Mechanical Strength and Rheological Properties of Tissue Adhesives With Regard to Colorectal Anastomosis

An Ex Vivo Study

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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.

Keywords: anastomotic leakage, colorectal surgery, surgical glue, tissue adhesives

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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,[1–4] and mesh fixation.[5] 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.[6] These experiments, mostly on animal models, provide insight into the effectiveness of tissue adhesives on surgical complication rates, particularly AL.[7]

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.[8,9] Modern-day CA are becoming less histotoxic and more flexible.[10] 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.[3,8] 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.[11] 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.[2] 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.[12,13]

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

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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 postapplication 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.[7] 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.

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.[15] 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 tap water, 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

TABLE 1. Included Tissue Adhesives

Adhesive Category	Commercial Name	Company	Composition
CA	Histoacryl Flex	B. Braun (Tüttlingen, Germany)	n-butyl-2-cyanoacrylate
	Glubran 2	GEM Italia (Viareggio, Italy)	n-butyl-2-cyanoacrylate and methacryloxysulfolane
	Omnex	Ethicon (J&J, Bridgewater, NJ)	2-octyl-cyanoacrylate and butyl lactoyl cyanoacylate
	Dermabond	Ethicon (J&J, Bridgewater, NJ)	2-octyl-cyanoacrylate
AB adhesives	Bioglue	Cryolife (Kennesaw, GA)	Glutaraldehyde-albumin glue
	Covabond	Covalent medical inc. (Ann Arbor, MI)	Albumin, aldehyde cross linker
	GRF	Cardial SA (St Etienne, France)	Gelatin-resorcinol-formaldehyde glue
PEG adhesives	DuraSeal Xact	Covidien (Mansfeld, MA)	PEG, trilysine amine, blue dye, N-hydroxy succinimide
	CoSeal	Baxter (Deerfield, IL)	PEG, hydrogen chloride and sodium phosphate-sodium carbonate
	DuraSeal	Covidien (Mansfeld, MA)	PEG, trilysine amine and blue dye
FG	Tissucol	Baxter (Deerfield, IL)	Fibrin glue, with aprotinin
	Evicel	Ethicon (J&J, Bridgewater, NJ)	Fibrin glue, without aprotinin

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.[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,

