Resistance and Stability of A New Method for Bonding Biological Materials Using Sutures and Biological Adhesives

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ABSTRACT: The valve leaflets of cardiac bioprostheses are secured and shaped by sutures which, given their high degree of resistance and poor elasticity, have been implicated in the generation of stresses within the leaflets, contributing to the failure of the bioprostheses. Bioadhesives are bonding materials that have begun to be utilized in surgery, although there is a lack of experience in their use with inert tissues or bioprostheses.

Tensile testing is performed until rupture in samples of calf pericardium, a biomaterial employed in the manufacture of bioprosthetic heart valve leaflets.

One hundred and thirty-two trials are carried out in three types of samples: intact or control tissue ($n = 12$); samples transected and glued in an overlapping manner with a cyanoacrylate ($n = 60$); and samples transected, sewn with a commercially available suture material and reinforced at the suture holes with the same cyanoacrylate ($n = 60$). Seven days after their preparation, 12 samples from each group, including the controls, are subjected to tensile testing until rupture and the findings are compared.

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In the stability study, groups of 12 each of the remaining 48 glued and 48 sutured and glued samples underwent tensile testing until rupture on days 30, 60, 90, and 120, after their preparation.

The results show that bonding with the adhesive provided a resistance ranging between 1.04 and 1.87 kg, probably insufficient for use in valve leaflets, but also afforded a high degree of elasticity. After 120 days, both the glued and the sutured and glued series show excellent elastic behavior, with no rigidity or hardening of the pericardium. These samples present reversible elongation, or strain, when they surpass their elastic limit at rupture. This finding may be due to a load concentration that is damaging to the pericardium, to the behavior of the tissue as an amorphous material, or perhaps to both circumstances.

These results need to be confirmed in future studies as they may be of value in the design and manufacture of cardiac bioprostheses.

KEY WORDS: bioadhesives, sutures, mechanical resistance, pericardium, bioprostheses.

INTRODUCTION

To date, experience in the utilization of adhesives in the medical field, and specifically in cardiovascular surgery, is limited. The use of biological adhesives in inert tissues, such as bioprostheses or implants, involves essential questions such as their mechanical resistance and stability over time. Their behavior would not be that of a bioadhesive employed to join live tissues while they heal [1]. Thus, the issue requires careful analysis and study.

Nonabsorbable sutures present great resistance to tensile stress, are compatible and inert, and have resolved the problem of securing implants to tissues or giving shape to bioprosthetic heart valve leaflets [2]. Suture materials are highly resistant to deformation, or strain, because they are much less elastic than the biomaterials used to make the implants or bioprostheses. Thus, they produce a strong interaction, or internal shear, with said biomaterials. This interaction can be considered a cause, although not the only one [3,4], of the limited durability of cardiac bioprostheses [5–7].

The substitution of sutures by other bonding materials is currently a theoretical issue, although biological adhesives and glues play an increasingly important role as auxiliary elements in vascular repair [8]. Their resistance and durability over time remain unknown.

A combination of sutures and bioadhesives is employed in the surgical repair of aortic dissection. The mechanical behavior of the biological glue utilized has not been established, and complications have even been reported. Its evaluation is difficult because of the risks and high mortality rate associated with this type of surgery [9].

In other branches of the medical field, such as ocular surgery, the experience is greater. One cyanoacrylate (*N*-butyl-2-cyanoacrylate), approved by the American Food and Drug Administration, has been compared with a commercially available suture material (polypropylene) for use in blepharoplasty, with excellent results in terms of quality [10].

In this study, we analyze the mechanical behavior and stability of a cyanoacrylate adhesive, over time (120 days) known for its hemostatic effect and instantaneous adhesion that has been commercialized for use in cardiac and vascular surgery. Assays are designed to determine the resistance to tensile stress of samples of calf pericardium, a material similar to that used in the manufacture of bioprosthetic heart valve leaflets [11,12], transected and glued with tissue overlap or transected and sewn with a commercially available suture material (Pronova 5-0), which was reinforced at the suture holes with the same adhesive.

The objective of the study is to determine whether the adhesive, alone or in combination with suturing, offered sufficient and stable resistance. We propose to assess the possible benefits of these techniques in the bonding of implants or in the design and manufacture of cardiac bioprostheses [11].

MATERIAL AND METHODS

Biomaterials Employed

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 age of 9 and 12 months. The tissue was transported in saline solution (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 between the right and left ventricles. Once cut open, in such as way as to leave the diaphragmatic ligament in the center and the sternopericardial ligaments on the circumference, the pericardial sacs measured approximately 15 cm long, from root to apex, and 10 cm wide. All specimens were procured in such a way as to guarantee that they would present a similar morphology. They 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.

