Mechanical effects of increases in the load applied in uniaxial and biaxial tensile testing: Part I. Calf pericardium

J. M. GARCÍA PÁEZ, E. JORGE, A. ROCHA, M. MAESTRO, J. L. CASTILLO-OLIVARES
Servicio de Cirugía Experimental, Clínica Puerta de Hierro, Madrid, Spain

I. MILLAN
Servicio de Bioestadística, Clínica Puerta de Hierro, Madrid, Spain

A. CARRERA, A. CORDON
Departamento de Mecánica Estructural y Resistencia de Materiales, Escuela Técnica Superior de Ingenieros Industriales, Madrid, Spain

G. TELLEZ, R. BURGOS
Servicio de Cirugía Cardiovascular, Clínica Puerta de Hierro, Madrid, Spain

The authors analyzed the mechanical behavior of the calf pericardium employed in the construction of valve leaflets for cardiac bioprostheses. Forty samples of pericardium were subjected to uniaxial tensile testing, 20 as controls and 20 exposed to loads increasing stepwise until rupture, with a return to zero load between each new increment. Another 20 samples were used similarly in biaxial tensile tests involving loads increasing stepwise until rupture, again returning to zero load between steps.

The ultimate stresses in the uniaxial study were very similar and were not influenced by the region of pericardial tissue being tested or the increments in load to which the tissue was exposed. The mean stresses at rupture in the stepwise biaxial assays were significantly greater ($p < 0.01$). Using morphological and mechanical criteria for sample selection, it was possible to obtain mathematical fits for the stress/strain relationship in both types of assays, with excellent coefficients of determination ($R^2 > 0.90$).

In uniaxial tests in which the selection criteria were not applied, the correlation improved as the load increased, a phenomenon that did not occur in the biaxial studies.

The values varied throughout the different cycles, adopting exponential forms when the strain was greatest. These variations, which demonstrate that the increase in the energy consumed is a function of the stress applied and of the strain produced, should be good parameters for assessing the changes in the collagen fiber architecture of pericardial tissue subjected to cyclic stress, and may help to detect early failure.

1. Introduction

Calf pericardium is being employed in the manufacture of valve leaflets for cardiac bioprostheses since the 1970s [1]. Given the good hemodynamic properties of these devices, as well as their low thrombogenicity, making long-term anticoagulation therapy unnecessary [2], they came to be considered the ideal replacement in cases of irreversibly damaged valve leaflets or severe dysfunction of a native valve [3]. Since then, their durability has been shown to be limited, sometimes resulting in early tears in the valve leaflets [4] and in other cases, in medium-term structural failure [5–8] or calcium deposition [9, 10], making their replacement necessary. Today, these bioprostheses are employed only as an alternative to mechanical prostheses which, although more costly and encumbered by the need for indefinite anticoagulation therapy and monitoring, offer greater guarantees of long duration. However, throughout the world, there are many countries that cannot provide their citizens with this type of attention [3].

On the other hand, it has been shown that bioprostheses implanted in tricuspid position [11, 12] or into elderly patients requiring aortic valve replacement [13] are capable of successful long-term function and thus, are now preferred over mechanical prostheses in these two circumstances. The long duration of the few bioprosthetic valve replacements implanted in tricuspid position can only be attributed to the low pressure and nonturbulent blood flow, coming from the vena cava, to which they are subjected [11, 12]. The good results in aortic position in elderly patients must be explained by the reduced ejection fraction, a situation that is directly related to aging [13] and is associated with a decrease in the mechanical stress that the leaflets have to withstand throughout the cardiac cycle. Both observations appear to indicate that the durability of a bioprosthesis is related to the balance between the degree and duration of the mechanical stress to which it is exposed and the device's capacity to withstand it [14].

Cardiac valve leaflets are subjected to two types of mechanical stress: tensile stress produced by the hydrostatic pressure associated with valve closure and shear stress produced by the bending of the leaflet during the cardiac cycle. Although the mechanical behavior of biomaterials is usually assessed by uniaxial studies, the more complex biaxial tests provide more precise information as the load conditions they reproduce are closer to the physiological situation [15]. On the other hand, calf pericardium, like other biomaterials, is a clearly anisotropic tissue [16]. When a biomaterial is subjected to a load, its collagen fibers, as the structures responsible for the resistance of the biomaterial, align themselves in the direction of said load [17], but this feature does not interfere with the inherent structural anisotropy. This behavior justifies the need to perform biaxial trials.

