The influence of chemical treatment and suture on the elastic behavior of calf pericardium utilized in the construction of cardiac bioprostheses

J.M. GARCÍA PÁEZ, E. JORGE HERRERO, A. CARRERA SAN MARTÍN*, J.V. GARCÍA SESTAFE**, G. TÉLLEZ***, I. MILLÁN, J. SALVADOR, A. CORDÓN*, J.L. CASTILLO-OLIVARES

*Servicio de Cirugía Experimental, *Servicio de Cirugía Cardiovascular, Clínica Puerta de Hierro; **Departamento de Mecánica Estructural, Escuela Superior Técnica de Ingenieros Industriales; ***Universidad Antonio Nebrija; Madrid, Spain*

Corresponding author: Dr J.M. García Páez, Servicio de Medicina Preventiva, Clínica Puerta de Hierro, San Martín de Porres, 4, 28035 Madrid, Spain

Poor mechanical properties of biological tissue are known to cause wear, leading to the failure of cardiac bioprostheses made of calf pericardium. Different chemical agents such as sodium dodecyl sulfate (SDS) are presently being tested as possible inhibitors of the calcification process. The objective of this report was to determine the mechanical behavior of calf pericardium treated with SDS for 24 h and the influence of the suture on the mechanical properties of the tissue. Forty-eight samples were tested: 24 subjected to a standard treatment with glutaraldehyde (12 sewn with 4/0 silk suture thread) and 24 incubated with SDS for 24 h (12 sewn with the same suture thread). Each sutured and nonsutured sample was cut into two strips to yield paired samples. All were subjected to tensile stress to breaking point. The mean stress at breaking point in the nonsutured series treated with glutaraldehyde alone was 16.42 and 13.85 MPa, depending on the region of the pericardium, while in the sutured samples subjected to glutaraldehyde the mean stress was 7.50 and 7.63 MPa, respectively, differences which were statistically significant ($p = 0.03$ and $p = 0.003$, respectively) when the means for nonsutured samples from equivalent regions treated with glutaraldehyde were compared. The stress at breaking point was lower in the SDS-treated series, ranging between 2.60 and 3.56 MPa. The mathematical functions that govern the stress/strain or deformation were obtained. In the series of pericardium treated with SDS, deformations of 10% were produced with stresses of under 0.4 MPa, an outcome that is intolerable from the constructive point of view. We established a regression model that enabled us to determine the mechanical behavior of a sutured sample by testing a contiguous piece of tissue, with a high correlation coefficient ($r > 0.99$). We consider this finding to be of interest in the selection of pericardium for use in the construction of leaflets for cardiac bioprostheses.

Introduction

The understanding of the mechanical behavior of the calf pericardium utilized in the construction of cardiac bioprosthesis leaflets is one of the main points of interest in the field of biological prostheses [1–3]. Calcification [4–7], mechanical wear [8–12] or both processes [13, 14] are known to cause the failure of these devices, although the interaction between the two phenomena is less well defined. The reduction or delay of calcification by means of chemical treatments has been the objective of numerous studies [15–18]. If this goal is accomplished, it is necessary to guarantee that the chemical treatment employed will not alter the mechanical properties of the biomaterial and that its viscoelastic behavior is similar or even better than that resulting from standard glutaraldehyde treatment.

On the other hand, valve leaflet sutures and the shearing or cutting effect they exert on the pericardium of which the leaflet is made have been accused of compromising the durability of the bioprostheses [9]. There is some evidence that phospholipids are involved in the calcification process affecting biological cardiac prostheses. Certain chemical treatments have been shown, in *in vivo* models, to be effective in eliminating the influence of these substances [6, 7, 15–19]. Organic solvents, such as chloroform-methanol use by our group, notably reduced the amount of calcium determined in a subcutaneous implantation model [6, 15–17], and

compared favorably with the use of sodium dodecyl sulfate (SDS) [6]. Recently, Vyavahare *et al*. tested ethanol as an alternative to treatment with chloroform/methanol, reporting similar results [20]. All these treatments, including SDS, may act by denaturing tissue collagen. However, they could also function by extracting lipids from matrix vesicles or membranous vesicles. Hirsch *et al*. [18] showed that the principal effect of SDS on biological tissue was phospholipid extraction. Incubation of porcine aortic valve specimens in a 1% SDS solution for 24 h significantly inhibited calcification after 21 days of subdermal implantation in 3-week-old male rats.