The samples were cut from the sacs in longitudinal, root-to-apex direction or, perpendicularly, in the transverse direction. They all had a length of 12 cm and a width of 2 cm (Figure 1).

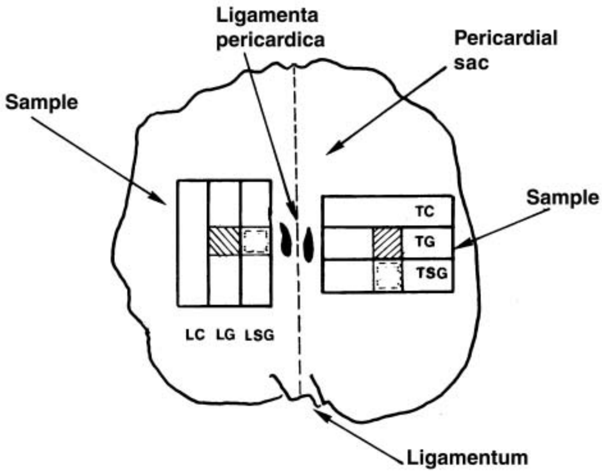

Figure 1. Diagram of the open calf pericardium showing the form in which the samples were cut. LC: longitudinal control samples; LG: longitudinal samples, cut and repaired by gluing with tissue overlap; LSG: longitudinal samples, cut and repaired by suturing and gluing. TC: transverse control samples; TG: transverse samples, cut and repaired by gluing with tissue overlap; TSG: transverse samples, cut and repaired by suturing and gluing.

Adhesive

The adhesive employed, Glubran 2 (GEM srl, Italy), a transparent, low-viscosity, instantaneous bioadhesive (*N*-butyl-2-cyanoacrylate) (monomer)/methacryloxysulfolane (monomer), has been authorized for medical use by the VSP norm (class VI). It is a monocomponent cyanoacrylate associated with rapid healing and, according to the manufacturer, a high resistance to tensile and shearing stresses. Moreover, it can be employed to bond different substrates.

Suture

Ethicon Pronova 5-0, a commercially available monofilament, non-absorbable suture produced by Johnson & Johnson was employed.

Sample Preparation

One hundred and thirty-two samples were tested (Table 1). Of the 12 samples used as controls, six were cut longitudinally (LC) and six transversely (TC). The remaining 120 strips, 60 cut longitudinally and 60 cut transversely, were transected for subsequent gluing (LG, $n = 30$, and TG, $n = 30$) or suturing and gluing (LSG, $n = 30$, and TSG, $n = 30$).

Table 1. Characterization of the series of calf pericardium samples employed in the study according to preparation and time elapsed until tensile testing.

Samples	Day 7 (*n*)	Day 30 (*n*)	Day 60 (*n*)	Day 90 (*n*)	Day 120 (*n*)	Total (*n*)
Controls	12	–	–	–	–	12
(LC + TC)	6	6	6	6	6	30
LG	6	6	6	6	6	30
LSG	6	6	6	6	6	30
TG	6	6	6	6	6	30
TSG						
Total	36	24	24	24	24	132

LC: longitudinal samples left intact
TC: transverse samples left intact
LG: longitudinal samples transected and repaired by gluing
LSG: longitudinal samples transected and repaired by suturing and gluing
TG: transverse samples transected and repaired by gluing
TSG: transverse samples transected and repaired by suturing and gluing

The glued series (LG and TG) were repaired with the adhesive Glubran 2, applied in such a way as to leave 0.5 cm of overlapping tissue, for a total surface area of 1 cm^2. Series LSG and TSG were joined, with a 1 cm^2 overlap, by a continuous suture, using Pronova 5-0, which surrounded the entire area of overlap. Glubran 2 was then applied to the holes produced by the suture.

Assays

Six samples from each of the repaired series (LG, TG, LSG, and TSG) were subjected to tensile testing 7, 30, 60, 90, and 120 days after their preparation ($n = 24$ at each time point), while the 12 controls (series LC and TC) were tested on day 7 (Table 1). The six samples from each of the four repaired series tested on day 7 were used for comparison with the controls, and, thus, these series will be referred to as LGC, TGC, LSGC, and TSGC. Until assay, the samples were preserved at 4°C in saline (0.9% NaCl) plus two antibiotics, streptomycin at a concentration of 333 μg/mL and penicillin at a concentration of 2000 U/mL.

The assay consisted of increasing uniaxial tensile testing, along the major axis, until rupture, confirmed by the loss of load and the morphological confirmation of the separation of the two portions of the samples when the adhesive failed, or the tears produced by the suture. These trials were performed on an Instron TTG4 tensile tester (Instron Ltd, High Wycombe, Buck, UK) which determines tensile stress and the strain or elongation it produces.