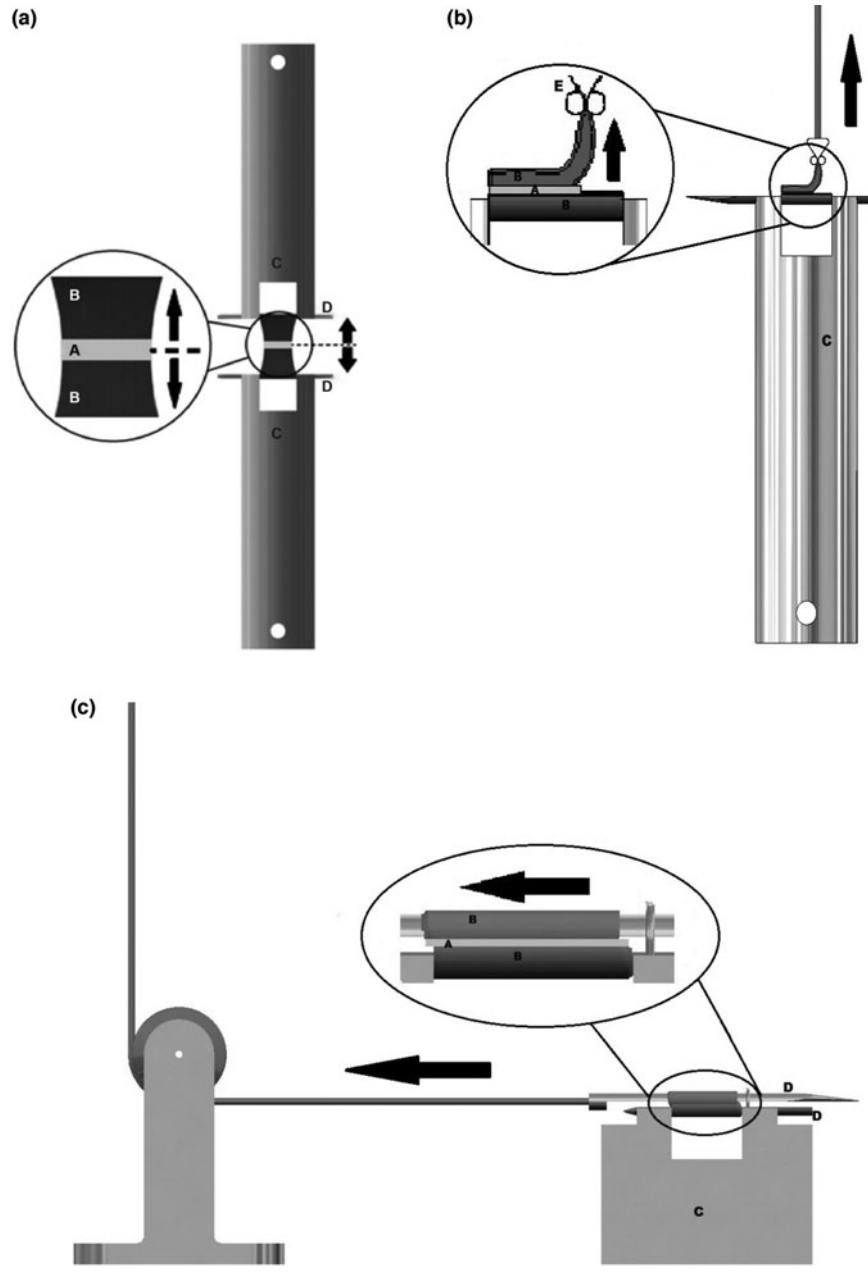
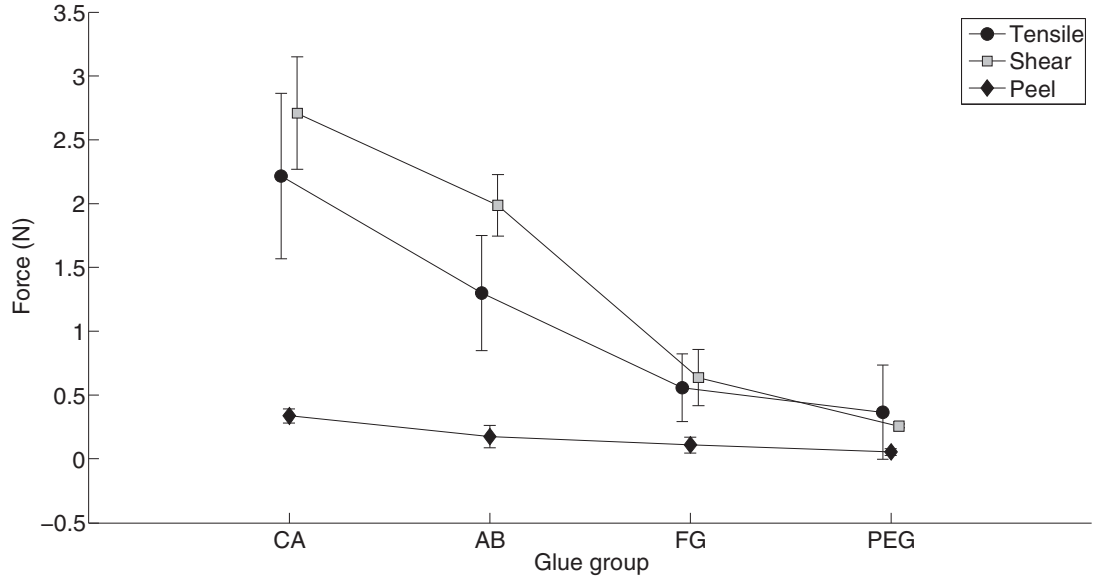


FIGURE 1. Experimental configuration, per test type. Arrows define direction of motion. (a), *Tensile strength test.* Legend: A: Glue. B: Colon segments. C: Cylinder. D: Colonic pin. (b), *Peel strength test.* Legend: A: Glue. B: Colon segments. C: Cylinder. D: Colonic pin. E: Clamp. (c), *Shear strength test.* Legend: A: Glue. B: Colon segment. C: Fixation device. D: Colonic pin.



Adhesive category	Tensile strength	Shear strength	Peel strength
CA vs AB	$t_{20} = 2.61$ ($P = 0.017$)	$t_{19} = 1.97$ ($P = 0.064$)	$t_{19} = 4.24$ ($P < 0.001$)
AB vs PEG	$t_{17} = -4.01$ ($P < 0.001$)	$t_{15} = -6.13$ ($P < 0.001$)	$t_{18} = -2.60$ ($P = 0.018$)
PEG vs FG	$t_{11} = 0.55$ ($P = 0.596$)	$t_{11} = 2.95$ ($P = 0.013$)	$t_{11} = 0.705$ ($P = 0.496$)

FIGURE 2. Mechanical adhesive strength between adhesive categories. Results of statistical analysis are shown in the table. CA indicates Cyanoacrylate; AB, Albumin based adhesives; PEG, Polyethylene glycol adhesives; FG, Fibrin glues.