We have carried out uniaxial and biaxial tests in contiguous samples of calf pericardium, analyzing the differences in the mechanical behaviors of this tissue when subjected to static tensile stress and to repeated loading until rupture. The biaxial tests were performed on a hydraulic simulator capable of measuring the stress and strain, or deformation, to which the sample of pericardium is subjected. We also employed a method tissue selection, applying mechanical and morphological criteria to paired samples [18] to ensure greater homogeneity in the fragments of biomaterial used and thus, in the outcome.

The purpose of this study was to compare the results of the two tests and the mechanical impacts of repeated or stepwise increments in loading.

2. Materials and methods

Calf pericardium was obtained directly from a local abattoir. The claves, which had been born and raised in Spain, were sacrificed between the ages of 9 and 12 months. The tissue was transported to the laboratory in cold isotonic saline (0.9% sodium chloride). Once the tissue was cleaned, each sac was mounted loosely on a 15 mm diameter ring, with the diaphragmatic attachment in the center and the sternopericardial ligaments on the circumference. The samples used in the trials were obtained according to the distribution appearing in Fig. 1, which shows the pericardial sac open, with the ligamentum in the lower part of the diagram and the pericardial ligaments in the center. Four rectangular membranes measuring $12 \times 2\,cm^2$ were cut along the root-to-apex axis, as shown in Fig. 1. In addition, two circular membranes measuring 2 cm in diameter were cut from each of the same pericardial sacs, again as shown in Fig. 1. These circular membrane pairs were comprised of one sample from the left side (C) and one from the right side (B) of the apex-to-root axis.

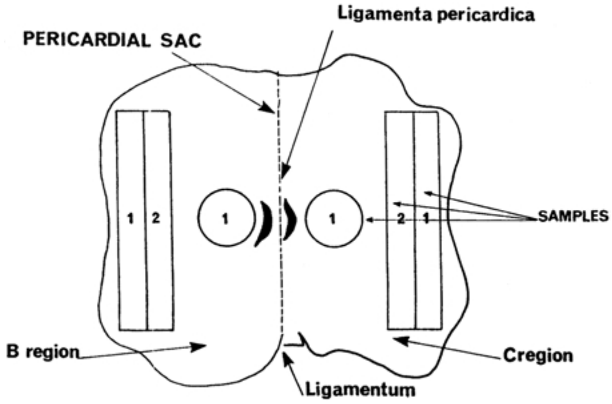

Figure 1 Open pericardial sac from which the samples were obtained. Circular sample (1) for stepwise biaxial trials: Rectangular samples (1 and 2) for control and stepwise uniaxial trials, respectively. Region B, pericardium corresponding to right ventricle, region C to left ventricle.

The thickness of each tissue fragment was determined by measuring a series of ten points, using a Mitutoyo micrometer (Elecount, series E:A33/8 Digital) with a precision at 20 °C of $\pm 3\,\mu m$. All of the trials were carried out at a room temperature of 22 to 24 °C.

All the membranes were 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.

2.1. Uniaxial trials

For the control study, 20 rectangular tissue samples, one from the left side of each sac (C) and one from the right (B), were subjected to tensile testing in the direction of the longitudinal axis (root-to-apex) until rupture. Another group of 20 rectangular tissue samples (one from the left side of each sac and one from the right) was subjected to a similar uniaxial tensile test the differed only in that the increase in load was stepwise (starting at a mean load of 2 kg and increasing to mean loads of 3, 4, 5, 6, 8, and 10 kg, after which the load was incremented until rupture), with a return to zero load between each step. These trials were preformed on an Instron TIG4 tensile tester (Instron Ltd., High Wycombe, Buck England) which records tensile stress under varying loads. The samples were clamped in such a way as to leave a free lumen of 45 mm. The results were recorded graphically, showing the load/strain (or load/deformation) diagram necessary to be able to calculate the stress/strain curve. The tensile stress of the pericardium was calculated taking into account the minor section.

2.2. Biaxial trials

The 20 circular membranes (one from the left side of each sac and one from the right) underwent stepwise increments in mean load (starting at 1.5 kg and increasing to mean loads of 3, 7, and 10 kg, and thereafter to rupture) produced by biaxial tensile stress applied to each sample in the directions of its two major axes by a

Figure 2 Clamping of the circular membranes for the biaxial trials.

