Porcine pericardial membrane subjected to tensile testing: preliminary study of the process of selecting tissue for use in the construction of cardiac bioprostheses

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The durability of cardiac bioprostheses is limited fundamentally by structural failure due to mechanical fatigue and calcification. In the present report, we analyze, using an *in vitro* hydraulic simulator to test tensile strength, the mechanical behavior of porcine pericardium for the purpose of establishing the criteria for selecting the biomaterial, taking into account both morphological criteria (thickness and homogeneity of the specimens) and mechanical criteria (stress at breaking point), using the epidemiological model of paired samples. The stress at breakage was found to range widely from 24.07 MPa to 100.29 MPa, although we observed no statistically significant differences when comparing the mean results in the different regions and zones of the pericardium being studies. The application of the selection criteria in the present series resulted in an excellent mathematical fit in terms of the stress/elongation ($R^2 > 0.95$), making it possible to establish, by means of linear regression, the prediction of the tensile strength in one zone on the basis of the values observed in its paired specimen.

1. Introduction

The materials most widely employed for the construction of biological cardiac prostheses at the present time are bovine pericardium and porcine valves [1]. In both cases, the durability of the implanted cardiac bioprosthesis is limited by calcification and mechanical fatigue, making its replacement with another similar one or a mechanical prosthesis necessary over the medium or long term [2–9]. A great deal of effort has focused on preventing or diminishing, by means of chemical treatments, the deposition of calcium on the valve leaflets of the bioprostheses and to improve the mechanical behavior of the biomaterials employed in their construction.

However, tissue calcification and primary tissue failure are the most important factors involved in bioprosthesis failure. Primary tissue failure is caused by the mechanical stress to which the tissue is subjected, an aspect that can also be corrected by improving the design. Another factor that plays a major role in the mechanical function and durability of cardiac bioprostheses is the interaction of the different materials used in their construction, making it necessary to take into consideration each and every one of the elements of which the leaflet is composed and their different behaviors when subjected to fatigue stress [10–17].

Further improvements in fixation techniques and in the bioprostheses themselves are being introduced to prolong the durability and enhance the functional characteristics of bioprosthetic heart valves. The development of a biomaterial capable of withstanding calcification and mechanical stress and, at the same time, as durable as a metallic prosthesis would make bioprostheses constructed of that material the replacement of choice by eliminating the need for anticoagulation therapy.

The purpose of the present report is to take a closer look at the reality of this mechanical behavior by testing the tensile strength of these biomaterials *in vitro*. We have used a hydraulic model developed in our laboratory that is capable of reproducing, in static as well as dynamic tests, the behavior of pericardial membrane subjected to flow pressure. Using this system for static testing to determine the stress/elongation ratio of a circular piece of porcine pericardial membrane, placed perpendicularly to the flow, and its resistance to rupture when subjected to increasing pressure. The final objective is to develop a new *in vitro* system involving

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a hydraulic simulator to establish the criteria for selecting a biomaterial based on the prediction of the mechanical behavior of the samples tested [18].

2. Material and methods

2.1. Materials

The porcine pericardium was obtained immediately after the animal was sacrificed, and was transported to the laboratory in ice-cold isotonic saline (0.9% sodium chloride). Once the tissue was cleaned, each sac was mounted loosely on a 10 mm diameter ring, with the diaphragmatic attachment in the center and the sterno-pericardial ligaments on the circumference. Six circular membrane discs measuring 2 cm in diameter were cut out of each sac according to the diagram in Fig. 1.

The pericardium was treated for 24 h with 0.625% gluteraldehyde prepared from a commercial solution of 25% glutaraldehyde (Merck) in 0.1 M sodium phosphate buffer (pH 7.4) at a ratio of 1/50 (w/v). The trial involved 3 series of 15 pairs from the aforementioned zones, for a total of 90 specimens, Each membrane was subjected to increasing stress until breakage, which was determined by the loss of stress and its subsequent physical confirmation. The thickness of each membrane was measured by serial readings at 10 different points using a digital Mitutoyo micrometer (Elecount series E:A:33/8) having a precision at 20 °C of $\pm 3\,\mu$.