The samples were secured by means of two double clamps consisting of a wooden inner piece which held the samples and an outer metallic piece to hold the wooden clamps. A free lumen of 50 mm remained. The results were recorded graphically, showing the load–elongation (or deformation) diagram necessary to allow the calculation of the stress–strain curves.

The tensile stress in the control samples (LC and TC) was calculated taking into account the minimal cross-sectional area. In the glued strips (LG, TG, LGC, and TGC), the calculation was based on the area of contact ($2\,cm \times 0.5\,cm = 1\,cm^2$). In the sutured and glued samples (LSG, TSG, LSGC, and TSGC), this calculation was carried out using the cross-sectional area of the thread multiplied by the number of stitches, that is, the total cross-sectional area of the continuous suture employed to repair the sample.

Selection Criteria

Selection criteria were established to ensure greater homogeneity of the samples. The purpose of these statistical criteria was to establish the probability that each sample tested actually belonged to the zone to which it was assigned in the initial macroscopic selection. Thus, those strips 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 samples in which the difference between the mean thickness in the corresponding series and the minimum thickness of the sample was greater than the mean value for this difference as determined in the series, plus one standard deviation, indicating a lack of homogeneity.

On the basis of the aforementioned criteria, the following 80 samples were selected, representing 60.60% of the 132 samples assayed:

Series tested on day 7 (controls): LC, samples nos. 1, 3, and 5; TC, samples nos. 1, 3, and 5; LGC, samples nos. 1, 4, and 5; TGC, samples nos. 1, 3, 4, and 6; LSGC, samples nos. 1, 2, and 5; TSGC, samples nos. 1, 3–6.

Series tested on day 30: LG, samples nos. 3 and 5; TG, samples nos. 2–4 and 6; LSG, samples nos. 1–3 and 5; TSG, samples nos. 1–6.

Series tested on day 60: LG, samples nos. 2–4; TG, samples nos. 3–5; LSG, samples nos. 2 and 3; TSG, samples nos. 3–5.

Series tested on day 90: LG, samples nos. 1, 2, 4, and 6; TG, samples nos. 2–5; LSG, samples nos. 2–5; TSG, samples nos. 1–6.

Series tested on day 120: LG, samples nos. 2–5; TG, samples nos. 3–5; LSG, samples nos. 2–5; TSG, samples nos. 2–4.

Statistical Study

Comparison of the mean values at rupture

To compare the mean values at rupture, both in MPa and in machine kg, of controls and repaired series tested on day 7 and the remaining repaired series tested on days 30, 60, 90, and 120, we used analysis of variance (ANOVA) and the Newman–Keuls test for multiple comparisons. The Shapiro–Wilk test was used to confirm the normal distribution.

Mathematical Fit of the Stress–Strain (Elongation) Ratio

The tensile stress (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 $y = a_1x + a_2x^2 + a_3x^3$, where y is the tensile strength or mechanical stress in MPa and x is the per unit elongation (strain). 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 mathematical fit of the machine kg/strain ratio was also studied for each series of samples.

The same fits were determined after sample selection according to the criteria described in the preceding section.

RESULTS

Stress at Rupture in Machine kg

The mean stress at rupture in machine kg for the different series tested at each time point are shown in Tables 2 and 3.

Table 2 shows the results in the series tested 7 days after preparation. The mean stress in control samples cut longitudinally (LC) was 13.35 machine kg. This value was significantly higher than those observed in the series cut in the longitudinal direction and glued (LGC, 1.56 kg; $p = 0.000$) or sutured and glued (LSGC, 8.80 kg; $p = 0.032$). In the series cut transversely, the controls (TC) reached a mean stress at rupture of 12.24 kg, which, again, was significantly greater than that of glued samples cut in transverse direction (TGC, 1.87 kg; $p = 0.000$), but showed no statistical significance when compared with the sutured and glued series (TSGC, 11.42 kg).

The findings in the repaired series tested 30, 60, 90, and 120 days after their preparation are compared in Table 3. The mean values at

Table 2. Tensile stress at rupture, expressed in machine kg, in samples tested 7 days after preparation (controls).

Series	*n*	Means (kg)	Standard Deviation	95% CI	*p*
Control					
– LC	6	13.35	2.71	10.51–15.19	*
– TC	6	12.24	3.67	8.38–16.09	**
– LGC	6	1.56	0.54	0.99–2.12	***
– LSGC	6	8.80	2.96	5.68–11.91	
– TGC	6	1.87	0.34	1.50–2.23	****
– TSGC	6	11.42	2.37	8.93–13.91	

*LC versus LGC: $p = 0.000$; LC versus LSGC: $p = 0.032$
**TC versus TGC: $p = 0.000$
***LGC versus LSGC: $p = 0.000$
****TGC versus TSGC: $p = 0.000$
LC: longitudinal samples left intact; TC: transverse samples left intact; LGC: longitudinal samples transected and repaired by gluing; LSGC: longitudinal samples transected and repaired by suturing and gluing; TGC: transverse samples transected and repaired by gluing; TSGC: transverse samples transected and repaired by suturing and gluing; CI: confidence interval.