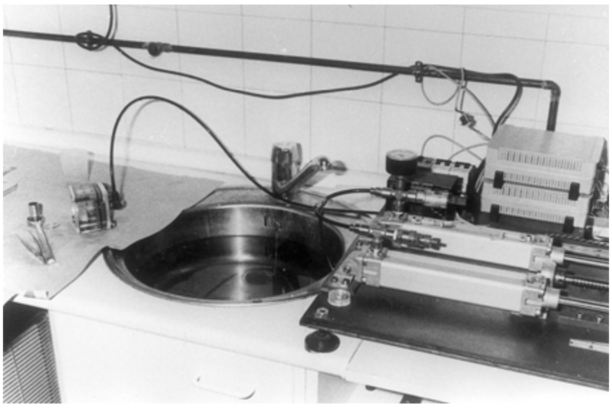

Figure 3 General view of the equipment used in the biaxial trials.

hydraulic simulator capable of delivering increasing stresses to the calf pericardial membranes secured with pressure clips (Fig. 2). The membranes were exposed to increasing hydrostatic pressure caused by the compression transmitted to saline solution by a piston. As the piston moved, the fluid deformed the membrane and a pressure gauge determined the pressure, ranging between 0 and 16 atm. The simulator consisted basically of a unit for measuring pressure equipped with a servomotor to drive the pump propelling the piston (Fig. 3).

Sample rupture was suspected when a loss of stress was observed, and was confirmed by the appearance of macroscopic tears in the tissue.

2.3. General description of the function of the system

A piston is activated by means of digital monitor based on a high-speed processor that controls the direct current electric servomotor. The piston compresses the fluid and the pericardial membrane resists the pressure. The biomaterial is subjected to loads that are incremented stepwise, with a return to zero load prior to each new step, and thereafter increased continuously until rupture. The controlling computer indicates the angular velocity of the activating system, which is maintained throughout the trial. The data acquisition system evaluates the fluid pressure and the movement of the piston at all times. The numerical data corresponding to these variables are transferred to a computer via a series interface, and stored for subsequent analysis.

2.4. Technical features of the hydraulic simulator

The most relevant technical specifications are as follows:

Amplifier. D-MOS technology; H-bridge configuration; maximum working voltage: 53 V; maximum intensity in steady state: 3 A.

Motor. Rated voltage: 24 DCV; rated output: 15 W; starting torque: 120 mN m; current in a vacuum: 21.7 mA; starting current: 3040 mA; maximum permanent torque: 30.46 mN m.

Incremental position sensor. Optical, two quadrature outputs and index impulse.

Quadrature processor. Programmable logic technology; two quadrature inputs; incremental/decremental monopolar impulse output; maximum working frequency of 4 MHz.

Digital compensator. Processor, RISC microcontroller, 24 MHz, 8 bits, 200 ns/instruction; maximum sampling frequency of 1 kHz; velocity range from 00 to 8 388 607 counts/sampling period $\times$ 256; proportional action coefficient (KP) of $-32\,768$ to 32 767; differential action coefficient (KD) of $-32\,768$ to 32 767.

Piston. 160-mm strokes; 32 mm in diameter; maximum pressure 16 atm.

Pressure sensor. Maximum pressure 16 atm; output signal: 4–20 mA.

Computer. Standard Pentium-75 configuration.

This system was developed by Sat Polar, S.L., a Spanish electronic engineering firm.

2.5. Tensile strength

Once the stress withstood by the pericardial membrane at each instant of the trial was known, its tensile strength was calculated using the Laplace equation for a thin-walled membrane subjected to pressure: $T_s = pr/2e$, where p is the pressure in $\mathrm{kg/cm^2}$, r the radius of the membrane expressed in cm, e the thickness of the membrane in cm and T_s the tensile strength in $\mathrm{kg/cm^2}$. To convert this value to MPa, we divided the result by 10.19.

2.6. Strain (deformation)

The movement of the piston indicated the variation in the fluid volume at every moment and for each different stress applied and thus, the changes in membrane geometry up to the moment of rupture. At that point, the shape was that of a round dome, the base of which must be a known circle (the frame on which the membrane to be tested was mounted). By measuring the changes in length of the longest arc of the dome, it was possible to determine the percentage of deformation, or strain, at each moment of the trial.

2.7. Statistical study and mathematical analysis

2.7.1. Comparison of means at rupture

The mean values at rupture for the series of samples were compared, taking into account the different regions (B and C), by means of analysis of variance (ANOVA) and the Newman–Keuls test for multiple comparisons.