2.2. Assay method

The assay was carried out on a hydraulic simulator capable of delivering increasing stresses to the pericardial membranes secured with pressure clips (Fig. 2). The membranes were exposed to compressed saline solution. The simulator consists basically of a unit for measuring pressure equipped with a servomotor to drive the pump propelling the piston (Fig. 3).

2.3. General description of the function of the system

A piston is activated by means of a digital monitor based on a high-speed processor that controls the direct current

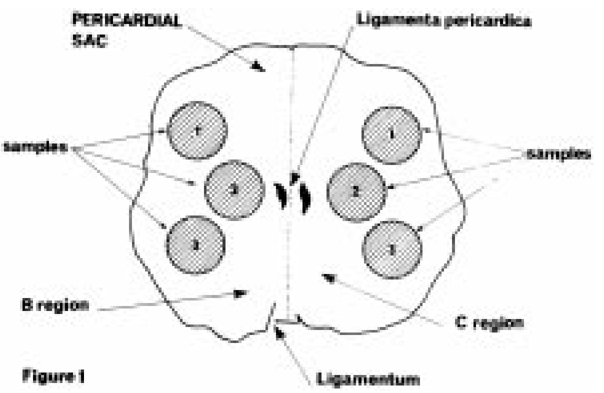

Figure 1 A pig pericardial membrane cut open, showing the different zones and regions to be tested. Six symmetrical circular discs were obtained from upper external, central and lower external zones 1, 2 and 3, respectively, in regions B and C, corresponding to the pericardium surrounding right ventricle (region B) and its symmetric equivalent from pericardium surrounding left ventricle (region C).

electric servomotor. The piston compresses the fluid and the pericardial membrane resists the pressure. The biomaterial is subjected to continuous increasing pressure 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 of the pressure pump at all times. The numerical data corresponding to these variables are transferred to a computer via a series interface, where they are stored for subsequent analysis.

2.4. Technical features of the system

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

Motor: Rated voltage: 24 Vdc; rated output 15 watts; starting torque: 120 mNm; intensity in a vacuum: 21.7 mA; starting current: 3040 mA; maximum permanent torque 30.46 mNm. Incremental position sensor: optoincremental type; two output channels in quadrature and index pulse.

Quadrature processor; programmable logic technology; two quadrature inputs; incremental/decremental monopolar impulses output; maximum working frequency of 4 MHz. Digital compensator: processor, RISC microcontroller, 24 MHz, 8 bits, 200 ns/instruction; maximum sampling frequency 1 KHz; velocity range from 00 to 838 8607 counts/sampling period *256; proportional action coefficient (KP) of − 32 768 to 32 767; differential action coefficient (KD) of − 32 768 to 32 767.

Piston: 160 mm stroke; 32 mm in diameter, maximum pressure 16 atm.

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

Computer: standard Pentium 75 configuration.

2.5. Tensile strength

Once the pressure withstood by the pericardial membrane at each instant of the trial was known, its tensile strength was calculated using the Laplace formula described by Timoshenko [19] for a thin-walled membrane subjected to pressure: $T_s = pr/2e$, where p is the pressure in 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 Kg/cm^2. To express this value in MPa we divided the result by 10.19.

2.6. Elongation

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

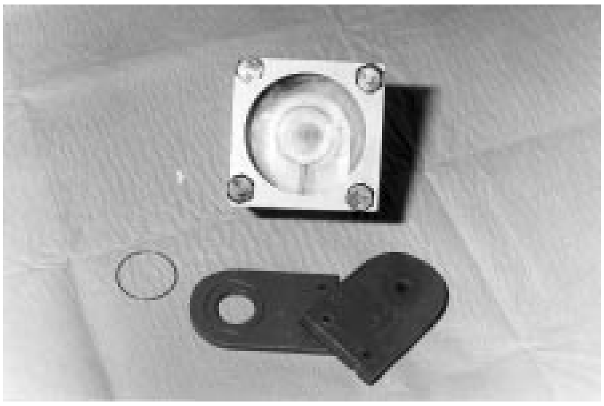

Figure 2 Close-up of the pressure clips employed to secure the pericardial membrane.