Table 3. Tensile stress at rupture, expressed in machine kg, in glued and in sutured and glued series assayed 30, 60, 90, and 120 days after preparation.

Series	*n*	Means	Standard Deviation	95% CI
30 days				
–LG	6	1.28	0.51	0.73–1.81
–LSG	6	7.25	2.16	4.99–9.53
–TG	6	1.10	0.66	0.41–1.79
–TSG	6	12.07	3.58	8.30–15.83
60 days				
–LG	6	1.04	0.23	0.79–1.29
–LSG	6	10.02	2.16	5.04–14.99
–TG	6	1.18	0.56	0.59–1.77
–TSG	6	10.67	3.22	7.29–14.04
90 days				
–LG	6	1.55	0.63	0.88–2.22
–LSG	6	8.18	1.71	6.39–9.98
–TG	6	1.10	0.89	0.17–2.03
–TSG	6	9.57	1.84	7.63–11.50
120 days				
–LG	6	1.67	0.62	1.02–2.32
–LSG	6	8.33	2.53	5.75–10.99
–TG	6	1.39	0.54	0.83–1.96
–TSG	6	9.68	2.21	6.93–12.43

LG: longitudinal samples transected and repaired by gluing; LSG: longitudinal samples transected and repaired by suturing and gluing; TG: transverse samples transected and repaired by gluing; TSG: transverse samples transected and repaired by suturing and gluing; CI: confidence interval.

rupture of the glued series (LG and TG) ranged between 1.04 (LG tested on day 60) and 1.67 kg (LG tested on day 120). There were no statistically significant differences between the mean values of the respective glued series when compared in terms of the direction in which they were cut (longitudinal or transverse) or the time elapsed between their preparation and tensile testing.

The mean values of the sutured and glued series (LSG and TSG) ranged from 7.25 (LSG tested on day 30) and 12.07 kg (TSG tested on day 60). The only statistically significant difference was observed at day 30, in the comparison between LSG (7.25 kg) and TSG (12.07 kg).

Stress at Rupture in MPa

In the control samples, this value represented the mean tensile stress being exerted on the pericardial tissue at the time of rupture, which was 24.73 MPa in the LC series and 28.16 MPa in the TC series. In the glued series compared with the controls on day 7 after preparation, these values, which represented the mean tensile stress at the time the two halves of a sample became detached or separated, were 0.15 MPa in the LGC series and 0.18 MPa in the TGC series. In the sutured and glued series tested on day 7, these values, which express the stress recorded in the suture thread when the shearing stress produces tears in the pericardial tissue, were 64.60 MPa in the LSGC series and 84.04 MPa in the TSGC series (Table 4).

Table 4. Tensile stress at rupture, expressed in MPa, in samples tested 7 days after preparation (controls).

Series	*n*	Mean	Standard Deviation	95% CI	*p*
Control					
– LC	6	24.73	5.68	18.76–30.69	*
– TC	6	28.16	8.76	18.96–37.35	**
– LGC	6	0.15	0.05	0.09–0.21	***
– LSGC	6	64.70	21.81	41.81–87.59	
– TGC	6	0.18	0.03	0.14–0.22	****
– TSGC	6	84.04	17.37	65.81–102.27	

*LC versus LSGC: $p = 0.000$; LC versus LGC: $p = 0.017$
**TC versus TSGC: $p = 0.000$; TC versus TGC: $p = 0.005$
***LGC versus LSGC: $p = 0.000$
****TGC versus TSGC: $p = 0.000$
LC: longitudinal samples left intact; TC: transverse samples left intact; LGC: longitudinal samples transected and repaired by gluing; LSGC: longitudinal samples transected and repaired by suturing and gluing; TGC: transverse samples transected and repaired by gluing; TSGC: transverse samples transected and repaired by suturing and gluing; CI: confidence interval.

Table 5. Tensile stress at rupture, expressed in MPa, in glued and in sutured and glued series assayed 30, 60, 90, and 120 days after preparation.

