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# Hysteresis of a biomaterial: Influence of sutures and biological adhesives

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**Abstract** We studied the changes in energy consumption of samples of calf pericardium, when joined or not joined by sutures and adhesives, by means of hysteretic cycles. Sixty-four samples were subsequently subjected to tensile stress until rupture. An overlapping suture sewn in the form of a rectangle presented an acceptable mean resistance to rupture of over 10 MPa, although lower than the mean values in an unsutured control series where the mean resistance surpassed 15 MPa. The contribution of an acrylic adhesive to the resistance to rupture was negligible. The sutured samples that were reinforced with adhesives and had not been subjected to hysteretic cycles prior to rupture showed an anisotropic behavior. This behavior appeared to be lost in all the samples that underwent hysteretic cycles. We found an inflection point in the stress/strain curve following the stepwise increase in the load, with a value greater than and proximate to the final load applied. This inflection should be analyzed by means of microscopy. Finally, the mathematical relationship between the energy consumed and the stress applied, the strain or deformation produced and the number of cycles of hysteresis to which the samples were subjected was established as the ultimate objective of this study. The bonding systems provoked a greater consumption of energy, with the greatest consumption corresponding to the first cycle in all the series assayed. An equation relating the energy consumption in a sample to the number of hysteretic cycles to which it was subjected was obtained. Its asymptote on the *x*-axis indicates the energy consumption for a theoretical number of cycles, making it possible to estimate the durability of the sample.

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## 1 Introduction

The energy consumed by a heart valve leaflet during each cycle is probably a good marker for its durability [1]. Second generation prostheses made of calf pericardium, which have an excellent hemodynamic behavior, can be recommended for implantation to replace aortic valves, especially in elderly patients [2].

When functioning properly, a native heart valve tends to consume a minimum of energy with the lowest possible pressure gradient in its movements since it must perform millions throughout the lifetime of an individual. Diseases that demand a greater cardiac output necessarily increase the heart rate or the pressure on the heart valves since they increment the force required for the left ventricle to pump blood, resulting in hypertrophy. In both cases, the energy consumption is greater, a circumstance that deteriorates cardiac function and leads to a loss of expectancy and quality of life [3].

The biomaterials employed in the design of a bioprosthetic heart valve leaflet are going to be subjected to a repetitive force alternating between flexural and tensile stresses that must be absorbed by their collagen fibers, with a minimum of energy consumption, if they are not to be severely damaged [4, 5]. We consider this to be the essence of durability. A recent study of the need for reintervention and of survival in patients with mechanical prostheses and porcine bioprostheses [6] demonstrated that there are no differences between the two types of devices, except for a greater risk of bleeding in patients with mechanical prostheses, for up to 8 years after implantation in mitral position and for up to 12 years in aortic position [7]. Bioprosthetic valve failure beyond those years is caused by structural failure due to a loss of mechanical resistance [8–10].

When we construct a bioprosthesis using calf pericardium, we bring into contact a number of different materials such as sutures, rings, pericardium, etc., in order to create a functional unit [11–14]. These materials usually have differing mechanical and elastic properties that generate internal shear stresses among them when they form part of a working bioprosthesis [15]. These internal stresses are known to damage the structure, with the resulting negative effects on its durability [16]. The absorbance of these stresses requires additional energy consumption on the part of the valve leaflet. If we accept the hypothesis that the amount of energy produced in a valve leaflet (hysteresis) in each cycle responds to an equation that is related to time (cycles), the asymptote of the curve expressed by said equation will indicate the durability of the leaflet.

The objective of this study was to evaluate the energy consumed in the hysteretic cycles of a biomaterial, calf pericardium, employed in the manufacture of cardiac bioprostheses. We also analyzed the effects of sutures and biological adhesives on that biomaterial during the hysteretic cycles, as well as its elastic behavior and resistance, for the purpose of devising a model for bonding the materials utilized in the construction of a heart valve leaflet.

We subjected 64 samples of calf pericardium to tensile stress with stepwise increases in load followed by unloading until the samples tore. Sixteen samples were cut in half and sewn with an overlapping suture and 32 received the same treatment but with reinforcement of the suture with one of two types of biological cyanoacrylate adhesives. The remaining 16 samples were left intact and were assayed without sutures or adhesives as a control group. The tensile testing was performed in samples cut in both longitudinal and transverse direction in all four series.

The purpose of these tests was to establish the mathematical expressions that relate the energy consumed by the pericardium thus prepared with the stress withstood, the deformation produced and the number of cycles carried out. The knowledge of these functions should help to determine more precisely the durability of the biomaterial.

## 2 Material and methods

### 2.1 Procurement of the calf pericardium

Calf pericardium was obtained directly from a local abattoir. The animals had been born and bred in Spain and were sacrificed between the ages of 9 and 12 months. The tissue was transported in cold isotonic saline (0.9% sodium chloride) to the laboratory, where it was carefully cleaned.

The sacs obtained corresponded to the parietal pericardium that covers the anterior portion of the heart. Once cut open, in such as way as to leave the diaphragmatic ligament in the center and the sternopericardial ligaments on the circumference (Fig. 1), the pericardial sacs measured approximately 15 cm long, from root to apex, and 20 cm wide.