2.7. Statistical study and mathematical analysis

2.7.1. Comparison of means at rupture

The mean values at rupture in all the different regions and zones of the membranes were compared using Student's *t* test.

2.7.2. Mathematical fit of the tensile strength/elongation ratio

The tensile strength (Mpa)/elongation (per unit) ratio was studied using the least squares method. The best fit corresponded to a third degree parabola 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 is the per unit elongation of the membrane. For biological considerations, the analysis was done for $b_0 = 0$ and $x < 1$.

2.7.3. Mean overall fit for regions B and C and zones 1, 2 and 3

The tensile strength/elongation ratio was also studied, using the values obtained for each region and each zone within the regions.

2.7.4. Selection criteria

The following selection criteria were established to ensure greater homogeneity of the samples. The purpose of these statistical criteria was to determine the probability that each membrane tested actually belonged to the region or zone to which it was assigned in the initial selection. Thus, those membranes whose minimum thickness fell outside the mean minimum thickness plus or minus one standard deviation of the corresponding series were excluded, as were those membranes in which the homogeneity (understood to be the greatest difference between the mean thickness for the series and the minimum thickness of the sample being studied) fell outside the mean plus one standard deviation of the values obtained for the corresponding series. We also excluded the pairs of membranes (one each from regions B and C) in which the stress for $x = 1$ in region B was greater or lesser than the mean plus or minus one standard deviation of said value for region B (assuming that the value for C would initially be unknown and the results for B would be projected over C, with region B being assayed for the purpose of selecting tissue from region C, that is $y_c = f(y_b)$, where the values for C are those of the dependent variable and the values for B those of the independent variable.

Figure 3 View of the entire simulator system. The piston pumps can be seen at the left.

2.7.5. Mean overall fit of the selected regions and zones

On the basis of the aforementioned criteria, the sample pairs selected for this fit from regions B and C were as follows: zone 1, pairs 7, 8, 10, 11 and 12; zone 2, pairs 3, 9, 10 and 12; and zone 3, pairs 1, 3, 4, 7 and 8. In all, 31.1% of the specimens assayed were selected.

2.7.6. Predictive study (predicting the values for region C on the basis of those for region B)

A predictive study of the values for region C was performed after the selection process had been carried out and the values for the equivalent region B samples (zones 1–3) were known. This determination involved the mathematical calculation of the values for the selected pairs according to the aforementioned criteria, using linear regression analysis, where the values for region B were known, or independent variables, and those of region C were the dependent, or predictive, variables. The stress (MPa) in region C (y_c) was estimated on the basis of that of region B (y_b), and the 95% confidence intervals were calculated.

3. Results

3.1. Rupture

The mean results at rupture in the assayed series (regions and zones) are shown in Table I. Although there are no significant differences among the different regions and zones (52.45 MPa versus 64.38 MPa), it is interesting to note the broad range of values (24.07 MPa to 100.29 MPa) when all the sample series were taken into account.