2.7.2. Mathematical fit of the tensile strength/strain ratio

The tensile strength (Mpa)/strain (per unit deformation) ratio was studied using the least squares method. The best fit corresponded to a third-order polynomial, the shape of which is expressed as $y = a_1x + a_2x^2 + a_3x^3$, where y is the tensile strength or stress in MPa and x is the per unit deformation (strain) of the membrane. The value of the constant a_0 was made to equal zero since due to biological considerations, the equation must pass through the origin (at zero tensile strength, there would be no deformation). For similar considerations, the analysis was done for $x < 0.20$ in the uniaxial study and for $x < 1$ in the biaxial study, since the behavior of the function when the membrane could have surpassed its elastic limit, entering the realm of irreversible deformation, was not considered to be of interest.

2.7.3. Mean overall fit for each set of samples in the two regions

The stress/strain ratio was also studied by region within each series of samples.

Selection criteria. Selection criteria were established to ensure greater homogeneity of the samples. The purpose of these statistical selection criteria was to determine the probability that each membrane tested actually belonged to the region 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 and region 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 of a given region in each series and the minimum thickness for the corresponding region was greater than the mean value for this difference as determined in the corresponding set, plus one standard deviation, indicating a lack of homogeneity.

In the control uniaxial trials, those samples in which the stress for a per unit deformation of 0.20 was greater than the mean plus or minus one standard deviation for said series were also excluded.

In the trials involving stepwise increases in load, those samples in which the tensile stress (MPa) reached in the first increment was higher than the mean value for the corresponding series, plus or minus one standard deviation, were excluded.

Mean overall fit for the selected samples. On the basis of the aforementioned selection criteria, the following 24 samples were selected for each assay, representing 40.0% of all the samples assayed:

TABLE I Mean stresses at rupture in control and stepwise uniaxial trials and stepwise biaxial trials

Type of trial	No. of samples	Region	Mean values at rupture (MPa)	Standard deviation	Range (MPa)
Uniaxial					
Control	10	B	28.72	5.45	22.74–38.56
	10	C	25.70	9.46	11.90–44.24
Stepwise	10	B	27.53	8.05	19.62–38.39
	10	C	26.61	10.16	12.06–38.72
Biaxial					
Stepwise	10	B	49.93	12.01	30.02–68.78
	10	C	71.26	34.47	27.08–126.01

Stepwise trials consisted of a specific number of stepwise increases in load separated by decreases to zero load, after which the load was increased steadily until sample rupture. Region B: pericardium covering right ventricle. Region C: pericardium covering left ventricle.

Control uniaxial trial. Samples nos. 6, 7, and 9 from region C (left side) and nos. 3, 4, 9, and 10 from region B (right side).

Stepwise uniaxial trial. Samples nos. 2, 4, 5, and 7 from region C and nos. 5, 6, and 8 from region B.

Stepwise biaxial trial. Samples nos. 2, 5, 6, 7, and 9 from region C and nos. 1, 4, 5, 8, and 9 from region B.

Hysteresis. The energy consumed in deformation with each increment in load and return to zero load is referred to as hysteresis, and is represented by the area under the stress/strain curve for each cycle or step. The analysis of the results of the biaxial trial included the estimation of the function of the ratio between the area under the stress/strain curve to the strain or the stress, respectively.

3. Results

3.1. Rupture

The stresses at rupture are shown in Table I. In the uniaxial trials, the mean values obtained at rupture ranged between 25.72 and 28.72 MPa. There were no statistically significant differences between the results in regions B and C with respect to type of assay (control or stepwise load increases). In the biaxial trial, the differences between the mean values at rupture for region B (49.93 MPa) and region C (71.26 MPa) were statistically significant ($p < 0.05$).

TABLE II Values for the equation fitting the stress/strain curve ($y = a_1x + a_2x^2 + a_3x^3$) and R^2 in the control uniaxial trial without and with the application of sample selection criteria

Samples	a_1	a_2	a_3	R^2
Region B				
Not selected	– 37.99	508.02	– 862.66	0.697
Selected	0.69	24.38	156.83	0.958
Region C				
Not selected	– 38.83	518.31	– 885.00	0.701
Selected	– 40.35	596.06	– 961.48	0.816

$y = a_1x + a_2x^2 + a_3x^3$, where y is the area under the stress/strain curve and x is the stress or the strain, respectively.

R^2: coefficient of determination. Region B: pericardium covering right ventricle. Region C: pericardium covering left ventricle.