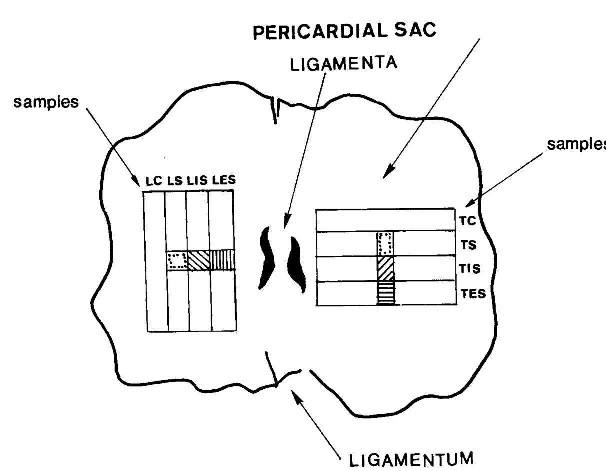


**Fig. 1** Open pericardial sac showing how the samples were obtained. Cut in longitudinal direction: LC, control; LS, sutured; LIS, sutured and glued with Glubran 2; LES, sutured and glued with Loctite 4011. Cut in transverse direction: TC, control; TS, sutured; TIS, sutured and glued with Glubran 2; TES, sutured and glued with Loctite 4011

### 2.2 Chemical treatment

The pericardium was treated for 24 h with 0.625% glutaraldehyde (pH 7.4) prepared from a commercially available solution of 25% glutaraldehyde (Merck) at a ratio of 1/50 (w/v), in 0.1 M sodium phosphate buffer. All the samples were stored at a temperature of 4 °C until they were to be tested.

### 2.3 Sutures

The suture material employed was 5–0 monofilament Prolene (polypropylene) from Braun-Dexon, Inc. (UK). The sutured samples were sewn using a model in which one centimeter of pericardium was made to overlap and the suture lines formed a rectangle. In those samples that were sutured and glued, the biological adhesive was applied over the suture lines.

### 2.4 Adhesives

Two transparent, low-viscosity, instantaneous, acrylic biological adhesives were employed: Glubran 2 (General Enterprise Marketing srl, Italy), or *N*-butyl-2 cyanoacrylate (monomer)/methacryloxysulfolane (monomer), and Loctite 4011, an ethyl cyanoacrylate. Both have been authorized for medical use by the VSP norm (class VI).

### 2.5 Sample preparation

Eight pericardial membranes were utilized. From each, eight samples measuring 120 mm × 20 mm were cut longitudinally, from root to apex, or transversely (Fig. 1). The 32 longitudinally cut tissue samples were grouped as follows: 8 intact controls (LC); 8 cut in half and sutured using the technique described above (LS); 8 cut, sutured and glued with Glubran 2 (LIS); and 8 cut, sutured and glued with Loctite 4011 (LES). Likewise, the 32 samples cut in transverse direction were grouped as 8 intact controls (TC); 8 cut in half and sutured (TS); 8 cut, sutured and glued with Glubran 2 (TIS); and 8 cut, sutured and glued with Loctite 4011 (TES).

### 2.6 Trials

The 16 control samples (LC and TC) and the 16 samples repaired by suturing alone (LS and TS) were subjected to tensile stress applied in the direction of the major axis with stepwise increasing loads of 1, 2, 3 and 4 kg, each followed by unloading, and ultimately an increase in load until rupture.

The 16 samples sutured and then glued with Glubran 2 (LIS and TIS) and the 16 sutured and glued with Loctite 4011 (LES and TES) were subjected to tensile stress at a fixed load of 1 and 0.5 kg, respectively; this was repeated five times in samples 1–7 of each series, with unloading between each loading. Sample no. 8 in each of the four series was subjected to tensile stress directly until rupture.

### 2.7 Assay method

The trial consisted of subjecting each sample to tensile testing as described above, always along the major axis, until rupture, which was confirmed by the loss of load and the morphological changes observed in the tissue. These trials were performed on an Instron TTG4 tensile tester (Instron Ltd., High Wycombe, Buck, England) which determines tensile stress and the strain, or elongation, produced.

The samples were clamped in such a way as to leave a free lumen of 40 mm. The results were recorded graphically, showing the load/elongation diagram necessary to allow the calculation of the stress/strain curves and the hysteresis (energy consumed in each loading and unloading cycle). The trials were carried out at a temperature of 23 ± 2 °C (UNE standard 53–509).

The thickness of each sample was determined by a series of measurements at 10 different points using a Mitutoyo digital micrometer (Elecount series E/A33/8), which has a precision at 20 °C of ±3 μm. The thicknesses ranged between 0.250 and 0.450 mm. The tensile stress in each sample was calculated taking into account the minimum cross-sectional area.

### 2.8 Morphological selection criteria

Two sets of selection criteria were established to ensure the greatest possible homogeneity of the samples. The purpose of these statistical criteria was to determine the probability that each membrane tested actually belonged to the zone to which it was assigned in the initial selection. Thus, those membranes with a minimum thickness greater than the mean value for the corresponding series plus one standard deviation or less than the mean value minus one standard deviation were excluded, as were those membranes in which the difference between the mean thickness and the minimum thickness of the tissue sample was greater or lesser than the mean value for this difference in the corresponding series, plus or minus one standard deviation, indicating a lack of homogeneity (non-uniform thickness).

