



BASIC RESEARCH

# Mechanical behavior of colonic anastomosis in experimental settings as a measure of wound repair and tissue integrity

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ues to anastomotic failure.

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## Abstract

**AIM:** To determine the mechanical properties of anastomotic colonic tissue in experimental settings and therefore give a measure of wound healing.

**METHODS:** Thirty-six male Wistar rats were used as experimental models of anastomotic tissue integrity. On the 5<sup>th</sup> post-operative day, the tensile strength was measured by application of an axial force, providing a quantitative measure of anastomotic dehiscence and leakage.

**RESULTS:** Diagrams of the load as a function of the time [ $P = P(t)$ ] and of the displacement also as a function of time [ $\Delta s = \Delta s(t)$ ] were recorded for each test, permitting the design of the load versus the displacement diagram and thus providing significant data about the critical values of anastomotic failure. Quantitative data were obtained concerning the anastomotic strength of both control specimens (healthy rats), as well as specimens from non-healthy rats for comparison.

**CONCLUSION:** This experimental model provides an excellent method of measuring anastomotic strength. Despite the relative small number of specimens used, this method provides an accurate way of measuring wound repair. More experimental measurements need to be performed to correlate emerging tensile strength val-

## INTRODUCTION

Investigating wound healing and attempting to improve its outcome necessitates process quantification<sup>[1]</sup>. Parameters for anastomotic repair and adhesion formation<sup>[2]</sup> may be mechanical, biochemical, or histological. Histology is not a primary tool for quantification when comparing various series of experimental anastomoses. Certainly it is very useful to describe the course and eventual result of the healing sequence at the tissue level. Also, the successive infiltration of various cells into the wound area may be followed, and obvious differences between anastomoses (e.g., ileal and colonic) will certainly be demonstrated this way. However, the measurement of choice to evaluate anastomotic repair and the effects of variations in surgical techniques, administration of drugs, or of any other modification to establish procedures, will mostly be either mechanical or biochemical or both.

The developing mechanical strength is, without doubt, a meaningful parameter to follow while investigating anastomotic healing. For this purpose, two fundamentally different approaches can be chosen. First, one can choose bursting strength, which is expressed either as bursting pressure or bursting wall tension, which is the measure of the resistance of the intestinal wall to increasing intraluminal pressure. Second, one can choose breaking strength, which reflects the resistance of the intestinal wall to forces exerted in a longitudinal direction<sup>[3-5]</sup>.

While both of these methods used to evaluate anastomotic healing have been investigated in the international literature, no paper has so far described

in detail the process itself, analyzing its advantages, disadvantages and parameters taken into consideration, therefore establishing the need for an in-depth presentation of the mechanical apparatus used and presenting not only the theoretical background behind the measurements but also the technical difficulties that arise.

## MATERIALS AND METHODS

Thirty-six male Wistar rats weighing 300-350 g were used, and were housed two per cage. They were fed a standard diet and water *ad libitum*. All experiments were approved by the Athens Prefecture, Directorate of Veterinary Services (License No. K/355/27-1-2005), according to the Presidential Decree No. 160/1991 (Governmental Gazette A' 64), with which Greece has conformed to the 86/609/EEC directive. Laparotomy<sup>[6]</sup> was performed through a midline 2 cm incision under anesthesia induced by ketamine (80 mg/kg) and xylazine (3 mg/kg). A colonic segment, 1 cm in length, 5 cm distal to the ileocecal junction was transected and the colon was re-anastomosed end-to-end using 5-0 Vicryl (Ethicon) sutures in single-layer interrupted fashion<sup>[7]</sup>. About 10 sutures were placed for each anastomosis to secure an inverted anastomosis without mucosal protrusion, which is regarded as a major cause of perianastomotic adhesions. The abdominal muscle wall was then closed with 5-0 Vicryl (Ethicon) sutures, followed by skin closure with 4-0 Silk (Medipac) sutures.

To obtain the test specimen, the rats were sacrificed with an overdose of ether, on the 5<sup>th</sup> post-operative day. The previous abdominal incision was reopened, and the anastomotic site identified and inspected for possible adhesions and leakage. An 8 cm segment of the colon with the anastomosis in the middle was resected. Care was taken not to detach adhesions from the anastomosis, but to dissect the surrounding tissues. The resected specimen was gently irrigated with saline to remove feces and was mounted on a table.