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

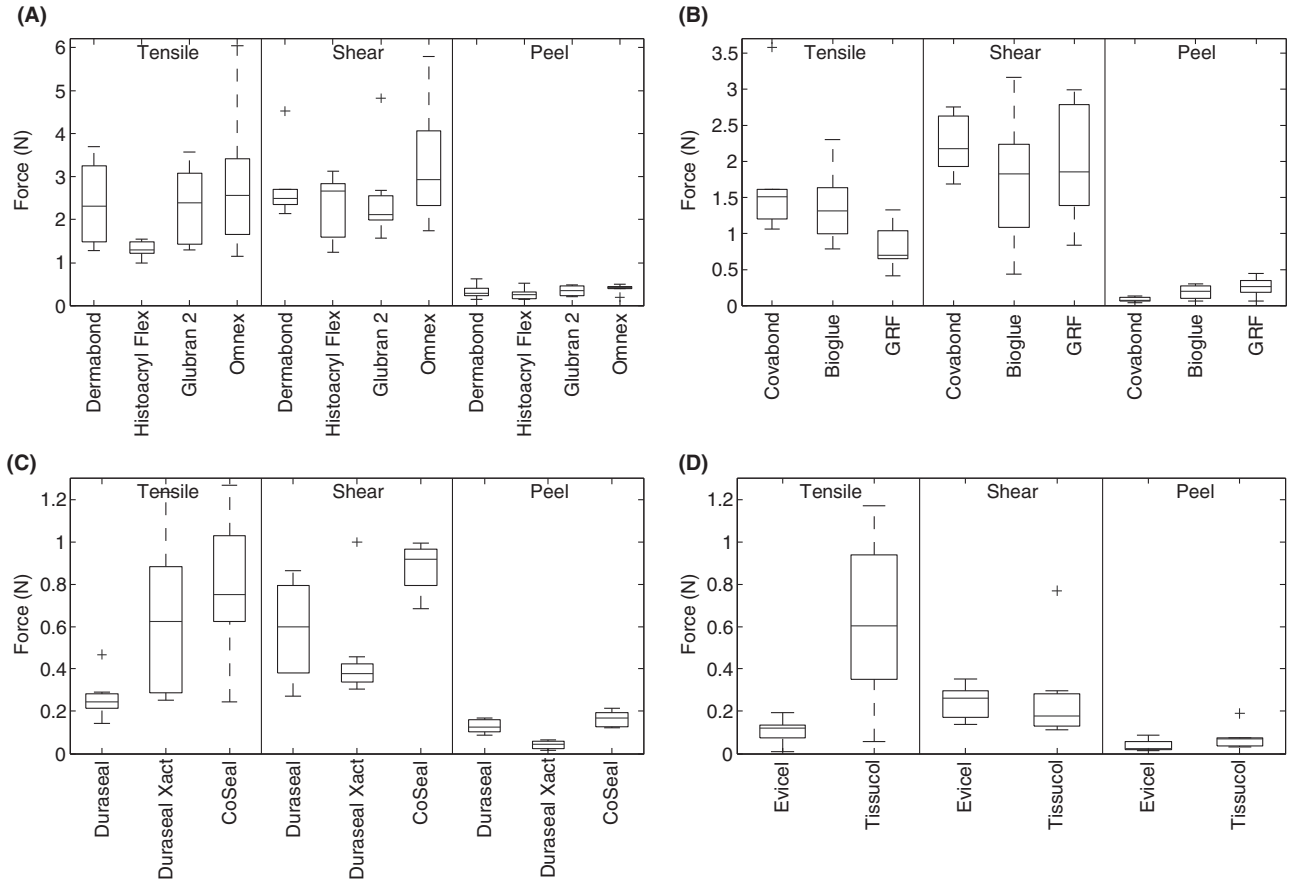
A paired *t* test was used to compare adhesive categories with each other with respect to their tensile, shear, and peel strength, and a 1-way analysis of variance with a post hoc Tukey-Kramer test was conducted to compare adhesives within categories. Pearson correlations were calculated between the 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



Adhesive categories	Tensile strength	Shear strength	Peel strength
CA	$F_{3,24} = 2.60$ ($P = 0.076$)	$F_{3,23} = 1.22$ ($P = 0.325$)	$F_{3,23} = 1.09$ ($P = 0.372$)
AB	$F_{2,22} = 5.61$ ($P = 0.011$)	$F_{2,19} = 0.74$ ($P = 0.491$)	$F_{2,18} = 6.23$ ($P = 0.009$)
PEG	$F_{2,15} = 5.17$ ($P = 0.020$)	$F_{2,14} = 5.29$ ($P = 0.020$)	$F_{2,17} = 32.68$ ($P = 0.001$)
FG	$t_6 = -3.19$ ($P = 0.019$)	$t_6 = -0.303$ ($P = 0.773$)	$t_6 = -1.53$ ($P = 0.176$)

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.