On the basis of these criteria, the following samples were excluded: series LC, 3 and 7; series TC, 3 and 8; series LS, 3 and 6; series TS, 1, 3 and 7; series LIS, 1 and 3; series TIS, 1, 3 and 5; series LES, 3 and 5; and series TES, 1, 4 and 7. Thus, a total of 19 samples (29.69%) were excluded and 70.31% were accepted.

### 2.9 Statistical study and mathematical analysis

#### *2.9.1 Mean values at rupture*

The values at rupture for each series in kg and MPa are expressed in terms of the mean, standard deviation and 95% confidence interval.

The assumption of a normal distribution was evaluated using the Kolmogorov–Smirnov test, and the Leverne test was employed to assess the homogeneity of variance.

For the interseries comparison of the results at rupture, ANOVA (analysis of variance) was used, followed by a Tukey multiple comparison method. All the tests were two-tailed and the level of significance was $P < 0.05$. The SPSS v.10.0 software package was used to carry out the statistical analysis.

#### *2.9.2 Mathematical fit of the tensile strength/elongation ratio*

After the stepwise increases in load, the samples were subjected to tensile stress until rupture.

The tensile strength (MPa)/strain (per unit elongation) ratio was studied using the least squares method. The best fit corresponded to a third-order polynomial, the shape of which is expressed as $y = b_1x + b_2x^2 + b_3x^3$, where $y$ is the tensile strength in MPa and $x$ the per unit elongation. (The value of the constant $b_0$ was made to equal zero since, due to biological considerations, the equation must pass through the origin; at zero tensile stress, there would be no elongation.)

The same fit was determined in sample no. 8 of series LIS, LES, TIS and TES, which were assayed directly until rupture.

#### *2.9.3 Hysteresis*

For the study of the hysteresis, a mathematical program was devised to calculate the area between the upslope and the downslope by summing the trapezoids formed by the different points. The difference between the areas represents the value for the energy consumed. To facilitate the management of the data, it is expressed in joules × $10^{-3}$.

In series LC, TC, LS and TS, the mathematical fit of the ratio of energy consumed in joules × $10^{-3}$ ($y$) to stress applied in MPa ($x$) and that of the ratio of energy consumed in joules × $10^{-3}$ ($y$) to percentage of elongation (%) ($x$) was then achieved using the least squares method. In both cases, the best fit corresponded to a third-order polynomial, the shape of which is expressed as $y = b_1x + b_2x^2 + b_3x^3$, where y is the energy in joules × $10^{-3}$ and $x$ the tensile stress in MPa or the percentage of elongation, respectively.

The least squares method was also employed in series LIS, TIS, LES and TES to achieve the mathematical fit of the ratio of energy consumed in joules ($y$) to the number of cycles ($x$). The best fit corresponded to a third-order polynomial expressed as $y = b_0 + b_1x + b_2x^2 + b_3x^3$, where $y$ is the energy in joules × $10^{-3}$ and $x$ is the number of cycles.

## 3 Results

### 3.1 Results at rupture in machine kilograms

Table 1 shows the force to which the samples were being subjected at rupture expressed in kg.

The control series (LC and TC) presented the highest mean values (16.77 and 15.26 kg, respectively), with no statistically significant differences between them. Nor were significant differences observed between samples cut longitudinally and those cut in transverse direction when the results in the two series corresponding to each of the different bonding methods

**Table 1** Mean force to which the samples of pericardium were being subjected at rupture expressed in kg

| Series | *n* | Mean | SD | 95% CI |
|---|---|---|---|---|
| LC | 6 | 16.77* | 6.06 | 11.70, 21.84 |
| TC | 6 | 15.26** | 5.87 | 9.82, 20.68 |
| LS | 6 | 11.15* | 2.68 | 8.90, 13.40 |
| TS | 5 | 10.91 | 3.55 | 7.93, 13.86 |
| LIS | 6 | 12.40 | 3.18 | 9.10, 17.80 |
| TIS | 5 | 9.86 | 3.77 | 5.10, 15.20 |
| LES | 6 | 10.77* | 3.21 | 8.08, 13.46 |
| TES | 5 | 8.97** | 2.82 | 6.36, 11.58 |

SD: standard deviation; 95% CI: 95% confidence interval. LC: longitudinally cut control samples; TC: transversely cut control samples; LS: samples cut longitudinally and sutured; TS: samples cut transversely and sutured; LIS: longitudinal samples sutured and glued with Glubran 2; TIS: transverse samples sutured and glued with Glubran 2; LES: longitudinal samples sutured and glued with Loctite 4011; TES: transverse samples sutured and glued with Loctite 4011

* LC vs. LS $P = 0.043$; LC vs. LES, $P = 0.028$

** TC vs. TES, $P = 0.040$ (see text)

were compared. When the longitudinal control (LC) samples were compared with the other longitudinal series, a significant loss of resistance to rupture was detected in series LS (11.15 kg; $P = 0.043$) and in series LES (10.77 kg; $P = 0.028$), but not in series LIS. The comparison of the transverse control (TC) samples with the other series cut transversely revealed a significant loss of resistance to rupture in series TES (8.97 kg; $P = 0.040$), but not in series TS or TIS.