TABLE III Values for the equation fitting the stress/strain curve ($y = a_1x + a_2x^2 + a_3x^3$) and R^2 in the stepwise uniaxial trial without application of sample selection criteria

Load changes	a_1	a_2	a_3	R^2
Region B				
1st increase	41.75	– 361.06	1014.41	0.860
Zero load	51.49	– 620.75	2002.88	0.906
2nd increase	– 9.67	2307.81	– 23 274.00	0.945
Zero load	99.23	– 2309.10	23 642.20	0.912
3rd increase	– 1.29	1712.84	– 11 848.01	0.950
Zero load	26.74	798.63	– 5542.20	0.915
4th increase	19.85	499.66	3043.57	0.972
Zero load	47.42	– 490.06	9941.65	0.955
5th increase	2.01	1717.48	– 9244.90	0.908
Zero load	38.13	823.50	– 2312.10	0.949
6th increase	33.05	308.02	3175.53	0.944
Zero load	140.96	– 2916.70	24 825.40	0.856
7th increase	31.51	555.67	1940.44	0.959
Zero load	1.12	1438.59	– 3989.50	0.967
8th increase/rupture	22.83	1156.73	– 2212.90	0.961
Region C				
1st increase	56.84	– 872.71	4741.18	0.826
Zero load	86.16	– 1561.40	8749.07	0.859
2nd increase	51.19	– 137.70	1782.19	0.900
Zero load	97.97	– 1847.40	17 285.70	0.900
3rd increase	66.20	– 512.32	7389.17	0.905
Zero load	78.89	– 427.84	498.76	0.859
4th increase	33.94	752.70	– 1572.70	0.944
Zero load	66.47	278.44	– 585.81	0.948
5th increase	4.48	2183.53	– 13 772.00	0.949
Zero load	106.70	– 433.39	418.92	0.913
6th increase	38.97	922.97	– 3335.80	0.945
Zero load	45.39	772.24	– 2720.50	0.946
7th increase	3.75	2236.84	– 11 697.00	0.966
Zero load	49.51	753.77	– 1144.40	0.953
8th increase/rupture	25.92	1491.43	– 3810.90	0.974

$y = a_1x + a_2x^2 + a_3x^3$, where y is the area under the stress/strain curve and x is the stress or the strain, respectively.

For a brief description of stepwise trials, see Table I. Loads: 2, 3, 4, 5, 6, 8 and 10 kg, and return to 10 kg/increase until rupture.

R^2: coefficient of determination. Region B: pericardium covering right ventricle. Region C: pericardium covering left ventricle.

TABLE IV Values for the equation fitting the stress/strain curve ($y = a_1x + a_2x^2 + a_3x^3$) and R^2 in the stepwise uniaxial trial with the application of sample selection criteria

Load changes	a_1	a_2	a_3	R^2
Region B				
1st increase	44.44	– 496.04	1725.31	0.877
Zero load	57.29	– 695.93	2458.80	0.937
2nd increase	– 22.05	2555.51	– 24 415.00	0.965
Zero load	51.54	– 773.22	11 304.10	0.962
3rd increase	4.35	1439.31	– 9537.60	0.993
Zero load	20.01	809.51	– 4808.10	0.968
4th increase	15.50	763.81	568.19	0.972
Zero load	43.30	– 430.43	9646.16	0.946
5th increase	9.24	1526.51	– 8385.00	0.888
Zero load	43.91	776.75	– 2916.40	0.938
6th increase	15.23	256.89	5715.45	0.985
Zero load	51.51	– 802.80	12 455.30	0.962
7th increase	25.79	194.69	6354.68	0.985
Zero load	36.20	70.48	6646.65	0.975
8th increase/rupture	14.03	1139.84	– 1915.90	0.974
Region C				
1st increase	31.85	– 320.27	1820.40	0.901
Zero load	54.56	– 781.24	4174.78	0.927
2nd increase	46.53	– 56.36	1355.94	0.945
Zero load	89.73	– 1442.60	11 885.10	0.946
3rd increase	52.57	– 229.22	7726.09	0.960
Zero load	40.30	510.23	– 2512.50	0.918
4th increase	27.74	421.45	5585.99	0.976
Zero load	69.93	– 190.28	6307.40	0.971
5th increase	15.16	1362.64	– 2342.00	0.977
Zero load	22.54	1358.66	– 4349.80	0.974
6th increase	– 5.24	1930.30	– 6129.70	0.985
Zero load	55.01	– 403.96	13 703.00	0.984
7th increase	4.01	1631.52	– 3597.40	0.981
Zero load	29.91	858.26	1411.06	0.969
8th increase/rupture	7.35	1935.86	– 5423.90	0.990

$y = a_1x + a_2x^2 + a_3x^3$, where y is the area under the stress/strain curve and x is the stress or the stress or the strain, respectively.

