of a long-term strong TA bond.\textsuperscript{14} Following the above-mentioned ex-vivo studies the best performing TAs were selected for in vivo evaluation. In Chapter 6, mechanical strength of TAs was investigated at one-week and one-month after application. In this novel model no colonic defects were made and two separate colonic segments were glued to one another. The distal segment was resected and the strength of the glue bond was tested using the same protocol as in Chapter 3, enabling a fair comparison of results. Results were consistent between the two studies, with the CAs (Histoacryl Flexible in particular) showing the highest mechanical strength. Lower mechanical strength was found for FG when compared to results of past in vivo studies\textsuperscript{17,18,20}. Importantly, mechanical strength was higher at 28 days than at 7 days, indicating that the strength of a TA bond increased after initial application. It should be noted that mechanical strength in the gelatine-resorcinol-formaldehyde (GRF) group was the highest at 28 days; this was, however, due to adhesion formation following severe inflammatory reaction.

In part III of this thesis the use of TAs as sealants of colonic defects was investigated. Mechanical strength in these studies was evaluated by ABP-testing. Chapter 7, in which colonic defects were sealed with TA in a sutureless fashion, showed that CAs were the strongest TAs and were similar to Tissucol, a FG. This finding implied that the curing process of Tissucol was altered when applied on an in vivo surgical wound, most probably due to the presence of blood, which might act as a catalyst in the fibrin clotting cascade which FG depends on for curing\textsuperscript{21}. In Chapter 8 a colonic resection and anastomosis were created 24 hours after the induction of a contaminated environment using a colon ligation and puncture model. ABP-testing was performed at day 3 and day 10. An increase in mechanical strength by TA was especially apparent at day 3, when all TAs (Histoacryl Flexible, Duraseal, Tissucol) exhibited significantly increased ABP compared to the control group which had no TA seal. Interestingly, at day 10, mechanical strength in the control group was comparable to Histoacryl Flexible and Duraseal. This high mechanical strength in the Tissucol and Duraseal group was surprising, as in our previous studies in which no colonic defect was created, Duraseal and Tissucol showed poor adhesive results. This finding indicated that the presence of a contaminated environment might increase mechanical strength in these TAs and might be an interesting subject for future research.

From the abovementioned studies it was found that the most promising TAs were the CAs, which combined high mechanical strength with an inert histopathological profile and a low complication rate. In Chapter 10 the three best performing CAs were selected and used to seal insufficient 4-suture colonic anastomoses in a rat model, comparing these to a 4-suture control group without a TA bond as well as a standard 12-suture anastomosis group. This study showed
a protective effect of CA sealing, which showed ABP similar to the standard anastomosis group and significantly higher than in the no TA seal control group.

Part III: Can tissue adhesives be safely used on living tissue?

Chapter 5
Next to the evaluation of the mechanical strength of TAs in part III of this thesis, the clinical and (immuno)histopathological effects of various TAs were investigated. Firstly in Chapter 5 our experience with an initial in vivo study on the mouse was presented. This study was based on one of the first colon AL models, as described by Komen et al. 23. Various TA categories were included: Omnex (cyanoacrylate), VascuSeal and Pleuraseal (polyethylene glycol, PEG), Cardial and BioGlue (albumin-based) and Evicel (fibrin glue). None of the used TAs were able to show a protective effect on the anastomosis. All tested TAs, except Evicel, showed signs of tissue toxicity, high ileus formation and increased mortality rate. BioGlue showed a 100% mortality rate. It was concluded that the mouse model was not suitable for TA research as it was too small to accurately dose and place the TA bond. In a recent review on experimental AL models, Pomergaard et al. concluded that mouse and pig models were superior to the rat, since the rat might be less sensitive to infection. According to the authors it was difficult to create a leaking anastomosis with clinical consequences in a rat model24. In our experience the rat model was a feasible and practical model for research on AL. In a recent study from our group a novel AL model was validated in the rat. In that study 5-suture colonic anastomoses were created after partial colectomy, resulting in an AL rate of 20-50%25.