Studies performed by other investigators involving intracardiac implantation of mitral and tricuspid valve replacements into young sheep have shown that preimplantation processing with SDS inhibits the calcification only on porcine aortic valve bioprostheses [19].

In this work, we have tested the mechanoelastic properties of calf pericardial tissue subjected to 24 h of treatment with SDS to evaluate the influence of this anionic detergent on the mechanical behavior in a model of stress/strain, comparing it to that of the same tissue exposed to treatment with glutaraldehyde. It is not clear, whether the mechanism of SDS anionic detergent involves a chemical modification of the tissue proteins, that impedes hydroxyapatite crystallization. It is currently under investigation by the FDA for approval for use as a commercially available treatment [21].

Material and methods

Tissue selection and preparation

The pericardium of young calves was obtained directly from a local slaughterhouse and transported in ice cold isotonic saline (0.9% NaCl). Once the tissue was cleaned, the sac was inspected visually, with the diaphragmatic attachment in the center and the sternopericardial ligaments on the circumference. Then it was cut into 12×2 cm strips of varying thickness. All the strips of pericardium that were less than 0.3 mm or more than 0.7 mm thick at any point were rejected to ensure a mean thickness of approximately 0.5 mm; only those strips that fulfilled this condition were selected. The strips were divided into two different groups according to the treatment they were to receive.

Twenty-four fragments of calf pericardium were obtained from regions B and C according to a modification of a scheme proposed by Purinyia [22] (Fig. 1). A total of 48 paired samples were obtained by cutting each piece of tissue cleanly into two strips, one of which was sewn up the middle with a running suture using silk 4/0 thread (Lorca Marin Inc., Spain) (Table I). Taperpoint needles were employed to avoid tearing the biological tissue during suturing.

Chemical treatments

The four control groups (G) were treated for 24 h with 0.625% glutaraldehyde 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 four SDS groups were pretreated for 24 h with 1% SDS (Sigma) in 0.15 M NaCl at a ratio of 1/30 (w/v), followed by the same treatment as the control group. The following subgroups were established:

a) 12 control strips from region B, 6 sutured (GBs) with silk 4/0 and 6 nonsutured (GB)

b) 12 control strips from region C, 6 sutured (GCs) with silk 4/0 and 6 nonsutured (GC)

c) 12 SDS-treated strips from region B, 6 sutured (SDS-Bs) with silk 4/0 and 6 nonsutured (SDS-B)

d) 12 SDS-treated strips from region C, 6 sutured (SDS-Cs) with silk 4/0 and 6 nonsutured (SDS-C).

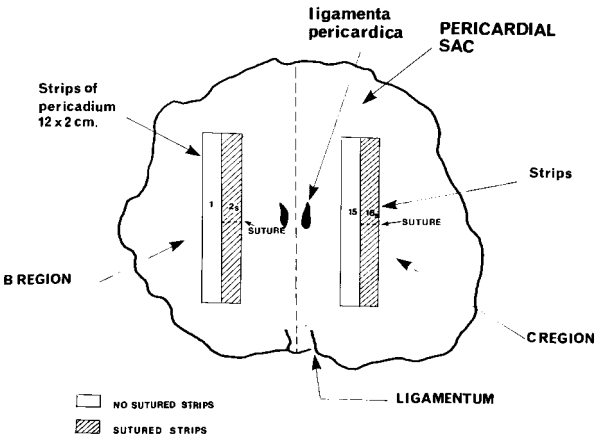

Figure 1 Diagram of the pericardium. Regions B and C, the source of the samples, are indicated and the procedure to obtain paired samples by cutting a single sample into two strips is shown.

TABLE I The different series of calf pericardium samples tested

Series	No. samples	Numeration	Type of suture
GB	6	B1,B3,B5....B-11	–
GBs	6	B2s, B4s...B12s	Silk 4/0
GC	6	C1,C3...C11	–
GCs	6	C2s,C4s...C12s	Silk 4/0
SDS-B	6	B13,B15..B23	–
SDS-Bs	6	B14s,B16s..B24s	Silk 4/0
SDS-C	6	C13,C15...C23	–
SDS-Cs	6	C14s,C16s...C24s	Silk 4/0

Testing of mechanical properties

All the samples were subjected to tensile testing to breaking point, using an Instron TTG4 tensile tester (Instron Ltd., High Wycombe, Buck, UK) (Fig. 2) which records tensile deformation under varying rates of strain. The samples were clamped in such a way as to leave a free lumen of 60 mm. All the samples were cut with the aid of a stencil to ensure the exact dimensions. The thickness was measured by a Mitutoyo digital micrometer (Elecount series E: A33/8) having a precision at 20 °C of $\pm$ 3 microns. Readings were made every 0.5 cm. The results were recorded graphically, showing the load/stretch diagram necessary to be able to calculate the stress/strain curve. The tensile stress of the pericardium was calculated taking into account the middle section.