The basic purpose of the present experimental protocol is the determination of the mechanical behavior of intestinal anastomoses and more specifically the response to tensile loading and the determination of the respective tensile strength. One can define the ratio of the applied force at the moment of failure,  $F_{cr}$ , over the surface,  $A$ , upon which the force acts normally<sup>[8]</sup>, as tensile strength (Figure 1). The ratio of the force over the respective area is known in engineering science as stress and therefore the tensile strength is the respective tensile stress at the moment of failure.

In the international scientific literature the mechanical behavior under tension, of specimens like the ones of the present protocol, has been studied in two ways: (1) By applying an internal hydraulic pressure,  $p$ ,<sup>[9-12]</sup>. In this case (and for points relatively far from the borders of the specimens) a stress state equivalent to the so-called biaxial tension appears on the surface of the concave cylindrical specimen. Assuming that the thickness of the specimens is much smaller in comparison to its diameter, the principal tensile stresses at the moment of failure are given by the

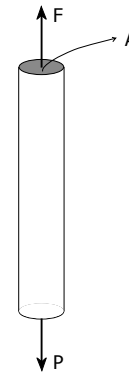


Figure 1 Typical forces applied on a standard specimen.

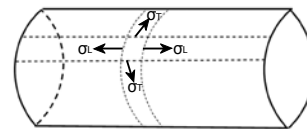


Figure 2 The application of hydraulic pressure creating a two-dimensional stress field.

relations (Figure 2)<sup>[13]</sup>:

$$\sigma_L = \frac{p_{cr}r}{2t}, \quad \sigma_T = \frac{p_{cr}r}{t}$$

where  $p_{cr}$  is the value of the hydraulic pressure at the moment of the first failure,  $r$  the radius of the intestine and  $t$  its wall thickness. The stress system is characterized as principal since no shear stresses can be generated by a pure hydraulic pressure.

(2) By applying directly an axial force,  $F$ ,<sup>[14,15]</sup>. In this case the critical value of the tensile strength is expressed as:

$$\sigma_{cr} = \frac{F_{cr}}{2\pi r t}$$

The procedures described above present both advantages and disadvantages. More specifically, the application of hydraulic pressure, although relatively easily realizable in the laboratory, creates a two-dimensional stress field, as shown in Figure 2. Therefore the conclusions drawn are not directly comparable to the respective ones of the experiments with uniaxial loading. Especially in the case of anisotropic materials (like the ones of the present study), it is impossible to define which one of the two stresses is responsible for the failure and therefore it is not possible to determine the critical value, since the direction at which failure will appear is not *a priori* known.

On the contrary, the application of an axial force is especially difficult from a practical point of view (as it will be seen in the next paragraph), but the results obtained are directly usable without reductions and additional assumptions.

### Experimental difficulties of the direct tension experiment

In the present study the second procedure (direct tension) was adopted. The most important difficulties stated above

are summarized as following: (1) The nature of the materials under study renders the gripping of the specimens, with the aid of conventional friction grips through compression loads, extremely difficult. In fact, since it is impossible to form “gripping heads” to the specimens (“dog-bone” specimens), it is given that the failure will appear in the portion of the specimen which is inside the grips or in their immediate vicinity. However, in this area the stress field is strongly triaxial and therefore the results obtained are invalid and should be rejected. On the other hand, the limited chances to obtain long specimens in combination with the low friction coefficient between the external surfaces of the specimens (intestines) do not allow the use of pulley-shaped grips, in which the holding force emanates from the friction of a number of successive layers of the material rolled around the periphery of the pulley. (2) The extremely low force which is necessary for the fracture of even intact and healthy specimens, which according to international literature is estimated at the value of a few tenths of Newton<sup>[14]</sup>, renders the conventional arrangements of applying axial tension practically useless. (3) The nature of the specimens under study, which are twisted and bended around different axes, due to adhesion formation around the anastomotic area, renders the measurements of length changes and therefore of reduced deformations (strains,  $\epsilon$ ) almost impossible. (4) Finally, the nature of the intestines once again, which under torsion are “self-configured” into the form of plane plates, results in an interaction between the walls of the specimens, making difficult the reduction of the external loads into stresses ( $\sigma$ ). Another factor making the situation more difficult is the non-constant thickness of the specimens throughout their length and their perimeter, which does not allow us to calculate the effective area of the loaded intestine.

In order to confront these difficulties in the present experimental study, the following procedures were adopted.

### Gripping the specimens

A specific gripping system was designed, consisting of a pair of light metallic pins of cylindrical cross-section of diameter equal to 5 mm, with rounded head which permits easy entrance of the intestine in the pin, without injuring the specimen walls, reducing thus the time required for the in-situ preparation of the specimens (Figure 3). The pins are grooved at their mid-length and a suture which holds the specimens in place is rolled up in this groove. The upper part of the pins is drilled through the thickness and the specimen is suspended through this hole from the upper plate of the loading frame. At the same time, the second pin is fixed to the immobile plate of the frame.