For a brief description of stepwise trials, see Table I. Loads: 2, 3, 4, 5, 6, 8 and 10 kg, and return to 10 kg/increase until rupture.

R^2: coefficient of determination. Region B: pericardium covering right ventricle. Region C: pericardium covering left ventricle.

3.2. Mathematical fit of the stress/strain curve

Table II shows the results of the mathematical fit in the control uniaxial trial both when sample selection criteria were not applied and when they were. After sample selection, the coefficients of determination (R^2) ranged between 0.697 and 0.958 in region B and 0.701 and 0.816 in region C. The mathematical fits of the stress/strain curves for each increment in load and the corresponding no-load interval until rupture, without and with the application of the selection criteria, appear in Tables III and IV, respectively. In the trials in which the selection criteria were not employed, the coefficients of determination (R^2) ranged between 0.826 and 0.974, the highest values corresponding to the final increments in load. Those observed with the application of the selection criteria ranged from 0.872 to 0.993 and showed no changes throughout the different load/no-load cycles.

Tables V and VI show the fits of the stress/strain curves for the biaxial trials without and with selection criteria, respectively. When these criteria were not applied, the coefficients of determination (R^2) ranged from 0.797 to 0.949. When the selection criteria were employed, these values ranged between 0.825, corresponding to the final increase to reach the ultimate load at rupture in samples from region C, and 0.973.

3.3. Hysteresis

The best fit for the curves representing the relationship between the area under the stress/strain curve and the stress and the strain, respectively, corresponded to the third-order polynomial $y = a_1x + a_2x^2 + a_3x^3$, with coefficients of determination (R^2) ranging from 0.999 to 1.000. These findings appear in Table VII and in Figs 3 and 4.

4. Discussion

The most rational approach to the design of safe devices involving biomaterials for use in health care is to characterize the mechanical behavior of the tissues. One of these materials, calf pericardium, has been employed in arterial repair [19] given its impermeability to flowing blood and its low risk of thrombogenicity,

TABLE V Values for the equation fitting the stress/strain curve ($y = a_1x + a_2x^2 + a_3x^3$) and R^2 in the stepwise biaxial trial without the application of sample selection criteria

Load changes	a_1	a_2	a_3	R^2
Region B				
1st increase	1.905	− 1.824	0.955	0.938
Zero load	0.000	0.816	0.062	0.935
2nd increase	2.725	− 2.980	1.354	0.938
Zero load	3.154	− 3.527	1.513	0.938
3rd increase	4.391	− 5.076	1.984	0.946
Zero load	4.199	− 4.925	1.946	0.942
4th increase	8.967	− 10.339	3.447	0.944
Zero load	9.367	− 10.901	3.569	0.949
5th increase/rupture	10.296	− 13.213	4.495	0.941
Region C				
1st increase	0.817	0.935	− 0.239	0.911
Zero load	− 3.824	7.132	− 2.280	0.892
2nd increse	− 5.177	6.687	− 1.360	0.878
Zero load	− 3.884	5.367	− 1.039	0.874
3rd increase	− 2.693	2.861	0.011	0.871
Zero load	− 1.687	1.829	0.251	0.866
4th increase	3.133	− 4.646	2.304	0.873
Zero load	4.774	− 6.193	2.573	0.858
5th increase/rupture	− 19.872	13.343	− 0.783	0.797

$y = a_1x + a_2x^2 + a_3x^3$, where y is the area under the stress/strain curve and x is the stress or the strain, respectively.

For a brief description of stepwise trials, see Table I. Loads: 1.5, 3, 7, and 10 kg, and return to 10 kg/increase until rupture.

R^2: coefficient of determination. Region B: pericardium covering right ventricle. Region C: pericardium covering left ventricle.

even in the absence of postoperative anticoagulation therapy. It has also been utilized with similar advantages in the manufacture of more complex structures such as leaflets for bioprosthetic heart valves.