3.2. Mathematical fit of the strength/elongation ratio

The individual equations for the cubic parabolas corresponding to each region and zone had determination

TABLE I Mean results at rupture in zones 1, 2 and 3 of regions B and C

Region/zone	No. of samples	Rupture stress MPa	Standard deviation	Range
B				
1	15	59.98	15.01	32.54–88.53
2	15	64.38	17.59	31.08–85.28
3	15	59.57	13.41	39.56–81.27
C				
1	15	60.63	16.95	32.56–100.29
2	15	52.45	15.06	24.07–78.95
3	15	59.11	14.36	38.86–89.42

TABLE III Overall mean fit of specimens selected from zones 1–3 in regions B and C

Region	b_1	b_2	b_3	R^2
B				
1	4.75	– 3.27	2.46	0.963
2	7.76	– 5.08	3.61	0.989
3	6.09	– 6.30	4.92	0.994
C				
1	10.74	– 13.60	9.89	0.977
2	9.56	– 13.70	9.87	0.978
3	8.67	– 10.79	7.57	0.974

$y = b_0 + b_1x + b_2x^2 + b_3x^3$
y: stress in MPa; x: per unit elongation.

coefficients (R^2) of over 0.95 in every case. The determination coefficients of the overall mean fit of the different regions (B and C) and zones (1–3) ranged between 0.873 and 0.921 (Table II). After application of the selection criteria presented in the material and methods section, the overall mean fit presented a clear improvement in the determination coefficients, which ranged between 0.963 and 0.994 in the series assayed (Table III).

3.3. Predictive study

According to linear regression analysis, the fit between regions B and C for each of the three zones showed an excellent correlation (R^2 ranging between 0.973 and 0.998) (Table IV). The practical application of these results made it possible to estimate the tensile strength for a sample from region C (y_c) when that of the corresponding sample in region B of the same membrane (y_b) was known. These results, together with the 95% confidence intervals, appear in Tables V–VII.

4. Discussion

Cardiac bioprostheses constructed of pig or calf pericardium have a limited duration. Calcification [6, 8] or mechanical fatigue, either together [9] or separately [11], are the processes blamed for the early failure of these structures [2]. The purpose of the present study was to characterize the mechanical behavior of pig pericardium. Another aim was to determine the degree to which this behavior was uniform in order to establish predictive criteria to aid in its selection [20].

In 1994, Sacks *et al*. [21] attributed the variability of the mechanical behavior of pericardium to the wide variation in the preferred direction of the collagen fibers contained in this tissue. Nevertheless, these authors considered that the pericardium covering anterior left ventricle might be a suitable material for section. In 1998, Hiester and Sacks [22, 23] analyzed the thickness and cartography of bovine pericardium, arriving at the conclusion that the anatomy did not guarantee the selection of a sufficiently homogeneous tissue or serve as the basis for predicting its fibrous structure and, thus, its mechanical behavior. That same year, Braile *et al*. [24] estimated that the better preservation of the collagen and elastic fibers of the pericardium covering right ventricle made this region more suitable for selecting the biomaterial to be employed in the construction of bioprostheses.

Two things appeared to be very clear: that the selection processes, which were fundamental to ensure homogeneous tissue, were controversial and that the results were often contradictory. Our hypothesis, according to which the selection was based on paired samples, warranted testing. For this purpose, we built a hydraulic simulator that was used to subject the pig pericardial membranes to increasing tensile strength until rupture, with the aim of establishing the aforementioned selection criteria.

The results at rupture demonstrated a high degree of resistance (range of the mean values of the different series: 52.45–64.38 MPa). However, when we analyzed the overall range (Table I), we found it to be very broad (24.07–100.29 MPa). The resistance at rupture is not the best parameter for comparing biomaterials [25]. In fact, we observed no significant differences when the mean

TABLE II Overall fit for the different regions (B and C) and zones (1–3)

Region	b_1	b_2	b_3	R^2
B				
1	6.96	– 3.07	2.13	0.904
2	12.27	– 18.13	13.57	0.928
3	8.59	– 11.27	8.42	0.873
C				
1	9.23	– 9.61	7.07	0.921
2	9.45	– 10.78	7.18	0.886
3	8.00	– 8.02	5.56	0.907

$y = b_1x + b_2x^2 + b_3x^3$
y: stress in MPa; x: per unit elongation.