The suspension and fixing of the pins is achieved with the aid of circular rings. In this way the maximum possible number of degrees of freedom is given to the specimen making possible the self-alignment and the “untwisting” of the intestine during tension without external limitations and therefore without, as much as possible, the development of parasitic tensions and disfigurations.

Despite the low total weight of the specimens gripping system (about 0.12 N), it was deemed appropriate to add the weight of the lower half, which is suspended



**Figure 3** The gripping system consisting of a pair of light metallic pins. The grooves at which the intestine is gripped are indicated by the arrows.

and therefore sustained by the specimen, at the value of the final failure load, taken into consideration that these values are relatively comparable (the mean value of the failure load, as it was obtained from a series of preliminary experiments is equal to about ranges between 1.00 N and 2.20 N).

### The load application system

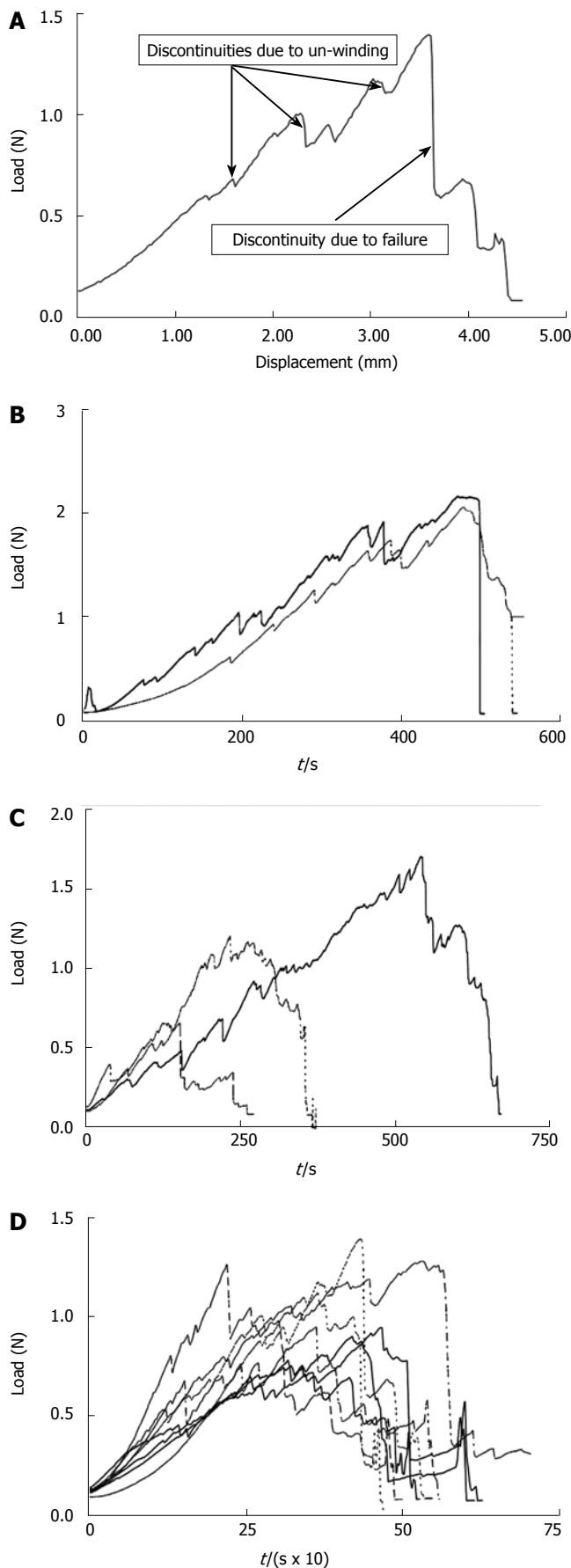
After the rejection of loading through the application of dead weights (water or lead grains), due to the induction of vibrations and oscillations, a special load cell of capacity of 5 N and sensitivity of  $10^{-3}$  N was used attached in a stiff electrical loading frame (Instron). This frame was selected, apart from its robustness, because it provides the ability of choosing the load application speed between wide ranges (from 0.5 mm/min to 500 mm/min). This characteristic of the frame is very important in case biological materials are to be studied, since their mechanical behavior exhibits viscoelastic nature, which is strongly dependent on the strain rate induced ( $d\epsilon/dt$ ).