### 3.2 Results at rupture in MPa

The tensile stress to which the samples were being subjected at rupture is shown in Table 2. Again the controls, series LC and TC, presented the highest mean values (34.36 and 27.54 MPa, respectively), with no statistically significant differences. As with the force expressed in kg, no significant differences were observed between longitudinal and transverse samples when the two series corresponding to each of the three bonding methods were compared. When the longitudinal control (LC) samples were compared with the other longitudinal series, a significant loss of resistance to rupture was detected in series LS (21.28 MPa; $P = 0.016$), in series LIS (20.21 MPa; $P = 0.008$) and in series LES (19.29 MPa; $P = 0.005$). The comparison of the transverse control (TC) samples with the other series cut transversely revealed a significant loss of resistance to rupture in series TES (15.80 MPa; $P = 0.038$), but not in series TS or TIS.

### 3.3 Results of the mathematical fit of the stress/strain (elongation) equation

The results of the mathematical fit and the coefficients of the curves that relate the stress to the elongation produced after application of the morphological selection criteria described in Material and Methods appear in Table 3. The stress and elongation obtained from the function are those of the pericardium and do not take into account the role of the other materials employed. The function is a third-order polynomial, the shape of which is expressed as $y = b_1x + b_2x^2 + b_3x^3$, where $y$ is the stress in MPa and $x$ the per unit elongation. The coefficients of determination ($R^2$) ranged between 0.835 and 0.967.

### 3.4 Results of the mathematical fit in the samples subjected directly to rupture

In series LIS, LES, TIS and TES, sample no. 8 was subjected directly to rupture, without undergoing cycles of stress. The results of the mathematical fit of the curve and the coefficients of the stress/strain (elongation) ratio are shown in Table 4. The coefficients of determination ($R^2$) ranged between 0.999 and 1.000.

### 3.5 G point (inflection point in assays until rupture following repeated loading)

In the samples subjected to stepwise increases in load, each followed by a return to zero load, and finally to

**Table 2** Mean tensile stress to which the samples were being subjected at rupture expressed in MPa

| Series | $n$ | Mean | SD | 95% CI |
|---|---|---|---|---|
| LC | 6 | 34.36* | 12.40 | 23.99, 44.74 |
| TC | 6 | 27.54** | 10.89 | 17.48, 37.61 |
| LS | 6 | 21.28* | 6.52 | 15.82, 26.73 |
| TS | 5 | 19.65 | 5.26 | 15.25, 24.05 |
| LIS | 6 | 20.21* | 6.25 | 14.32, 32.91 |
| TIS | 5 | 16.95 | 8.52 | 9.37, 36.19 |
| LES | 6 | 19.29* | 5.25 | 14.90, 23.67 |
| TES | 5 | 15.80** | 4.23 | 11.88, 19.71 |

SD: standard deviation; 95% CI: 95% confidence interval. LC: longitudinally cut control samples; TC: transversely cut control samples; LS: samples cut longitudinally and sutured; TS: samples cut transversely and sutured; LIS: longitudinal samples sutured and glued with Glubran 2; TIS: transverse samples sutured and glued with Glubran 2; LES: longitudinal samples sutured and glued with Loctite 4011; TES: transverse samples sutured and glued with Loctite 4011

* LC vs. LS, $P = 0.016$; LC vs. LIS, $P = 0.008$; LC vs. LES, $P = 0.005$

** TC vs. TES, $P = 0.038$ (see text)

**Table 3** Coefficients of the equation for the mathematical fit of the stress/strain (elongation) curve after applying the criteria for tissue selection

| Series | $b_1$ | $b_2$ | $b_3$ | $R^2$ |
|---|---|---|---|---|
| LC | –41.70 | 780.11 | –1179.20 | 0.872 |
| TC | –33.05 | 477.73 | –407.74 | 0.835 |
| LS | –2.26 | 17.84 | 649.56 | 0.819 |
| TS | 0.39 | –2.28 | 320.83 | 0.851 |
| LIS | –20.28 | 322.70 | –470.93 | 0.913 |
| TIS | –10.75 | 226.85 | –289.90 | 0.900 |
| LES | 0.47 | –3.39 | 375.39 | 0.967 |
| TES | –1.88 | 32.58 | 82.63 | 0.930 |

$y = b_1x + b_2x^2 + b_3x^3$, where $y$ is the stress in MPa and $x$ is the per unit strain (elongation)

$R^2$: Coefficient of determination

LC: longitudinally cut control samples; TC: transversely cut control samples; LS: samples cut longitudinally and sutured; TS: samples cut transversely and sutured; LIS: longitudinal samples sutured and glued with Glubran 2; TIS: transverse samples sutured and glued with Glubran 2; LES: longitudinal samples sutured and glued with Loctite 4011; TES: transverse samples sutured and glued with Loctite 4011

**Table 4** Fit of the curves at rupture in samples not subjected to cycles of loading and unloading (sample no. 8 of each series)

| Series | $b_1$ | $b_2$ | $b_3$ | $R^2$ |
|---|---|---|---|---|
| LIS | 2.51 | 114.91 | 220.76 | 1.000 |
| TIS | 2.79 | 25.29 | 603.39 | 0.999 |
| LES | –0.54 | 43.48 | 175.72 | 0.999 |
| TES | –4.18 | 66.53 | 605.76 | 0.999 |

$y = b_1x + b_2x^2 + b_3x^3$, where $y$ is the stress in MPa and $x$ is the per unit strain (elongation)

$R^2$: Coefficient of determination

LIS: longitudinal samples sutured and glued with Glubran 2; TIS: transverse samples sutured and glued with Glubran 2; LES: longitudinal samples sutured and glued with Loctite 4011; TES: transverse samples sutured and glued with Loctite 4011

tensile stress until rupture, an inflection point was observed in the diagrams obtained directly from the tensile testing machine (kg/elongation), which we refer to as G point. This occurred at a load just beyond that of the final step, and was not observed in the samples subjected directly to rupture. Table 5 shows the mean values in kg that the samples were withstanding at that point. Figures 2 and 3 represent the diagrams obtained from the tensile tester for sample 4 of series LC and sample 3 of series TES and for sample 8 of series LES and sample 8 of series TES, respectively, for their comparison.