Series	n	Mean	Standard Deviation	95% CI
30 days				
– LG	6	0.13	0.05	0.07–0.17
– LSG	6	53.25	15.84	36.63–69.88
– TG	6	0.11	0.07	0.04–0.18
– TSG	6	88.68	26.33	61.05–116.31
60 days				
– LG	6	0.11	0.02	0.08–0.13
– LSG	6	73.73	34.86	37.15–110.32
– TG	6	0.12	0.05	0.06–0.17
– TSG	6	78.73	23.61	53.95–103.51
90 days				
– LG	6	0.15	0.06	0.08–0.22
– LSG	6	60.14	12.55	46.96–73.31
– TG	6	0.11	0.09	0.01–0.20
– TSG	6	70.30	13.56	56.07–84.53
120 days				
– LG	6	0.17	0.06	0.10–0.23
– LSG	6	61.30	18.54	41.83–80.76
– TG	6	0.14	0.05	0.08–0.19
– TSG	6	71.48	16.14	51.44–91.52

Table 5 shows the mean tensile stress at the moment of rupture of the repaired samples tested 30, 60, 90, and 120 days later. In the glued samples, the mean values ranged between 0.11 MPa, recorded in TG at days 30 and 90 and LG at day 60, and 0.17 MPa in LG on day 120.

In the series that were sutured and glued, they ranged from 53.25 MPa in the LSG series tested on day 30 to 78.73 MPa in the TSG series tested on day 60. All the values corresponding to series cut in transverse direction (TSG) were greater than those of series cut longitudinally (LSG), although the difference was statistically significant only at day 30 after preparation ($p = 0.018$). In no case were there significant differences in terms of the time elapsed until tensile testing.

Mathematical Fit of the Stress–Strain Ratio

Table 6 shows the coefficients of the stress–strain ratio, expressed by the equation $y = a_1 x + a_2 x^2 + a_3 x^3$, in the series tested on day 7, both

Table 6. Fit of the stress–strain curves (MPa/per unit elongation) in control samples without and with application of the tissue selection criteria.

Controls	a_1	a_2	a_3	R^2
LC				
without	−52.38	742.31	−1554.10	0.594
with	−8.40	134.81	8.88	0.930
TC				
without	−17.98	238.09	18.65	0.845
with	−23.49	349.96	−118.50	0.904
LPC				
without	−0.03	3.73	−6.89	0.827
with	−0.15	3.37	−3.81	0.914
TPC				
without	0.01	5.33	−12.79	0.844
with	0.08	4.06	−3.33	0.917
LSPC				
without	−4.86	94.84	−113.49	0.881
with	−5.44	102.58	−154.80	0.907
TSPC				
without	−61.86	970.17	−1427.00	0.761
with	−42.65	740.44	−688.43	0.789

$y = a_1x + a_2x^2 + a_3x^3$, where y is the stress in MPa and x is the per unit elongation; R^2: coefficient of determination.

LC: longitudinal samples left intact; TC: transverse samples left intact; LGC: longitudinal samples transected and repaired by gluing; LSGC: longitudinal samples transected and repaired by suturing and gluing; TGC: transverse samples transected and repaired by gluing; TSGC: transverse samples transected and repaired by suturing and gluing.

when the criteria for tissue selection were not applied and when they were. The coefficients of determination (R^2) obtained when these criteria were not applied ranged between 0.594 and 0.881, versus a range of 0.789–0.930 when they were.

Tables 7 and 8 show these coefficients for the glued (LG and TG) and glued and sutured (LSG and TSG) series, respectively, tested on days 30, 60, 90, and 120 after preparation. The coefficients of determination (R^2) after application of the selection criteria in LG and TG ranged between 0.866 and 0.995. In LSG and TSG, they ranged from 0.921 to 0.989 after tissue selection.

Figures 2–4 illustrate these stress–strain curves. Figure 2 corresponds to the control series. Figure 3 shows, by way of example, that of glued, longitudinally cut samples (LG) on days 7 (control), 30, 90, and 120 after preparation, while Figure 4 corresponds to sutured and glued, transversely cut samples at those same time points.

Table 7. Fit of the stress–strain curves (MPa/per unit elongation) in glued samples, tested 30, 60, 90, and 120 days after preparation, without and with application of the tissue selection criteria.

Series	a_1	a_2	a_3	R^2
30 days				
LG				
without	−0.07	5.69	−14.08	0.822
with	−0.06	5.42	−6.30	0.909
TG				
without	0.17	−1.42	24.22	0.913
with	0.02	2.02	4.39	0.995
60 days				
LG				
without	−0.03	3.40	−8.20	0.865
with	−0.08	4.95	−11.54	0.916
TG				
without	−0.05	3.55	−8.65	0.817
with	−0.18	5.66	−16.70	0.866
90 days				
LG				
without	0.09	1.24	3.05	0.896
with	0.01	2.89	−1.70	0.876
TG				
without	0.74	−7.21	23.95	0.597
with	0.16	0.20	5.96	0.950
120 days				
LG				
without	0.19	−0.53	9.93	0.893
with	−0.07	2.88	−2.66	0.891
TG				
without	−0.25	6.86	−16.31	0.809
with	−0.04	3.91	−8.71	0.890

$y = a_1x + a_2x^2 + a_3x^3$, where y is the stress in MPa and x is the per unit elongation; R^2: coefficient of determination.
LG and TG: glued series cut longitudinally and transversely, respectively.