The controversy surrounding these biological devices concerns their long-term resistance, or durability [20, 21]. To address this question, we have analyzed the mechanical behavior of calf pericardium, a tissue that has been employed for more than 20 years in the construction of cardiac bioprostheses and other objects for health care use [3, 5–7, 19]. Our methodology involves the analysis and comparison of pairs of contiguous samples [18] to ensure the homogeneity necessary for the manufacture of these implants.

TABLE VI Values for the equation fitting the stress/strain curve ($y = a_1x + a_2x^2 + a_3x^3$) and R^2 in the stepwise biaxial trial with the application of sample selection criteria

Load changes	a_1	a_2	a_3	R^2
Region B				
1st increase	1.577	− 1.076	0.617	0.952
Zero load	0.000	1.237	− 0.209	0.951
2nd increase	0.794	− 0.613	0.675	0.947
Zero load	1.305	− 1.223	0.839	0.948
3rd increase	3.398	− 4.042	1.758	0.942
Zero load	3.554	− 4.266	1.810	0.937
4th increase	8.323	− 9.908	3.429	0.938
Zero load	9.608	− 11.371	3.753	0.945
5th increase/rupture	3.909	− 6.964	3.044	0.911
Region C				
1st increase	2.523	− 2.133	1.165	0.967
Zero load	0.043	1.311	0.000	0.967
2nd increase	5.969	− 6.926	2.816	0.971
Zero load	6.240	− 7.276	2.916	0.971
3rd increase	10.061	− 11.954	4.296	0.973
Zero load	10.643	− 12.722	4.512	0.973
4th increase	14.825	− 17.512	5.835	0.970
Zero load	14.028	− 16.480	5.396	0.931
5th increase/rupture	9.083	− 13.425	5.183	0.825

$y = a_1x + a_2x^2 + a_3x^3$, where y is the area under the stress/strain curve and x is the stress or the strain, respectively.

For a brief description of stepwise trials, see Table I. Loads: 1.5, 3, 7 and 10 kg, and return to 10 kg/increase until rupture.

R^2: coefficient of determination. Region B: pericardium covering right ventricle. Region C: pericardium covering left ventricle.

TABLE VII Values for the equation fitting the curves representing the relationship between the area under stress/strain curve and the stress and the strain, respectively and R^2 in the stepwise biaxial trial

Curve	a_1	a_2	a_3	R^2
Stress				
Region B	0.688	− 0.003	5×10^{-5}	1.000
Region C	1.279	− 0.013	8×10^{-5}	0.999
Strain				
Region B	12.267	− 12.743	3.554	1.000
Region C	1.631	− 5.261	2.973	1.000

$y = a_1x + a_2x^2 + a_3x^3$, where y is the area under the stress/strain curve and x is the stress or the strain, respectively.

R^2: coefficient of determination. Region B: pericardium covering right ventricle. Region C: pericardium covering left ventricle.

In the uniaxial trials, the stresses at rupture were very uniform, ranging between 25.70 and 28.72 MPa, and the comparison of the means revealed no statistically significant differences (Table I). The tensile strength of the tissue was not influenced by the region of pericardial tissue being tested (that covering right ventricle or that covering left ventricle) and when compared with control samples that underwent continuously increasing stress until rupture, there was no loss of resistance in tissue subjected to repeated cycles of stepwise increments in load until rupture.

In biaxial trials, the mean ultimate stresses were significantly different from those observed in the uniaxial studies ($p < 0.01$) and also differed significantly ($p < 0.05$) depending on the region from which the sample had been harvested (Table I). The pericardium covering left ventricle (region C) was more resistant than that covering right ventricle (region B). Left ventricle is exposed to greater mechanical stress and its cardiac muscle is much stronger than that of right ventricle. This observation suggests that the composition and direction of the fibers in these two regions must be different.

The selection method employed, based on the application of morphological and mechanical criteria to pairs of samples [18], led to the exclusion of 60% of the harvested specimens, ensuring a marked homogeneity among those remaining.