Chapter 6
In Chapter 6 effects on tissue healing and clinical outcomes of seven TAs from various clinically available categories were evaluated in a novel rat model. This rat model is unique because it enabled the application of TA on the colon, attaching two colonic segments, while maintaining the anatomical configuration and functionality of the colonic tract during follow-up. Furthermore, this was the first study on TAs implementing immunohistochemical analyses. Clinical complications were only seen in the case of GRF and Bioglu at 7 and 28 days. Tissucol (FG) and Duraseal Xact (PEG) showed the smallest amount of adhesions and the lowest complication rates at both 7 and 28 days, indicating safe use on the bowel. These findings were in line with previous research on the use of PEG adhesive26. The use of CA adhesives was not associated with an increase in clinical complications.

Histopathological evaluation showed that Bioglu and GRF elicited the most severe inflammatory response, leading to bowel wall necrosis. Glubran 2 was associated with mild local muscle lysis while the other CAs and sealants were inert, not showing any signs of toxicity or foreign body reaction.

In this study the use of immunohistochemistry was implemented to better understand the response of the adaptive immune system of the host and the regulation of the inflammatory response, which, in turn, affects the degradation of the TA. At day 7 Bioglu showed the highest scores for CD4, CD20 and CD68, which are markers of T-helper cells, B-cells and macrophages respectively. These findings indicated that this TA was associated with the most intense short-term inflammatory response. At 28 days GRF showed an ongoing (chronic) inflammatory response. This was confirmed by a high amount of CD4, CD8, CD20 and CD68. The cell
proliferation marker Ki67 was found to be the highest in Tissucol at 7 days. Taken together with its inert tissue reaction this finding indicates that physiological tissue healing may take place early on in the presence of this TA. The CA adhesives were overall inert without any evidence of toxic reactions or ongoing immune-response.

A new insight arisen from this study was that the best TA was not necessarily the one with the most inert immune response but should rather have elicited a minimal to moderate immune response to initiate high tensile strength without presence of an ongoing inflammatory response and subsequent clinical complications. This was based on the presence of macrophages (as depicted by CD68 staining), which stimulate collagen formation. Based on this theory it was found that the included CAs, in particular Histoacryl Flexible and Omnex, showed the most promising immunological profile.

Part IV: Prevention of colorectal AL with tissue adhesives

Chapter 7
In the final part of this thesis the focus of each previous part was pooled into one, examining the clinical relevance of TA sealing by evaluating the capability of a TA to stop leakage of a colonic defect or anastomosis. In Chapter 7, colonic defects were created and then sealed with the same set of TAs as used in Chapter 6. No additional sutures or staples were used, so the only barrier protecting the rat from fecal peritonitis was the TA seal. TA sealing led to a significant decrease in fecal peritonitis when compared to the control group. In that study the CAs Histoacryl Flexible, Glubran 2 and Omnex, as well as the FG Tissucol, showed the most promising results. Albumin-based (Bioglude, GRF) and PEG (Duraseal Xact) TAs were associated with the most leakage-related complications. Histological testing showed that CAs were the most inert, with Histoacryl Flexible showing increased collagen formation at day 10 when compared to the other TAs, implying viable tissue regeneration. It should be noted that Tissucol, despite being clinically inert, showed the highest levels of inflammation at day 10.

Chapter 8
In Chapter 8 the sealing capability of three TAs was evaluated when used to seal colonic anastomoses in a contaminated environment created with the cecal ligation and puncture model. Performing a primary colon anastomosis in a contaminated environment is known to result in high leakage rate, as shown by a decrease in AL in case of use of defunctioning (temporary protective) colostomy in clinical studies. In the current study a protective effect on the incidence of AL was found for each of the used TAs (i.e. Tissucol, Histoacryl Flexible, Duraseal). Despite previous reports from literature in which CAs were associated with poor outcomes when used in high-risk anastomosis (as in contaminated environment), it was found that Histoacryl Flexible was linked to an inert foreign body reaction with no toxic effects on the tissue. The foreign body reaction after adhesive application was moderate in all tissue adhesive groups.

Chapter 9
In the last part of this thesis, in Chapter 9 three CAs (Histoacryl Flexible, Glubran 2, Omnex) were used as colonic sealants in an AL model. In that model colonic anastomoses were created with 4 sutures and then sealed externally. Histoacryl Flexible showed the lowest rate of AL and
consisted only of punctiform abscess formation around the anastomosis, which did not have any clinical consequences. The histological evaluation showed that this TA resulted in the least inflammation and the highest level of collagen formation and healing of the TA groups. The use of Glubran 2 was associated with poor outcomes as reflected by a high rate of AL and mechanical ileus. Histological outcomes showed that Glubran 2 led to more inflammation than the other TAs and was associated with the presence of premature collagen, indicating impaired healing capability.