Strain (Elongation)

In the four series tested 120 days after sample preparation, the type of strain (reversible and irreversible) was quantified and qualified after rupture. Table 9 shows the percentages of strain in the longitudinal series (LG and LSG). The overall mean strain, or elongation, as measured by the tensile tester prior to unclamping the tissue was 23.8% for the LG series and 30.25% for the LSG series ($p = 0.018$). Once the

Table 8. Fit of the stress–strain curves (MPa/per unit elongation) in sutured and glued samples, tested 30, 60, 90, and 120 days after preparation, without and with application of the tissue selection criteria.

Series	a_1	a_2	a_3	R^2
30 days				
LSG				
without	41.30	77.12	514.69	0.652
with	−45.84	869.89	−1136.50	0.948
TSG				
without	−42.83	817.17	−984.21	0.954
with	−42.83	817.17	−984.21	0.954
60 days				
LSG				
without	−48.23	800.03	−171.71	0.826
with	10.10	−13.25	1353.60	0.992
TSG				
without	22.91	−104.52	1187.81	0.946
with	4.90	77.87	971.18	0.987
90 days				
LSG				
without	−77.71	1140.21	−1542.70	0.858
with	−58.36	838.70	−885.22	0.921
TSG				
without	−17.42	384.65	386.25	0.925
with	−17.42	384.65	386.25	0.925
120 days				
LSG				
without	−33.55	730.75	155.18	0.884
with	1.54	277.61	967.52	0.973
TSG				
without	−16.24	639.50	18.51	0.930
with	10.78	232.40	1733.76	0.989

$y = a_1x + a_2x^2 + a_3x^3$, where y is the stress in MPa and x is the per unit elongation; R^2: coefficient of determination.
LSG and TSG: sutured and glued series cut longitudinally and transversely, respectively.

samples were freed and measured again, it was possible to characterize the strain as reversible or irreversible. In the LG series, the mean rate of reversibility was 14.51% versus 13.03% in LSG. The mean rate of irreversibility in the LG series was 10.51% versus 17.34% in the LSG series. Neither of these differences was statistically significant.

The percentages recorded in the samples cut transversely appear in Table 10. The overall mean strain, according to the tensile tester, was 20.97% in the TG series and 33.63% in the TSG series ($p = 0.016$). The mean rate of reversibility in the TG series was 12.43% versus 15.92% in

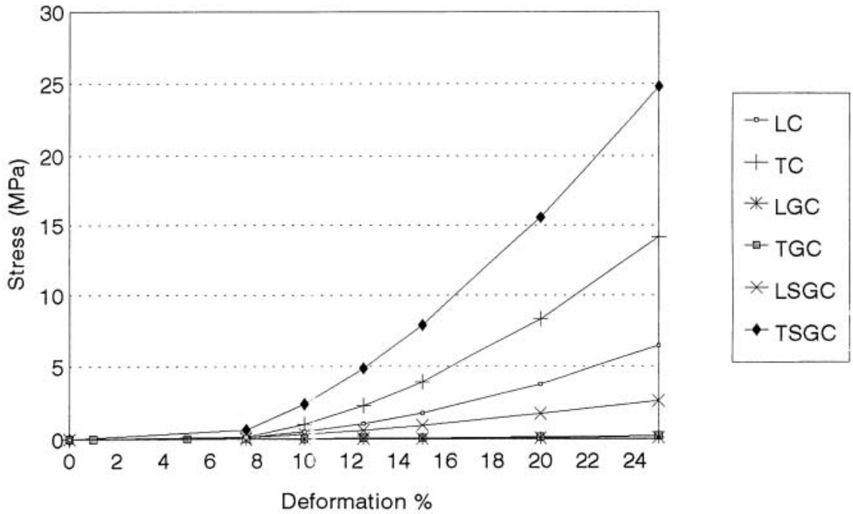

Figure 2. Stress–strain curves of control samples preserved in glutaraldehyde until testing 7 days after preparation. LC and TC: longitudinal and transverse series, respectively, left intact; LGC and TGC: series repaired by gluing with tissue overlap; LSGC and TSGC: series repaired by suturing and gluing.