In the first phase of the experimental project an especially low tension speed (1 mm/min) was selected, and therefore the loading can be considered as static or at least quasi-static. As a result the overall duration of each test usually exceeds 10 min. It is planned, in a second phase, to study the effect of loading rate by employing dynamic or quasi-dynamic protocols.

### Calibration of the apparatus

Before starting the main series of experiments, a number of preliminary tests were carried out, in order to define the range of the expected values of both the failure load and the elongation of typical specimens, and to calibrate the apparatus in the specific range of values.

The calibration of the loads was achieved with the safest method of the suspension of standardized (certified) weights from the load cell. Both the absolute reading values of the load cell as well as their linearity at the range of the expected loads were checked. The deviations detected for the absolute values of the loads did not exceed in any case the limit of 0.2% set by the “Quality Assurance System” of the Laboratory of Testing and



**Figure 4** Load versus time and displacement diagrams for characteristic tests. **A:** Load versus displacement for a typical test of the preliminary series; **B:** Load versus time using intact specimens from healthy rats; **C:** Load versus time using specimens from healthy rats after colonic anastomosis; **D:** Load versus time using specimens from non-healthy rats after colonic anastomosis.

Materials of the National Technical University of Athens (NTUA/LTM), as it is described in the respective "Quality Assurance Manual" according to ISO9000/2000 system.

On the other hand, the linearity of the values of the loading cell in relation to the respective ones of the standard weights exceeded 99.8% for the whole range of interest, as it was concluded from a linear interpolation in the experimental data, using the least square method.

The calibration of the readings of the load frame for the displacements was achieved with the aid of three LVDT's (Linear Voltage Displacement Transducers), which have been verified with a standard micrometric vernier of an accuracy of 1  $\mu$ m. Apart from the absolute values of the displacements, the parallel of the motion of the loading frame was also checked. The deviations detected did not exceed in any case the limits set by "Quality Assurance System" of the NTUA/LTM. Finally, the time recording device of the data acquisition and storage system was also calibrated with the aid of a prototype chronometer. The deviations were not measurable.

#### Data acquisition and storage system

The data to be recorded during the experiments include the values of the load as a function of the time [ $P = P(t)$ ] and the values of the displacement of the moving plate of the loading frame also as a function of time [ $\Delta s = \Delta s(t)$ ]. The data acquisition system includes a special multi channel "bridge" (National Instruments, type SCXI-1000), with the ability of adjusting the sampling rate. The system includes, also, a personal computer with suitable commercial software (LabVIEW-8). From the functions  $F = F(t)$  and  $\Delta s = \Delta s(t)$  recorded, one can eliminate the time obtaining the function of the applied force as a function of the displacement induced and therefore as a function of the elongation of the intestine, i.e.  $F = F(\Delta s)$ .

After the preliminary experiments, it was deemed appropriate to add to the data acquisition system a video device, in order to monitor the specimen during the experiment in a mode synchronous to the recording of the values of the load and the displacement. This was considered necessary, since the records of the load versus presented oscillations, due to two different reasons: (1) The "un-twisting" of the twisted parts or the "un-folding" of the folded parts of the intestine, which lead to a sudden length increase of the specimen, and therefore to instantaneous unloading, that is to a fall in the recorded load, as it is shown characteristically in the diagram of Figure 4A. (2) Local failures of parts of the specimen and especially in the case of anastomosed intestines failure of the anastomotic area itself or of directly neighboring areas, due to the tearing of the material from the anastomotic suture.

Since one cannot distinguish between these two discontinuities of the  $F = F(t)$  diagram, the synchronous video-recording of the experiment was considered necessary. In this way it is possible to locate the discontinuities of the diagram due to the "un-twisting" or to the "un-folding" of the specimen, until the discontinuity due to the anastomotic failure or failure of its immediate neighboring area. Therefore the loading corresponding to this discontinuity can be safely considered as the crucial anastomotic failure load.

## RESULTS

Three different classes of specimens were tested using the system described in the previous paragraphs.