**Conclusion**

Our research, as presented in this thesis, revealed that certain tissue adhesives (TAs) are promising candidates for the prevention of AL in colorectal surgery. Large differences existed between commercially available TAs in terms of mechanical strength, tissue toxicity and clinical complications. We found that the most promising TAs were the cyanoacrylates, which combined high mechanical strength with a relatively inert histopathological profile, resulting in low tissue toxicity and few clinical complications. Of the cyanoacrylate TAs Histoacryl Flexible was found to be the most promising TA. Based on the results presented in this thesis Histoacryl Flexible can be safely used on colonic tissue and, when used to seal a colonic anastomosis externally, may protect from AL. As the abovementioned studies were performed in rodent models, testing of this TA in a porcine model prior to clinical testing is strongly recommended.

**Future perspectives**

One of the most important aspects that can be derived from this thesis is the need for a more systematic and methodologically sound approach in surgical research on TAs. Despite years of research, it has not been able to produce homogenous results, impairing progression towards the clinical testing phase. As explained in part I a lack of standardization in animal testing protocols has led to large differences in results between researchers testing the same TAs and thus to a lack of consensus on the efficacy of the tested TA.

In other scientific fields like (bio-)engineering validated protocols are in use for the testing of biomedical materials. For example the American Society for Testing and Materials (ASTM) has provided standards for the testing of industrial tissue adhesives. These protocols have, however, not been implemented into surgical research. In this sense, collaboration between surgeons and engineers should be stimulated. Furthermore, a standardization protocol: the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines, has recently come into effect, providing a scaffold for authors to design, perform and report animal studies, hopefully increasing quality of future experimental studies. This is an important step in the field of (surgical) experimental research.

In addition to general guidelines on animal research, there is a need for a set of specific guidelines relating to TA testing. These guidelines should provide information on the minimal requirements regarding experimental methodology and reporting of animal studies.

Another important aspect is the definition of AL. Based on our studies two different forms of AL could be identified, which do not necessarily share the same pathophysiology. Firstly anastomotic dehiscence leading to fecal peritonitis is the most serious form of AL, which may be based on various factors including technical failure and anastomotic ischemia. Secondly the presence of macroscopic abscess formation around the anastomosis can be considered a
specific condition. In Chapters 7, 9 and 10 the presence of small peri-anastomotic abscesses did not lead to any clinical complications and was not considered to be AL. In our opinion, these two types of AL should not be considered to be the same entity and should be reported on separately.

In this thesis mostly rat models were used to evaluate TAs on the colon. The rat has proved to be a useful model with the development of several AL models and with an anatomy large enough to be able to apply the TA to colon with relative ease, especially when compared to our previous mouse models. Rat models do have several setbacks as research has shown that these animals are especially resistant to peritonitis. Furthermore, these models are not large enough to test human applicators or surgical staplers. Future research, as a final step prior to clinical testing, should focus on the testing of TAs in porcine models in which colonic resections and surgical staplers may simulate the clinical situation. These models may also be used to test novel applicators, which will be imperative in future clinical use, notably in laparoscopic surgery.

Regarding TA application, there is still much room for improvement. Future research may focus on novel curing techniques to facilitate the ease of TA use. One may imagine a photosensitive TA, in which the curing/polymerization cascade is triggered by a UV-light after its application. Furthermore, an ‘anastomotic tape’ made up of TA may work to facilitate TA application without the risk of accidental gluing to structures in close proximity to the glue site. Integration of TA into (circular) surgical staplers may be a promising technique, combining a stapled anastomosis with a TA seal in an easy manner.

As described in the conclusion of this thesis the CA Histoacryl Flexible was found to be the most promising TA. It was not linked with negative effects on tissue healing, tissue toxicity or premature degradation and was one of the strongest TAs. The authors believe that there is enough evidence to warrant clinical testing based on these results. However, as described in this chapter, the accurate application of TA remains a challenge. Prior to clinical testing, the authors therefore strongly recommend a follow-up study in a porcine model simulating colonic resection and anastomotic sealing with TA, focusing on the accurate application and correct dosage of this TA.