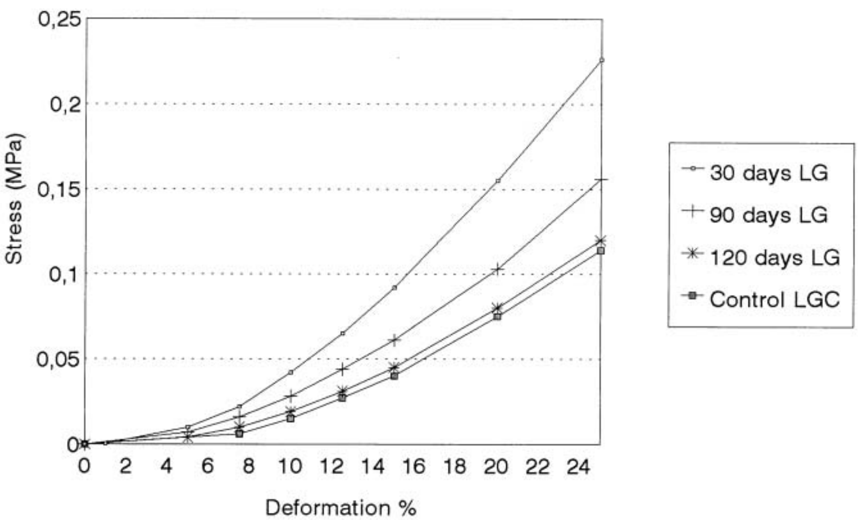

Figure 3. Stress–strain curves of longitudinal series repaired by gluing with tissue overlap (LG) after 7 (control), 30, 90, or 120 days of preservation in glutaraldehyde.

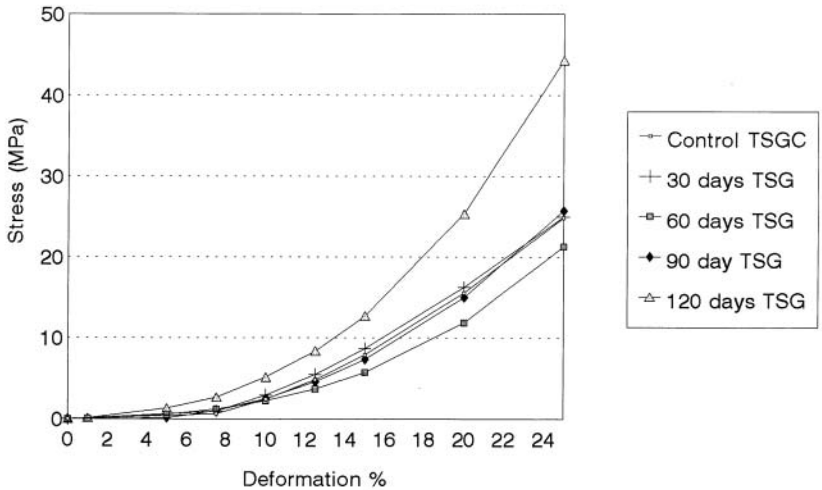

Figure 4. Stress–strain curves of transverse series repaired by gluing with tissue overlap (TSG) after 7 (control), 30, 90, or 120 days of preservation in glutaraldehyde.

Table 9. Percentages of irreversible and reversible elongations in longitudinal series 120 days after preparation.

Elongation	*n*	Mean (%)	Standard Deviation	95% CI	*p*
Irreversible					
– Glued	6	10.13	7.95	1.78–18.47	
– Sutured and glued	6	17.34	8.87	8.02–26.64	NS
Reversible					
– Glued	6	14.51	7.41	6.73–22.28	
– Sutured and glued	6	13.03	7.28	5.38–20.68	NS
Total					
– Glued	6	23.89	3.48	20.24–27.55	
– Sutured and glued	6	30.25	4.25	25.79–34.71	0.018

CI: confidence interval. NS: not statistically significant.

the TSG series. The mean rates of irreversibility in the TG and TSG series were 8.55 and 17.70%, respectively. Again, in neither case were the differences statistically significant.

DISCUSSION

Although prostheses made of biological materials, or bioprostheses, offer less of a guarantee in terms of medium-term safety, their

Table 10. Percentages of irreversible and reversible elongations in transversal series 120 days after preparation.

Elongation	*n*	Mean (%)	Standard Deviation	95% CI	*p*
Irreversible					
– Glued	6	8.55	9.49	1.42, 18.51	
– Sutured and glued	6	17.70	8.09	9.20, 26.21	NS
Reversible					
– Glued	6	12.43	5.86	6.27, 18.58	
– Sutured and glued	6	15.92	7.54	8.01, 23.83	NS
Total					
– Glued	6	20.97	4.23	16.53, 25.41	
– Sutured and glued	6	33.63	9.83	23.30, 43.95	0.016

CI: confidence interval. NS: not statistically significant.

hemodynamic behavior is excellent and their placement does not require a strict follow-up, circumstances that make them of great interest and utility [11,13].