Figure 2 Overall view of the installations showing the Instron tensile strength tester.

The results were subjected to statistical study and mathematical analysis. For the assays carried to breaking point, Student's *t* test was applied to compare the means. The functions that establish the relationship between the stress applied in MPa (y) and the strain produced expressed per unit (x) were fitted. This fit was calculated according to the equation $y = ax^2 + bx$. The excellent determination coefficients (R^2) obtained confirmed the goodness of fit (see the Results section). The average curve was obtained for each series (Figs. 3 and 4). The functions that defined the stress strain curves in terms of their discrepancies were compared (paired assays) for values under the elastic limit (values for x ranging between 0.05 and 0.1). For this comparison, the quadratic minimums were used.

Results

Breakage

The results of the tests taken to breaking point appear in Table II. The comparison of the series, showed no significant differences among them, although in the series treated with glutaraldehyde, in terms of the mean stress at breaking point, there were statistically significant differences between the nonsutured samples (GB and GC) and the sutured samples (GBs and GCs (16.42 and 13.85 MPa versus 7.50 and 7.63 MPa, respectively); $p = 0.03$ and $p = 0.003$). These differences were not detected in the series treated with SDS, in which the mean values ranged between 2.60 and 3.56 MPa (Table II).

TABLE II Mean stress at breaking point in each of the series being assessed

Series	Mean (MPa)	Standard deviation	σ_{n-1}
GB*	16.42	4.61	5.05
GBs*	7.50	2.31	2.53
GC**	13.85	5.38	5.88
GCs**	7.63	2.23	2.45
SDS-B	3.56	1.40	1.54
SDS-Bs	2.60	0.84	0.92
SDS-C	3.36	1.58	1.73
SDS-Cs	2.75	1.56	1.71

σ_{n-1}: centered standard deviation.
*GB/GBs, $p < 0.03$; **GC/GCs, $p < 0.003$ (see text).

The results of the analysis of the viscoelastic behavior of the pericardium treated with glutaraldehyde alone after being subjected to tensile stress are shown in Fig. 3. The mean values obtained showed a slightly greater resistance to stress in the pericardium obtained from region C; this difference was more marked in the sutured series, again with higher values in region C. These findings were not confirmed in the series of SDS-treated pericardium (Fig. 4) where stresses ranging between 0.2 and 0.4 MPa produced deformations of 10%.

Mathematical analysis of the stress/strain curves

Mathematical analysis demonstrated that when the samples underwent tensile testing, the functions with the best fit were quadratic parabolas expressed by: $y = ax^2 + bx + c$, and coinciding at their origin ($c = 0$). Tables III, IV, V and VI show the values for coefficients ''a'' and ''b'', as well as the values for the determination

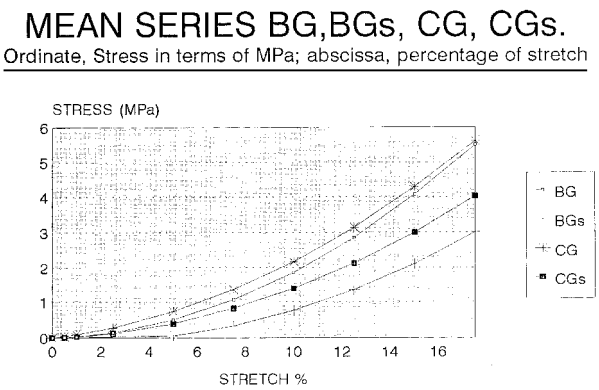

Figure 3 The mean functions for the series treated with glutaraldehyde alone. GB: control group obtained from region B. GBs: sutured samples (4/0 silk thread) from region B. GC: Control group obtained from region C. GCs: sutured samples (4/0 silk thread) from region C. Stress is expressed on the ordinates. Percentage of stretch on the abscissas.