Lastly, as recent research has shown, suturing abdominal wall incisions with a ‘small bite’ suturing technique in which many small and closely placed sutures are used instead of fewer larger stiches, may prevent the onset of incisional hernia by a better distribution of mechanical forces. These results may also be useful for the colonic anastomosis, in which high pressures may also lead to local anastomotic failure. The presence of a TA seal around the anastomosis may also better distribute mechanical forces, leading to a decrease in anastomotic leakage. This theory should be further investigated in future research.
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Nederlandse Samenvatting
Naadlekkage van een gehechte of gestapelde darmnaad (‘anastomose’) is een regelmatig voorkomende complicatie in de colorectale chirurgie die leidt tot hoge morbideiteit en mortaliteit in patiënten. Het doel van het onderzoek beschreven in dit proefschrift is om te evalueren of het gebruik van weefsellijmen binnen de colorectale chirurgie kan leiden tot verminderde complicaties, in het bijzonder naadlekkage.

In **hoofdstuk 1** wordt een algemene inleiding gegeven over de colorectale chirurgie en naadlekkage als een van de meestvoorkomende complicaties. Het concept van weefsellijmen wordt uitgelicht en het onderzoek in dit proefschrift wordt aan de hand van de verschillende hoofdstukken geïntroduceerd. Daarnaast beschrijft dit hoofdstuk de doelstellingen behorende bij dit proefschrift.

In **hoofdstuk 2** wordt een overzicht van de literatuur gegeven met betrekking tot de preventie van naadlekkage met weefsellijmen. Uit dit overzicht bleek dat er veel heterogeniteit was tussen studies wat betreft experimentele methodologie en communicatie van de resultaten.

In **hoofdstuk 3** wordt er specifiek gekeken naar het gebruik van één groep weefsellijmen, de cyanoacrylaten, op de colorectale anastomose. Het gebruik van deze lijmsoort liet positieve resultaten laten zien, voornamelijk in varkensstudies. Deze studie liet eveneens zien dat er grote verschillen waren tussen studies met betrekking tot experimentele methodologie en lijm-evaluatie.

In deel II van dit proefschrift richtten we ons op de evaluatie van de mechanische sterkte van weefsellijmen. **Hoofdstuk 4** beschrijft ex-vivo onderzoek naar de rheologische eigenschappen en de adhesieve kracht van verschillende weefsellijmen. Deze studie liet zien dat er grote verschillen ontstaan tussen de verschillende categorieën weefsellijmen met als sterkste en meest homogene groep de cyanocrylaten. Fibrinlijm en polyethyleen glycol (PEG) lijm waren de zwakste weefsellijmen.

In **hoofdstuk 5** gebruikten we Dermabond, een cyanocrylaat, om insufficiënte colon-anastomosen te versterken in een ex-vivo varkensmodel. Met een dunne laag weefsellijm konden we de mechanische kracht van de anastomose verhogen tot dat van een normale anastomose.

In deel III van dit proefschrift kijken we naar de effecten van weefsellijm op levend weefsel. In **hoofdstuk 6** worden verschillende weefsellijmen gebruikt om insufficiënte colon-anastomosen te verstevigen in een levend diermodel. Het gebruikte muismodel bleek te klein om de lijmen op een betrouwbare manier te doseren en te appliceren, waardoor er veel complicaties werden gezien. Na deze studie werd er gekozen om verder onderzoek te continueren in rattenmodellen.

In **hoofdstuk 7** beschrijven we een nieuw model, waarin twee lijmverbindingen worden gemaakt in het colon, zonder dat er een defect wordt aangebracht. Hierdoor konden we het directe effecten van de weefsellijm evalueren op wondhealing en mechanische kracht van de verbinding. Deze studie liet zien dat GRF (gelatine-resorcinol-formaldehyde) -lijm en Bioglue leidden tot hoge mate van toxiciteit en klinische complicaties. De cyanoacrylat-lijmen lieten
de hoogste mechanische kracht zien zonder tekenen van weefseltoxiciteit. De PEG-lijm en de fibrinlijm Tissucol waren de meest inerte weefsellijmen, maar waren tevens ook de zwakste.

In het laatste deel van dit proefschrift, deel IV, gebruiken we weefsellijmen om colon-defecten te verstevigen om zo naadlekkage te voorkomen. In hoofdstuk 8, wordt er een nieuw model beschreven waarin twee colon-defecten met een lijmlaag worden gerepareerd, zonder bijkomende hechtingen. Hierdoor was het mogelijk om de beschermende effecten van de weefsellijmen te testen. De cyanoacrylaatlijmen lieten de beste resultaten zien, met een vermindering van klinische complicaties en een stimulering van weefselregeneratie.