In the series subjected to stepwise 1–kg increases in load up to 4 kg (LC, TC, LS and TS), the mean values at G point ranged between 4.36 ± 0.24 kg and 4.80 ± 0.49 kg. In the series undergoing repeated fixed loads of 1 kg (LIS and TIS), the values at G point ranged from 1.26 ± 0.16 kg to 1.33 ± 0.16 kg, while in those subjected to repeated fixed loads of 0.5 kg (LES and TES), the G point values ranged between 0.66 ± 0.05 kg and 0.67 ± 0.06 kg.

**Table 5** Force at G point, or the inflection point, in kg

| Series | $n$ | Mean | SD | 95% CI |
|---|---|---|---|---|
| LC | 6 | 4.80 | 0.49 | 4.34, 5.26 |
| TC | 6 | 4.52 | 0.31 | 4.23, 4.81 |
| LS | 6 | 4.36 | 0.24 | 4.16, 5.56 |
| TS | 5 | 4.55 | 0.52 | 4.11, 4.98 |
| LIS | 6 | 1.33 | 0.16 | 1.18, 1.47 |
| TIS | 5 | 1.26 | 0.16 | 1.11, 1.40 |
| LES | 6 | 0.66 | 0.05 | 0.61, 0.71 |
| TES | 5 | 0.67 | 0.05 | 0.62, 0.73 |

SD: standard deviation; 95% CI: 95% confidence interval. LC: longitudinally cut control samples; TC: transversely cut control samples; LS: samples cut longitudinally and sutured; TS: samples cut transversely and sutured; LIS: longitudinal samples sutured and glued with Glubran 2; TIS: transverse samples sutured and glued with Glubran 2; LES: longitudinal samples sutured and glued with Loctite 4011; TES: transverse samples sutured and glued with Loctite 4011

### 3.6 Energy/stress ratio

The coefficients of the mathematical fit of the ratio of the energy consumed to the stress applied in the control series (LC and TC) and the series repaired by suture alone (LS and TS) can be seen in Table 6. The coefficients of determination ($R^2$) ranged between 0.881 and 0.981. These results are illustrated graphically in Fig. 4.

### 3.7 Energy/strain (elongation) ratio

Table 7 displays the coefficients of the mathematical fit of the ratio of the energy consumed to the elongation produced in the control series (LC and TC) and the series repaired by suture alone (LS and TS). The coefficients of determination ($R^2$) ranged between 0.934 and 0.968. These results are also presented in Fig. 5.

### 3.8 Energy/cycles ratio

Table 8 shows the coefficients of the mathematical fit of the ratio of the energy consumed to the number of hysteric cycles for the series repaired by suturing and gluing (LIS, TIS, LES and TES). The coefficients of determination ($R^2$) ranged between 0.712 and 0.920. These results are illustrated graphically in Fig. 6.

## 4 Discussion

The manufacture of cardiac bioprostheses with a guaranteed durability of more than 10 years is as yet an unresolved challenge [6, 8–10, 17]. To achieve this, it will be necessary to increase the resistance of the biomaterials used to avoid tissue degradation and secondary calcification [18]. Moreover, we should carefully select the calf pericardium employed in the construction of valve leaflets in those bioprostheses in which this material is used [19–21]. It will probably be necessary to find new materials capable of withstanding the mechanical stress [1, 22]. Finally, we will need to design cardiac bioprostheses with engineering in mind in order that their valve flows and gradients keep energy consumption to a minimum, thus conferring them a greater durability [23].

It is known that primary degeneration of the valve leaflet in the absence of calcification is produced by mechanical fatigue, the so-called primary failure. The rupture of collagen fibers is caused by stress, a dynamic stress that accelerates proteolysis, facilitating tissue destruction [24]. Bioprostheses that are subjected to