TABLE IV Predictive study (B/C)

Region	b_0 (95% CI)	B_1 (95% CI)	R^2
B over C			
1	0.263 (0.071, 0.455)	1.185 (1.038, 1.332)	0.973
2	0.129 (0.019, 0.239)	0.893 (0.823, 0.964)	0.989
3	0.045 (0.002, 0.088)	1.279 (1.240, 1.318)	0.998

$y = b_0 + b_1x$
y = MPa; x = per unit.

TABLE V Predictive study (B/C). Zone 1. Estimate of the stress (y_c)

Elongation*	Stress (MPa) y_b	Stress (MPa) y_c(real)	Stress (MPa) y_c (predicted)	95% CI
0.05	0.24	0.50	0.55	0.12, 0.99
0.10	0.50	0.95	0.86	0.44, 1.28
0.15	0.78	1.34	1.18	0.77, 1.60
0.20	1.06	1.68	1.52	1.11, 1.93
0.25	1.35	1.99	1.86	1.45, 2.27
0.30	1.65	2.26	2.22	1.80, 2.63
0.35	1.95	2.51	2.56	2.14, 3.01
0.40	2.26	2.75	2.94	2.49, 3.40

*Per unit elongation. CI: confidence interval.

TABLE VII Predictive study (B/C). Zone 1. Estimate of the stress (y_c)

Elongation*	Stress (MPa) y_b	Stress (MPa) y_c (real)	Stress (MPa) y_c (predicted)	95% CI
0.05	0.29	0.41	0.41	0.33, 0.51
0.10	0.55	0.77	0.75	0.67, 0.83
0.15	0.79	1.08	1.05	0.97, 1.13
0.20	1.01	1.36	1.33	1.25, 1.41
0.25	1.21	1.61	1.59	1.50, 1.67
0.30	1.39	1.83	1.83	1.74, 1.91
0.38	1.57	2.04	2.05	1.97, 2.14
0.40	1.74	2.23	2.27	2.18, 2.36

*Per unit elongation. CI: confidence interval.

values of the series from region B (pericardium covering right ventricle) were compared with those recorded for region C (pericardium covering left ventricle). However, on an individual basis, low resistance of a bioprosthesis can lead to its failure [26]. The dilemma that always arises is that the samples tested with satisfactory results can no longer be employed and, thus, the material used in the construction of a safe bioprosthesis can not be tested previously. The selection criteria described in the present study led to an improvement in the overall mathematical stress/elongation ratio in all the series studied, identifying 31% of the samples as suitable (Table III). Linear regression established the predicted tensile strength of samples from region C when the values were known for the paired samples in region B, with excellent correlations (Table IV). Tables V–VII show these predictions for estimated per unit elongations of up to 0.4. To achieve this value in zones 1–3, the real stress (y_c) was 2.75, 2.26 and 2.23 MPa, respectively and that estimated according to our prediction was 2.94, 2.38 and 2.27 MPa, respectively.

We feel we have established reliable selection criteria, morphological and mechanical in one sample and morphological in its mate, the former to be tested and, when the results are satisfactory, the latter for use in bioprosthesis construction. Our next proposal is to subject the selected pairs to dynamic fatigue testing [27]. This dynamic trial will serve to definitively determine whether our hypothesis is valid.

TABLE VI Predictive study (B/C). Zone 2. Estimate of the stress (y_c)

Elongation*	Stress (MPa) Y_b	Y_c (real)	Y_c (predicted)	95% CI
0.05	0.38	0.44	0.46	0.23, 0.69
0.10	0.73	0.83	0.79	0.56, 1.00
0.15	1.06	1.16	1.08	0.86, 1.29
0.20	1.38	1.44	1.36	1.14, 1.57
0.25	1.68	1.69	1.62	1.41, 1.84
0.30	1.97	1.90	1.88	1.66, 2.11
0.35	2.24	2.09	2.13	1.90, 2.36
0.40	2.52	2.26	2.37	2.13, 2.62

*Per unit elongation. CI: confidence interval.

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