In hoofdstuk 9 wordt er specifiek gekeken naar het gebruik van weefsellijmen in een gecontamineerde, reeds geopereerde omgeving. De geteste lijmen lieten een beschermend effect zien op de colon-anastomose.

In hoofdstuk 10 worden de meest veelbelovende weefsellijmen uit het voorgaand onderzoek gebruikt in een rattenmodel om insufficiënte colon-anastomosen te versterken. Dit onderzoek liet eveneens positieve effecten zien van het gebruik van de geïncludeerde weefsellijmen. Hoofdstuk 11 en 12 vormen de samenvatting van de resultaten van de studies beschreven in dit proefschrift, alsmede een algemene discussie en aanwijzingen voor toekomstig onderzoek.
Appendices
PhD Portfolio
About the author
Dankwoord
Oral presentation Erasmus MC 'staff designing and supervising a master's thesis medical student elective

for researchers' (GCP) course, Erasmus MC,
# PhD Portfolio

| Name PhD student: | K.A. Vakalopoulos |
| Erasmus MC Department: | Surgery |
| Research group: | REPAIR Research group |
| PhD period: | 2010-2016 |
| Promotor: | Prof. dr. J.F. Lange |
| Copromotor: | Prof. G.J. Kleinrensink |
| | Prof. dr. J.J. Jeekel |
| | Dr. D. Dodou |

## 1. PhD training

<table>
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<tr>
<th>Courses</th>
<th>Year</th>
<th>Workload (ECTS)</th>
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<tr>
<td>Tropical health course (STOLA) Rotterdam, NL</td>
<td>2008</td>
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<td>Course in basic didactics, Desiderius school, Erasmus MC, Rotterdam, NL</td>
<td>2009</td>
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<td>Good clinical practice for researchers’ (GCP) course, Erasmus MC, Rotterdam, NL</td>
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<td>Laboratory animal science (Art. 9 course), Rotterdam, NL</td>
<td>2010</td>
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<td>Course ‘networking for medical professionals’ KNMG, Utrecht, NL</td>
<td>2011</td>
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<td>Johnson &amp; Jonhson Ethicon biosurgery innovation meeting, Rotterdam, NL</td>
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<td>Rotterdam Interactive Congress on Hernia (RICH), NL</td>
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<td>Oral presentation Research day Sint-Franciscus Gasthuis, Rotterdam, NL</td>
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<td>Oral presentation morning rounds Erasmus MC, Rotterdam, NL</td>
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## 2. Teaching activities

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<tr>
<td>Supervisor MSc program ‘Projects in Advanced Products’ in medical devices TU Delft (10 MSc students during 6 months)</td>
<td>2011</td>
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<td>Supervisor of junior medical students’ research projects Erasmus MC</td>
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<tr>
<td>Supervision TU Delft MSc student (Mr. A. Füzy)</td>
<td>2011-12</td>
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<tr>
<td>Designing and supervising a master’s thesis medical student elective research program (21 weeks, Jul - Dec, Dr. M. Ahieyets)</td>
<td>2011</td>
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<tr>
<td>Designing and supervising a master’s thesis medical student elective research program (21 weeks, Nov - Jun, Dr. L.F. Kroese)</td>
<td>2012</td>
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About the author

Konstantinos Aristotelis Vakalopoulos was born on the 27th of November 1987 in Athens, Greece to a Greek father and Dutch mother. He is the oldest of three brothers. After having spent the first eight years of his life in Greece, the family moved to New York, USA where he attended elementary and middle school. When he was 15, the family moved to Leiden, the Netherlands, where Konstantinos attended high school. From a young age he wanted to become a surgeon, an ambition which led him to the Erasmus Medical Centre, where he started his medical studies in 2006. During his studies he showed a special interest in scientific research, and helped in various research projects in his early student years. He performed his 6-month master thesis with the REPAIR research group (Prof. dr. J.F. Lange, Prof. dr. J.J. Jeekel, Prof. dr. G.J. Kleinrensink) on the link between Advanced Glycemic Endproducts (AGE's) and abdominal wall hernias. In 2012, before continuing with his clinical rotations, he started working as a PhD candidate within the REPAIR research group to investigate the use of tissue adhesives in colorectal surgery, which eventually led to this thesis. During his PhD research he published numerous studies in esteemed scientific journals and presented his research at various national and international conferences. He obtained his medical degree in 2014 from the Erasmus Medical Centre in Rotterdam. He is currently living in Geneva, Switzerland, where he is a surgical resident at the Hôpitaux Universitaires de Genève.
Dankwoord