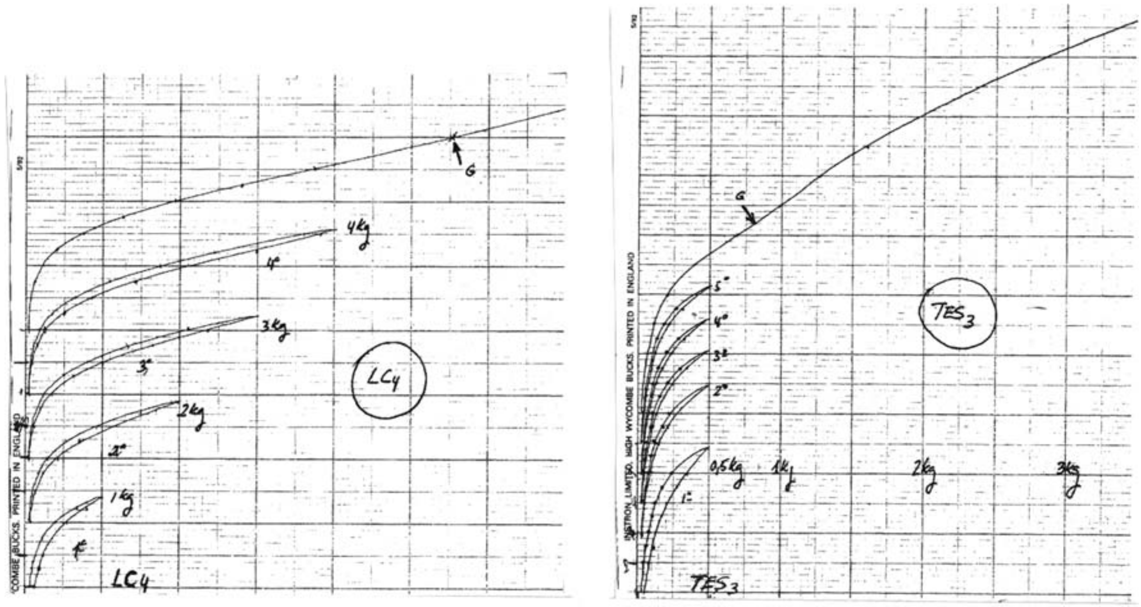


**Fig. 2** G point indicated in diagrams produced by the tensile testing machine. Left: sample 4 of the longitudinally cut control series ($LC_4$). Right: sample 3 of the transversely cut series sutured and glued with Loctite 4011 ($TES_3$)

mild stresses and flows and to very moderate pressures, such as those implanted in tricuspid position, do not fail [25].

In this study, we have assessed, by means of hysteretic cycles, the changes in energy consumption by samples of calf pericardium with and without bonding systems involving sutures and adhesives.

The samples were subjected to tensile stress in which the loads were applied along the principal axis until rupture. Cycles of stepwise or fixed loading followed by unloading were carried out to study the hysteresis, or energy consumption, throughout the trial. Finally, all the samples were subjected to tensile testing until rupture.

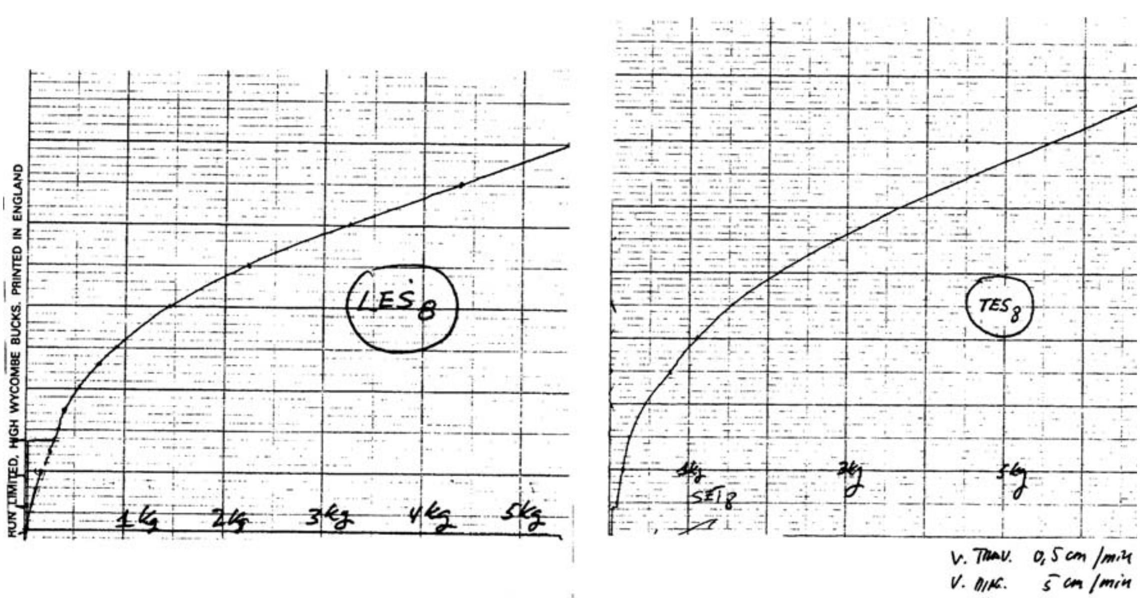


**Fig. 3** Diagrams produced by the tensile testing machine in assays until rupture in samples that were not subjected to cycles of loading and unloading. Left: sample 8 of the longitudinally cut series sutured and glued with Loctite 4011 ($LES_8$). Right: sample 8 of the transversely cut series sutured and glued with Loctite 4011 ($TES_8$)

**Table 6** Coefficients of the equation for the mathematical fit of the ratio of energy consumed to stress applied in control samples and sutured samples

| Series | $b_1$ | $b_2$ | $b_3$ | $R^2$ |
|---|---|---|---|---|
| LC | 0.0168 | –0.0029 | 0.0001 | 0.981 |
| TC | 0.0159 | –0.0024 | 0.0001 | 0.881 |
| LS | 0.0088 | 0.0022 | –0.0002 | 0.972 |
| TS | 0.0247 | –0.0010 | –0.0001 | 0.925 |

$y = b_1x + b_2x^2 + b_3x^3$, where $y$ is the energy in joules × $10^{-3}$ and $x$ is the stress in MPa

$R^2$: Coefficient of determination

LC: longitudinally cut control samples; TC: transversely cut control samples; LS: samples cut longitudinally and sutured; TS: samples cut transversely and sutured