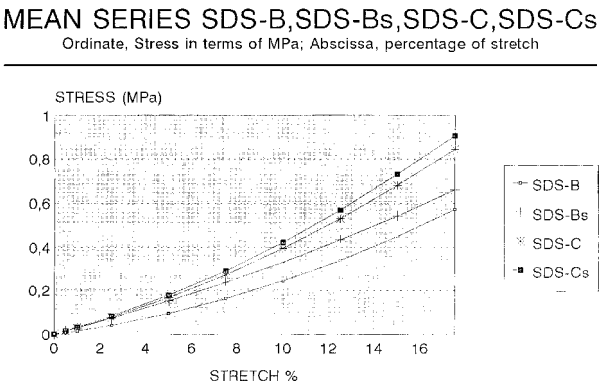

Figure 4 The mean functions for the series treated with sodium dodecyl sulfate (SDS). SDS-B: SDS-treated samples obtained from region B. SDS-Bs: sutured (4/0 silk thread) SDS-treated samples from region B. SDS-C: SDS-treated samples obtained from region C. SDS-Cs: sutured (4/0 silk thread) SDS-treated samples from region C. Stress is expressed on the ordinates. Percentage of stretch on the abscissas.

TABLE III Functions obtained for each sample (coefficients a, b and R^2 of the expression $y = ax^2 + bx$) for controls obtained from region B (nonsutured and sutured sample pairs)

Samples	a	b	R^2
B1	121.608	9.294	0.998
B3	217.454	− 14.489	0.997
B5	133.845	25.209	0.995
B7	172.341	3.886	0.999
B9	208.065	− 17.737	0.991
B11	179.675	1.795	0.996
B2s	113.474	− 0.486	0.999
B4s	118.199	− 2.035	0.998
B6s	137.136	− 7.825	0.999
B8s	134.834	− 5.122	0.999
B10s	68.867	− 16.050	0.985
B12s	198.375	− 0.021	0.995

TABLE IV Functions obtained for each sample (coefficients a, b and R^2 of the expression $y = ax^2 + bx$) for controls obtained from region C (nonsutured and sutured sample pairs)

Samples	a	b	R^2
C1	224.834	13.629	0.989
C2	145.969	5.787	0.998
C5	246.624	− 4.864	0.998
C7	92.904	16.554	0.996
C9	87.150	− 7.781	0.994
C11	40.110	22.832	0.994
C2s	160.388	− 3.417	0.997
C4s	128.199	5.444	0.997
C6s	142.666	11.889	0.997
C8s	60.006	14.889	0.995
C10s	120.203	− 9.603	0.995
C12s	113.159	− 8.046	0.992

TABLE V Functions obtained for each sample (coefficients a, b and R^2 of the expression $y = ax^2 + bx$) for SDS-treatment group from region B (nonsutured and sutured sample pairs)

Samples	a	b	R^2
B13	23.875	− 0.045	0.979
B15	19.775	1.211	0.998
B17	8.566	0.952	0.988
B19	0.210	2.725	0.980
B21	7.585	2.010	0.991
B23	6.345	1.146	0.996
B14s	5.338	1.249	0.996
B16s	6.947	2.570	0.998
B18s	6.190	3.137	0.994
B20s	− 0.205	3.163	0.992
B22s	8.254	3.097	0.997
B24s	9.474	3.069	0.998

coefficients (R^2). The mean values for each series were as follows:

series GB, $y = 172.165x^2 + 1.326x$
series GBs, $y = 128.481x^2 - 5.257x$
series GC, $y = 139.582x^2 + 7.692x$
series GCs, $y = 120.770x^2 + 1.859x$
series SDS-B, $y = 11.059x^2 + 1.333x$
series SDS-Bs, $y = 5.999x^2 + 2.714x$
series SDS-C, $y = 11.856x^2 + 2.755x$
series SDS-Cs, $y = 12.994x^2 + 2.923x$

where y is the stress expressed in MPa and x the per unit stretch.