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 ($t_6 = -3.19$, $P = 0.019$). 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.

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).

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 2×10^8 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 1×10^5 Pa to 1×10^6 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

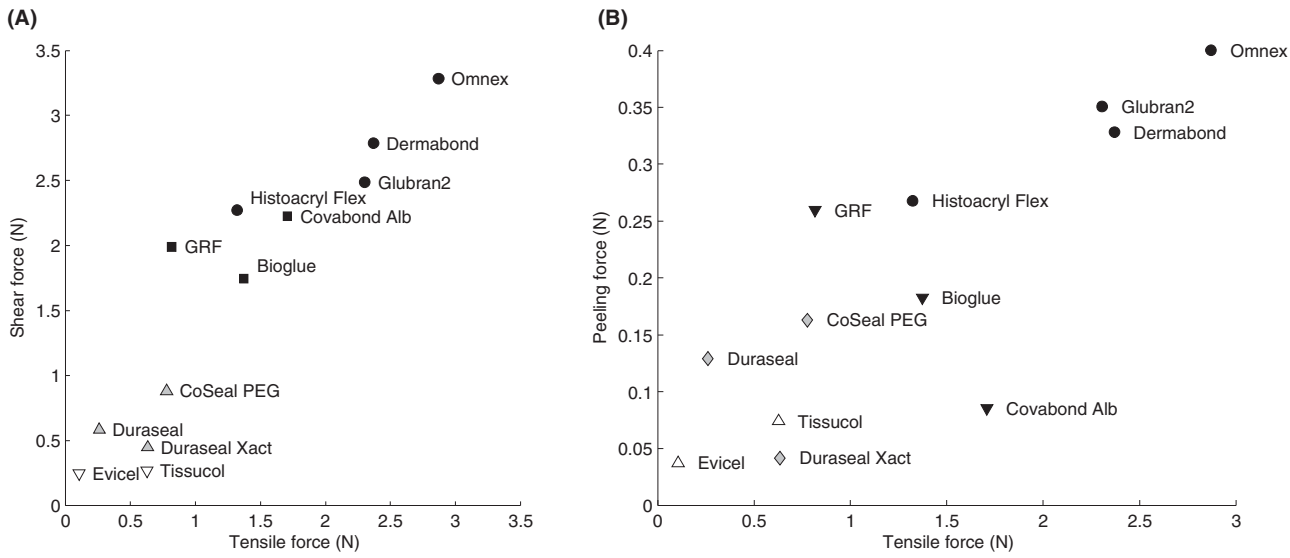


FIGURE 4. Correlation analyses. A, Tensile strength test vs shear test; correlation coefficient $r = 0.504$ ($P < 0.001$). B, Tensile strength vs peel test; $r = 0.578$ ($P < 0.001$).