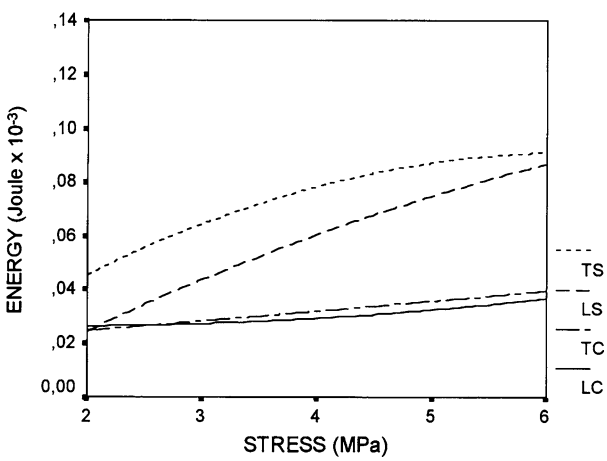


**Fig. 4** Graphic representation of the energy/stress equations applied in the control series (LC and TC) and the sutured series (LS and TS); $y$ is the energy consumed in joules and $x$ is the tensile stress in MPa

**Table 7** Coefficients of the equation for the mathematical fit of the ratio of energy consumed to strain (elongation) in control samples and sutured samples

| Series | $b_1$ | $b_2$ | $b_3$ | $R^2$ |
|---|---|---|---|---|
| LC | 0.0018 | 0.00002 | 0.000004 | 0.934 |
| TC | 0.0000 | 0.00009 | 0.000005 | 0.934 |
| LS | –0.0052 | 0.00080 | –0.00002 | 0.934 |
| TS | –0.0139 | 0.00140 | –0.00003 | 0.968 |

$y = b_1x + b_2x^2 + b_3x^3$, where $y$ is the energy consumed in joules × $10^{-3}$ and $x$ is the percent strain (elongation)

$R^2$: Coefficient of determination

LC: longitudinally cut control samples; TC: transversely cut control samples; LS: samples cut longitudinally and sutured; TS: samples cut transversely and sutured

The results of these final assays, expressed in machine kg and MPa and taking into account the minimal cross-sectional area of the samples, are shown

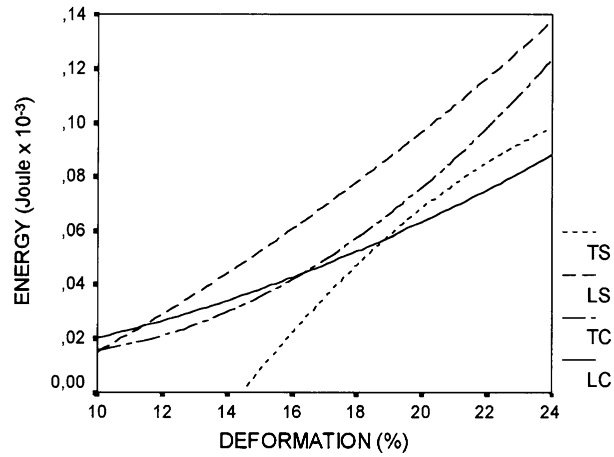


**Fig. 5** Graphic representation of the energy/elongation equations applied in the control series (LC and TC) and the sutured series (LS and TS); $y$ is the energy consumed in joules and $x$ is the percentage of elongation

**Table 8** Coefficients of the equation for the mathematical fit of the ratio of the energy consumed to the number of hysteric cycles for the series repaired by suturing and gluing

| Series | $b_0$ | $b_1$ | $b_2$ | $b_3$ | $R^2$ |
|---|---|---|---|---|---|
| LIS* | 0.0512 | –0.0404 | 0.0114 | –0.0010 | 0.796 |
| TIS* | 0.0587 | –0.0500 | 0.0149 | –0.0014 | 0.920 |
| LES** | 0.0269 | –0.0233 | 0.0073 | –0.0007 | 0.712 |
| TES** | 0.0238 | –0.0175 | 0.0049 | –0.0004 | 0.834 |

* Cycles of 1 machine kg

** Cycles of 0.5 machine kg

$y = b_0 + b_1x + b_2x^2 + b_3x^3$; where $y$ is the energy in joules × $10^{-3}$ and $x$ is the number of cycles of loading and unloading

$R^2$: Coefficient of determination

LIS: longitudinal samples sutured and glued with Glubran 2; TIS: transverse samples sutured and glued with Glubran 2; LES: longitudinal samples sutured and glued with Loctite 4011; TES: transverse samples sutured and glued with Loctite 4011

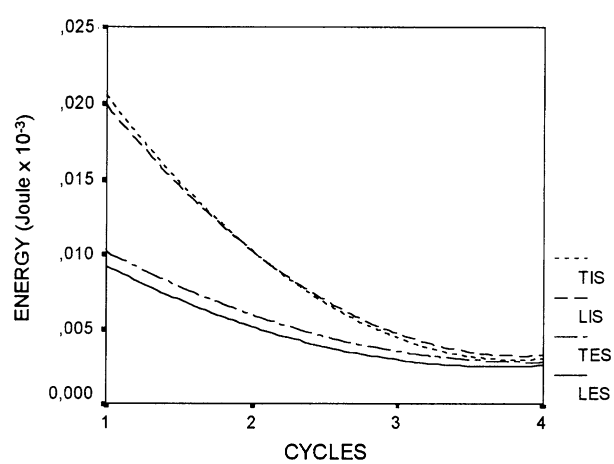


**Fig. 6** Graphic representation of the energy/cycles equations applied in the sutured and glued series (LIS, TIS, LES and TES); $y$ is the energy in joules and $x$ is the number of cycles

in Tables 1 and 2. When the mean values for both measurements in the longitudinally and transversely cut control series (LC and TC) are compared with their corresponding sutured and sutured and glued series, a loss of resistance is observed in all the repaired forms. In the comparison involving their behavior in response to tensile stress (MPa), the difference is statistically significant in all three repaired longitudinal series, while significant losses are observed only in the transverse series sutured and subsequently glued with Loctite 4011 (TES).