The differences between the sutured samples and their corresponding nonsutured samples were also assessed

TABLE VI Functions obtained for each sample (coefficients a, b and R^2 of the expression $y = ax^2 + bx$) for SDS-treatment group from region C (nonsutured and sutured sample pairs)

Samples	a	b	R^2
C13	6.854	1.843	0.985
C15	50.561	1.330	0.998
C17	1.252	3.448	0.994
C19	− 1.691	3.740	0.987
C21	5.937	3.645	0.992
C23	8.222	2.525	0.995
C14s	33.560	− 0.229	0.989
C16s	21.419	0.975	0.997
C18s	3.905	1.539	0.996
C20s	0.082	4.111	0.991
C22s	− 0.070	3.689	0.994
C24s	19.021	7.453	0.995

(paired samples). To illustrate this, the results are presented of the comparison in terms of the squared minimum values (regression curve fit) and the correlation coefficients (r) obtained:

GB....GBs: B1....B2s: $y = -0.220 + 0.602x$, $r = 0.9992$
GC....GCs: C1....C2s: $y = -0.339 + 0.438x$, $r = 0.9989$
SDS-B....SDS-Bs: B13...B14s: $y = 0.046 + 0.575x$, $r = 0.9978$
SDS-C....SDS-Cs: C13...C14s: $y = -0.119 + 1.678x$, $r = 0.998$

(comparison of the first samples of each series; paired samples).

Discussion

The use of different chemical treatments to improve the mechanical and biological properties of tissues used in the construction of cardiac prostheses, in the attempt to prevent calcification and degradation of the tissue, is one of the objectives of research in this field. Some of the new approaches, involve the search for an alternative chemical treatment to crosslinking with glutaraldehyde. Others focus on combining the use of glutaraldehyde with exposure to different chemical treatments that alter the biological properties of the tissue to eliminate or retard the mineralization process [23], including efforts aimed at the extraction of tissue phospholipids. Some groups are investigating the effects of organic solvents, such as chloroform-methanol, DMSO [23], ethanol [20] or anionic detergents (SDS) along these lines. However, any valid approach must address the problem of mineralization while conserving the mechanoelastic properties of the tissue.

SDS has been shown to have marked antimineralization properties in an *in vivo* model involving subcutaneous implantation [6], resulting in low levels of calcium deposition. The aim of this study was to determine the mechanical behavior of calf pericardium treated with SDS for 24 h and the influence of the suture (4/0 silk) on these treated samples. For this purpose, sutured and nonsutured samples were paired to avoid any error due to selection of the biomaterial. The control group consisted of pairs of sutured and nonsutured samples of glutaraldehyde-treated calf pericardium. Preincubation with SDS was effective in inhibiting calcification in aortic valves made of porcine pericardium [18]. Cutting stress has been implicated in the process of wear leading to breakage, and the suture is suspected of being responsible for this shearing effect

[9, 24]. Thus, we designed this trial for the purpose of learning more about the causes of the failure [13, 14] and their possible attenuation. The differences, between the sutured and nonsutured samples of calf pericardium subjected to standard treatment, in terms of their mean tensile stress at breaking point were statistically significant ($p < 0.03$ and $p < 0.003$ for regions B and C, respectively), there being a loss of resistance to breakage and a decrease in the elastic moduli [25]. As can be seen in Table II, the mean stress at rupture in series GB and GC (nonsutured samples treated with glutaraldehyde alone) was 16.42 and 13.85 MPa, much greater than the findings in the sutured series undergoing the same treatment: GBs and GCs (7.50 and 7.63 MPa, respectively). In the case of the pericardium treated with SDS, this does not appear to be the cause since the results at breaking point are very low in both the sutured and nonsutured series (between 3.56 and 2.60 MPa). The prolonged preincubation undoubtedly was harmful to their mechanical behavior.

Another objective of this study was to assess paired strips of pericardium obtained by cutting a single piece into two identical samples, one of which was sutured, to determine the degree of correlation between the sutured and the nonsutured samples and the possible influence of the chemical treatment. For this purpose, a study of the differences between the sutured and nonsutured samples was carried out, including their comparison by means of the quadratic minimums, which yielded an excellent fit and the corresponding regression curves (see the Results section). This finding, in our opinion, is highly interesting when it comes to selecting pericardium for the construction of cardiac valve leaflets [26].

In conclusion, given the excellent correlation obtained, a sample can be tested to determine the behavior of the contiguous region and the influence of the suture. These results are valid both for pericardium subjected to a standard treatment and that treated with SDS, applying the regression curves obtained, although we do not recommend such long preincubations (24 h) with the latter substance.

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