Allereerst wil ik mij richten tot mijn promotoren. Beste Professor Lange, Beste Johan, toen ik als vierdejaars student tijdens elke REPAIR-vergadering weer eens veel te lang aan het woord was, kon ik mij niet voorstellen dat ik een aantal jaren later u als mijn promotor in het dankwoord van mijn proefschrift zou mogen bedanken. U nam een risico door mij als jonge student te steunen en mij deze ongelooflijke kans te geven. Ik zie nu pas in hoe mijn tijd in de REPAIR mij gevormd heeft als mens, als dokter en als onderzoeker. U leerde mij systematisch te zijn, en `to the point`. In feite leerde u mij hoe een chirurg zich hoort te gedragen. Ik zal uw steun en uw wijsdom nooit vergeten. Ik hoop dat wij in de toekomst nog vaak zullen samenwerken, en de banden tussen Rotterdam en Genève kunnen aanhalen.

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Beste Dimitra, zonder jou had ik hier nooit gestaan. Een synergie dat ons onderzoek een nieuwe impuls en visie heeft kunnen geven. Door jouw ‘ingenieursvisie’ hebben we het wiel op nieuw uitgevonden en de manier van lijمونderzoek doen, veranderd. Jouw betrokkenheid en onuitputtbare focus is iets wat mij keer op keer verbaasde. Altijd klaar om te helpen. Een onderzoeker in hart en nieren, ik weet zeker dat jou nog hele grote dingen te wachten staan. Ook al laat je het niet merken, jouw Griekse kant helpt ook een handje mee 😊


Beste Joris, als vierdejaarsstudent heb je mij onder je hoede genomen. Wat begon als een klein projectje waarvoor we op zaterdag ochtend in de zeilmakerij stonden, is uitgegroeid
tot een waar promotietraject. Zonder jou had dit allemaal nooit plaatsgevonden, iets wat ik nooit vergeten zal.

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Cher professeur Morel et Professeur Bühler. Je vous remercie de m’avoir offert votre confiance dans le cadre de la formation de charge de la chirurgie viscérale. C’est à cette occasion que, par le billet du PhD, je souhaiterais vous témoigner de mon investissement scientifique auprès de vos équipes. Ces travaux sont pour moi le gage de notre collaboration présente et future ainsi que le gage de mon implication au sein des HUG en faisant ainsi une complémentarité parfaite pour ma formation chirurgicale.

Professeur Toso, merci pour votre présence en ce jour si important de ma carrière. J’ai la plus grande admiration pour votre personne et votre travail scientifique. Je me réjouis et me sens honoré de pouvoir travailler à vos cotés les années à venir.

Cher Frédéric, c’est avec beaucoup d’amitié que j’apprécie notre collaboration scientifique, et je garde à l’esprit le souvenir de celui qui m’a donné envie de rejoindre les HUG alors même que je n’étais qu’un stagiaire. Je suis par ailleurs très fier et honoré de la confiance et de l’investissement que tu portes à ma formation et à nos échanges scientifiques. J’ai à cœur de pouvoir satisfaire ton investissement et tes attentes dans les différents domaines qui nous incombe. C’est tel en élève assidue fasse un Mentor attentif que j’envisage c’est prochaines années de formation à tes côtés.

Finally, my family. The most important thing. I cannot express in words how lucky I am to have parents who have always supported me and pushed me through the hard times. I love you with all my heart and I hope, one day, to be as good a parent as you are to me. From Greece, to New York to Holland, we went through a lot of changes as a family, but the one thing that always stayed the same was our solid bond. Mike and Alex, my bro’s. My best friends. Everything I do, I do for you. Even though we live far apart for the time being, our
connection is as strong as ever. I am so proud of the things you do and even prouder that I can call you my brothers.
Tissue adhesives in colorectal surgery: a stepwise approach

Konstantinos A. Vakalopoulos