The overlapping suture technique guarantees an acceptable resistance to tearing, to which the contribution of the two acrylic adhesives tested is negligible.

In the study of the elastic behavior of the samples that had been sutured and glued and subjected directly to tensile stress until rupture, without undergoing repeated loading and unloading (sample no. 8 in each series; Table 4), we observed similarity between those cut in the same direction, regardless of the adhesive employed (LIS and LES; TIS and TES), while the behavior differed when the comparison was made between samples repaired using the same adhesive but cut in a different direction (LIS and TIS; LES and LIS). This finding seems to indicate that the bonding elements respect anisotropy to a certain degree [26, 27].

However, in the study of the elastic behavior of the samples of pericardium subjected to tensile stress until rupture after the hysteretic cycles described in the Material and methods section (Table 3), this characteristic does not appear to be present, although this supposition should be confirmed by microscopy studies. Anisotropy has a considerable influence in the construction and function of bioprostheses. The native mitral valve is structurally and mechanically anisotropic [28]. Clark and Finke observed reinforcing fibers running in circumferential direction, parallel to the free edge of the leaflet [29]. This anatomical structure reduces the stress on the functioning leaflet since it permits greater radial extensibility of the leaflet. Christie and Medland confirmed these findings using finite element analysis [30].

Observing the shape of the load/elongation curves in the diagram produced by the tensile testing machine, when the biomaterial was assayed until rupture following the cycles of loading and unloading, we find a small notch or indentation and a change in slope near the point of the last load applied (Fig. 2). We had not observed this inflection point in previous trials in which the tissue samples were not subjected to stepwise increases in load [13], nor did it occur in the samples subjected directly to rupture in the present study (Fig. 3). We decided to refer to this as the G point when a laboratory technician called our attention to this circumstance. We are unable to provide a precise explanation for this phenomenon, and it would be necessary to repeat the trials and analyze the behavior of the collagen fibers involved in the absorption of the programmed stress under the microscope [4]. The rupture or disruption of the collagen fibers can be assessed by scanning electron microscopy [18].

Table 5 shows the mean values for G point in the different series assayed in kg. They are higher and proximal to the final load, regardless of the force of said load. This finding leads us to suspect that by subjecting the biomaterial to cyclic fatigue, we condition its subsequent mechanical behavior. The load reorients the collagen fibers but does not interfere with the inherent anisotropy of the tissue, as was demonstrated by Zioupos et al. [26]. Can a biomaterial bonded by a suture and an adhesive and subjected to cyclic loading and unloading preserve this property?

The objective of this study was to relate the energy consumed to the stress withstood (tensile strength), the elongation produced and the number of cycles. In the control series (LC and TC) and in the sutured series (LS and TS), we were able to relate the energy to the first two parameters. Table 6 shows the coefficients for the equations that relate the energy consumed to the stress withstood by the samples, with excellent coefficients of determination ($R^2$). These relationships are illustrated in Fig. 4, in which a greater amount of energy is seen to be consumed by the sutured samples. Table 7 shows the coefficients of the equations that relate the energy consumed to the elongation produced in these same series, again with excellent coefficients of determination ($R^2$). Figure 5 demonstrates this relationship, showing a marked increase in energy consumption as the samples become more elongated or deformed.

The series that were sutured and reinforced with an adhesive were subjected to fixed loads of 1 or 0.5 kg, followed by unloading, making it possible to establish the equations that relate the energy consumed to the number of hysteretic cycles. The values of the coefficients of these equations and their graphic representation are shown in Table 8 and Fig. 6, respectively. With a limited number of cycles, coefficients of determination ($R^2$) ranging between 0.712 and 0.920 were obtained. The calculation of the asymptote parallel to the $x$ axis would make it possible to determine the energy consumption for a theoretical number of cycles and to estimate the durability. The path taken, which confirms the line of previous studies [31], should lead us to a better understanding of the mechanical behavior of the biomaterial employed. Morphological studies using microscopy techniques after similar trials at lower and thus less damaging loads should confirm these findings.

We can conclude that, because of the internal shear stress that they produce, bonding systems provoke a greater energy consumption in biomaterials and that this has a negative effect on their durability. Acrylic biological adhesives do not contribute to the resistance to tearing of the bonded tissues, but we do not know the role they play in the elastic behavior of the biomaterial at lower degrees of load.

The samples sutured and reinforced with Loctite 4011 showed a lower energy consumption during the initial cycles than those glued with Glubran 2.

Given the importance of the anisotropic behavior of the tissues employed in the manufacture of valve leaflets for bioprostheses and the need to utilize bonding materials, it will be necessary to determine whether this property persists in calf pericardium after cycles of loading and unloading when the samples are joined by suturing.

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