When the selection criteria were applied, the mathematical fit of the stress/strain curve [15] was excellent both in the uniaxial study (Tables II and IV) and the biaxial trials (Table VI), with coefficients of determination (R^2) of over 0.90 in nearly every case. As shown in Table III, in the stepwise uniaxial study without sample selection, the fit improved, with increasing R^2, as loads of greater magnitude were applied ($R^2 > 0.96$). The collagen fibers responsible for the resistance of the biomaterial [17] align themselves in the direction of the load [16] as they receive each new stimulus, apparently making selection unnecessary for the homogeneity of the

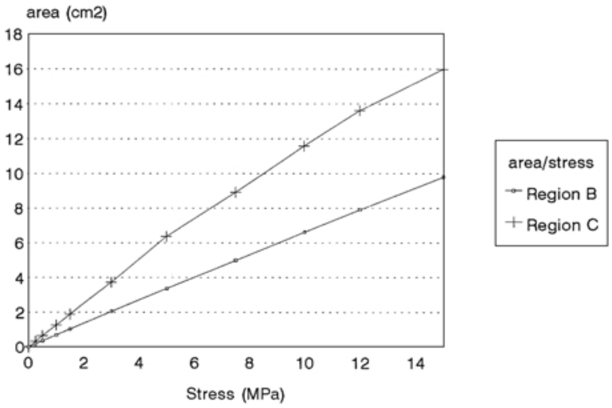

Figure 4 Relationship between the stress applied and the area under the stress/strain curve, or energy consumed by the subsequent deformation, in stepwise biaxial trials.

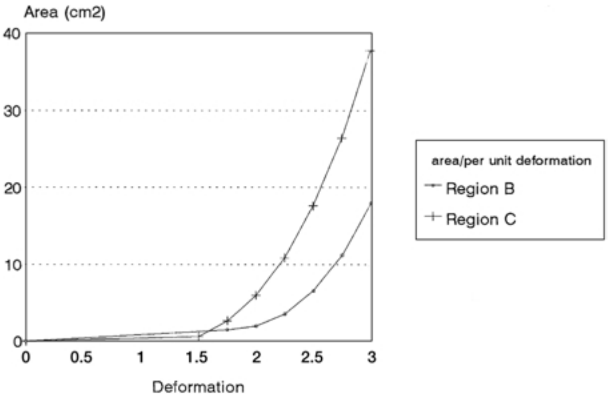

Figure 5 Relationship between the strain, or deformation, and the area under the stress/strain curve, or energy consumed by said deformation, in stepwise biaxial trials.

results. The load itself seems to render the response to the mechanical stimulus uniform. This phenomenon is not observed in the biaxial study. Collagen fibers receiving stimuli applied along two perpendicular axes would logically be unable to respond by aligning themselves in any one direction. The greatest deformation would be produced in the most elastic direction, that is, in the radial direction [16]. This greater elasticity reduces the stress in pericardium employed in a functioning cardiac valve leaflet, or in native leaflets, as well, as Clark demonstrated in 1973 [22]. This observation is further supported by the analysis of stress using finite element methods [23].

There are clear variations in the coefficients of the equation for the stress/strain ratio in the biaxial study after sample selection (Table VI), with increases in a_1 and a_3 and a decrease in a_2, indicating changes in the stress/strain curve after each stepwise increase in load followed by a return to zero load. This corresponds to the variation in hysteresis, defined as the energy consumed or dissipated with each deformation cycle [14]. In 1987, Trowbridge and Crofts demonstrated that the hysteresis in bovine pericardium was independent of the extension rate [24]. Our findings indicate that an increasing rate of elongation or deformation is associated with a change in the energy consumed during the deformation, and that this variation can be estimated using a mathematical model. Table VII shows the functions that relate the area under the stress/strain curve with the stress applied and with the strain produced, respectively; these results are also presented in Figs 4 and 5. They illustrate the exponential nature of the energy consumed to produce a great deformation, beyond the elastic limit of the pericardium, partly in internal shear stress [15] and partly in the destruction of collagen fibers [17]. This suggests that there is a limit to the energy consumed with each stress/strain cycle and that, beyond this limit, microfractures secondary to internal shear stress are produced in the fibers. These microfractures play a role in the loss of resistance over time in a stabilized biological tissue such as calf pericardium.

One of the conclusions that can be reached as a result of this study is that biaxial trials better simulate the actual physiological work to which a biomaterial will be subjected. Another is that the method for sample selection employed ensures the homogeneity of the results of the biaxial study [25]. We can also conclude that the deformation of the pericardium covering left ventricle, which is more resistant to tears, consumes more energy as compared to the pericardium on the right.

Physiological dynamic biaxial trials [26], that is, those carried out at a rate of one cycle per second [27], will be necessary before the pericardium over the left ventricle can be recommended for use in the manufacture of bioprosthetic cardiac valve leaflets. The change in energy consumed after deformation has taken place may be the key to the assessment of the short-term results of these trials.

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