The first one included a number of “intact” specimens, i.e. specimens from healthy rats without anastomoses. The results of these tests are to be used as a measure that will permit the characterization of the quality of the anastomosis, at least from the point of view of mechanical strength. Two characteristic examples of these tests are shown in Figure 4B. The data obtained from these tests for the failure force exhibited very small scattering (as it was perhaps expected) and the average value was of the order of:

$$F_{cr}^{intact} = 2.09 \text{ N} \pm 0.6 \text{ N}$$

Taking into account that the thickness of the wall of the intestine of the rats after the 8th week of their life is stabilized to about 1.1 mm while its perimeter varies in the range 9-12 mm<sup>[16]</sup>, it is concluded that the tensile failure strength of the “intact” specimens ranges between:

$$160 \text{ kPa} \leq \sigma_{fail}^{intact} \leq 210 \text{ kPa}$$

The second class of experiments included the control tests, namely it was carried out using specimens obtained from healthy rats but after having been subjected to colon anastomoses. The scattering of this series of tests was obviously higher compared to that of the “intact” specimens and it was considered necessary to study the acceptability of the results based on statistical experiments. The Chauvenet criterion was adopted and a number of tests were excluded from the analysis. In Figure 4C the results of three tests of this series are shown, corresponding to the ones with the lowest and highest acceptable failure forces and to a third one with failure force almost equal to the average value. The average value for the failure force was determined equal to:

$$F_{fail}^{control} = 1.35 \text{ N} \pm 0.42 \text{ N}$$

Similarly the failure strength ranges between:

$$100 \text{ kPa} \leq \sigma_{fail}^{control} \leq 135 \text{ kPa}$$

It can be concluded that the present procedure for the anastomotic operation results in a decrease of the mechanical strength of the colonic segments under study of the order of only 35% in comparison to the intact specimens.

As a final step, a third series of tests was carried out with specimens obtained from non-healthy rats after having been subjected to colon anastomotic surgery. It was strange to observe that the scattering of the results was rather lower in this case and the application of the Chauvenet criterion yielded the exemption of only one test. A number of characteristic tests of this class experiments is shown in Figure 4D. The average value for the failure force, for this series of tests was determined equal to:

$$F_{fail}^{non-healthy} = 1.09 \text{ N} \pm 0.19 \text{ N}$$

In this case failure strength ranges between:

$$82 \text{ kPa} \leq \sigma_{fail}^{non-healthy} \leq 110 \text{ kPa}$$

The decrease of the mechanical strength compared to the intact specimens is of the order of about 50%, while if the comparison is carried out on the basis of the results of the control tests, is of the order of about 19%.

## DISCUSSION

Wound leakage, the major concern for every surgeon performing intestinal anastomosis, is considered a multifactorial process, upon which many factors act, accelerating or inhibiting its metabolic pathway<sup>[17,18]</sup>. Numerous clinical entities and metabolic abnormalities can alter the course of tissue repair. Amongst them diabetes mellitus, hypothyroidism, immunocompetence, infection and other diseases are proven to be detrimental to anastomotic healing, while other factors like the surgical technique, advanced age, malnutrition, obesity, inadequate perfusion and/or oxygenation are considered risk factors for impaired wound healing<sup>[19-22]</sup>.

Taking the 5<sup>th</sup> post-operative day as a crucial time point upon which anastomotic failure is mostly recognized in clinical practice, the authors tried to give a measure of the anastomotic strength by taking advantage of its mechanical behavior. While both bursting pressure and tensile strength are used to describe the mechanical properties of viscoelastic materials like the ones under study, the authors preferred to evaluate the second and correlate it to the healing of colonic anastomosis. This is because tensile strength appears to be a better standard to evaluate the biological aspects of healing. Tensile strength is an important determinant of anastomotic strength, in contrast to the bursting pressure, which can evaluate the overall anastomotic integrity, but may reflect healing less accurately.

The authors in this paper described not only a system for gripping the specimens, the load application, the data acquisition and storage system, but also a detailed view of the theoretical background behind the forces applied in the tissues under study, as well as the experimental difficulties of the direct tension experiment.

The values of the load as a function of the time [ $P = P(t)$ ] and the values of the displacement of the moving plate of the loading frame also as a function of time [ $\Delta s = \Delta s(t)$ ] were recorded, giving the load versus the displacement curve for each measurement and therefore providing the recorded discontinuities due to the anastomotic failure.

While the tests performed were used only for a preliminary series of measurements, since the number of specimens was relatively small, significant conclusions can be made regarding wound strength and tissue regeneration. The decrease of the axial force required causing mechanical failure from 2.09 N in case of the “intact” specimens to about 1.35 N for the control tests and to 1.09 N for the specimens from non-healthy rats is an excellent index of the quality of the anastomotic operation. Of course, a larger number of measurements need to be carried out, so as to provide a more rigid approach to tissue leakage, its quantitative expression through the tensile strength experiments, and its clinical correlations with pathological entities that delay wound healing or with factors that promote anastomotic integrity and repair.

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