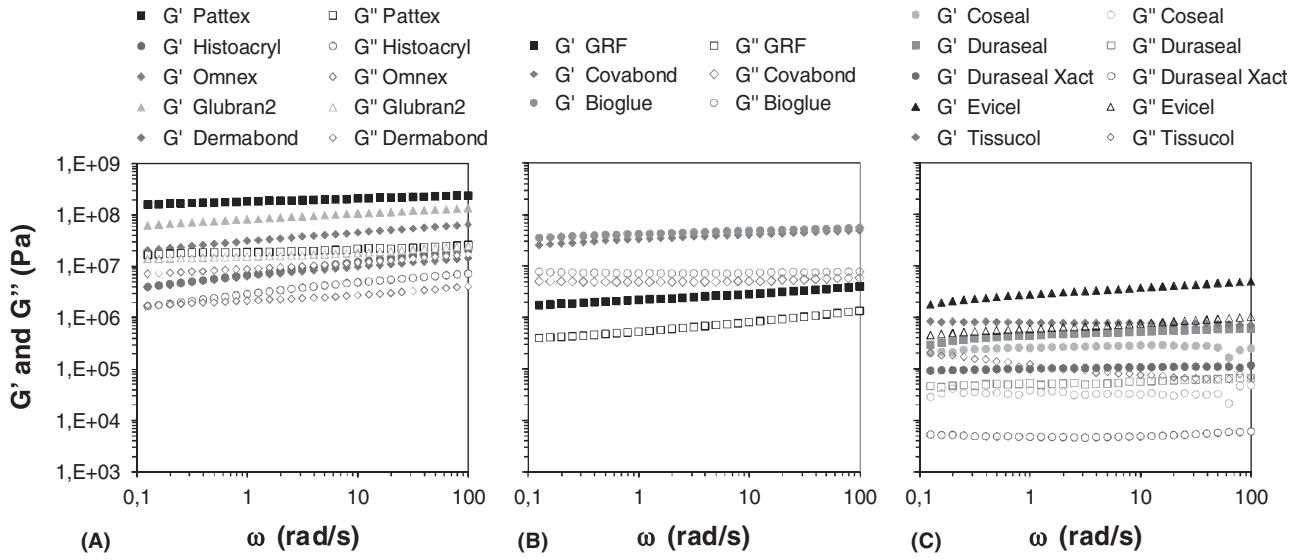


FIGURE 5. Frequency (ω) dependency of the real (G′) and imaginary (G″) shear modulus components for the following: A, Pattex, Histoacryl, Omnex, Glubran, and Dermabond. B, GRF, Covabond, and Bioglue. C, Coseal, Duraseal, Duraseal Xact, Evicel, and Tissucol.

that this gel contains 90.1% water. Although the solid content of the 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).

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 1×10^6 Pa to 1×10^7 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: $r_{G'} = 0.637$; $r_{G''} = 0.692$).

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.[4,5,19] 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

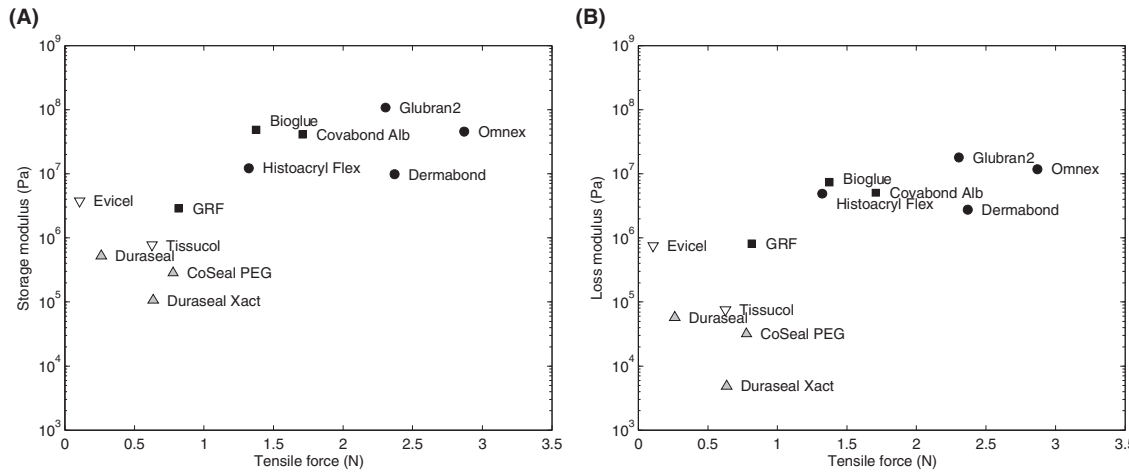


FIGURE 6. Correlation analysis between rheological results and tensile strength test. A, Storage modulus vs tensile strength; correlation coefficient $r = 0.711$ ($P < 0.001$). B, Loss modulus vs tensile strength; $r = 0.716$ ($P < 0.001$).

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 of 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 Bioglue in a model using periosteum from human cadavers. Azadani et al[22] compared the mechanical strength of several FG and Bioglue 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.[24] 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 Bioglue 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.[25] To ensure reproducible and correct application, we used the application procedure described previously by Nishimori et al[26] 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.[27] 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 Histoacryl Flex and Dermabond were the most flexible adhesives, yielding rheological profiles comparable to the AB. The increased flexibility of n-butyl cyanoacrylate–based (Histoacryl) 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 Bioglue 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 Bioglue 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 Evicel was almost similar to the PEG-based DuraSeal (9.8% compared with 9.9%), Evicel 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.[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 noninterchangeable 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.

CONCLUSIONS

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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