Unfortunately, after being in use for three decades, the unlimited durability of these biological implants, or at least its predictability in each particular case, has yet to be achieved [13,14]. Among the major causes of their reduced durability and subsequent failure are the low mechanical resistance of the biological materials employed, the difficulties inherent in obtaining homogeneous tissue samples through unresolved selection processes [12,15,16], calcium deposition and hardening of the valve leaflets [17,18], and interactions with other biomaterials or organs of the recipient [19].

Despite their high degree of mechanical resistance, the sutures that give shape to bioprosthetic valve leaflets or secure vascular grafts have very limited elasticity and produce harmful effects in the very structures in which they are employed. They generate internal stresses that result in different strains in the different types of materials that they join [19–21]. The collagen fibers, the backbone of the resistance of a biological tissue, must absorb these stresses and ultimately fail in this task [22].

The objective of this study is to attempt to improve the bonding method through the use of bioadhesives, either alone or in combination with the conventional sutures.

Having ruled out the use of fibrin glues and biological glues, we employed an adhesive composed of a cyanoacrylate. Fibrin glues were considered unsuitable because of their low adhesive strength and the fact that they are absorbable, and biological glues because, to work effectively, they require a dry field, a condition that is difficult to achieve in glutaraldehyde-treated pericardium, which usually has to be kept moist [23,24].

The results of tensile testing to rupture, in machine kg, show an excellent behavior of the samples sutured and reinforced with glue. The time elapsed between their preparation and their assay has no influence on these findings (Tables 2 and 3 and Figure 4), which demonstrate the persistence of stability after 120 days of storage. The series that were only glued present a great loss of resistance, with values slightly above 1 machine kg (Tables 2 and 3).

When expressed in MPa, the findings demonstrate the different stresses generated in a repaired structure composed of pericardium, a suture material, and an adhesive (Tables 4 and 5 and Figure 2). Each stress is associated with a different degree of strain.

For the stress values recorded in the suture at the time of rupture of the sutured and glued samples (LSG and TSG series), which range between 53.25 and 84 MPa (Tables 4 and 5), and applying the stress–strain equation of a Prolene suture (similar to the Pronova suture) reported by us [25], $y = 57810.42x^2 + 3650.51x$ (where y is the stress expressed in MPa and x is the per unit elongation), the strain exhibited by the suture would be less than 3%, while that of the pericardium, had it not been torn, would have been over 25%.

The Prolene suture breaks at a tensile stress of 430.00 ± 18.34 MPa [25], while carefully selected pericardium tears at a tensile stress of less than 30 MPa, showing a nearly 15-fold lower resistance to the same type of stress.

Native heart valve leaflets exhibit an anisotropic behavior, which is necessary for their physiological function [26,27]. This behavior is apparently maintained in the sutured and glued samples, according to the analysis of the results at rupture (Tables 4 and 5). These findings should be examined in future trials with a larger number of samples, which would enable the assessment of statistically significant differences that could confirm or refute the existence of the anisotropic behavior of this bonding system. If the preservation of this behavior by the suture–glue combination were to be conclusively demonstrated, this circumstance should be taken into account in the design of bioprosthetic heart valve leaflets.

The mathematical fit enables us to establish the equations that relate stress and strain, which are illustrated by way of example in Figures 2–4. Figure 2 shows the different mechanical behaviors of the three types of samples assayed as controls on day 7 (intact, transected, and repaired by gluing, and transected and repaired by suturing and gluing). It also indicates the differences, with the exception of the glued series (LGC and TGC), between samples cut longitudinally (LC and LSGC) and the corresponding samples cut in transverse direction (TC and TSGC). Figure 3 shows there is no difference between the behaviors of the glued series cut longitudinally and tested on day 7 (LGC) and the LG series tested on day 120, a finding that demonstrates the stability of the adhesive, at least during that period of time. Likewise, Figure 4 illustrates the excellent mechanical behavior of the transversely cut samples repaired by suturing and gluing (TSG) tested 120 days after preparation.

In these trials, we have observed no loss of elasticity resulting from a hardening process secondary to the use of the adhesive, an event that would be undesirable in the manufacture of valve leaflets. One hundred and twenty days after preparation, the elastic behavior of the four series to which the adhesive was applied is maintained or even improved, indicating that the use of this substance must not be harmful.

The analysis of the strain in the glued and the sutured and glued series was of utmost interest in those tested 120 days after their preparation (Tables 9 and 10). We found reversible elongation after rupture, that is, after having widely surpassed the elastic limit [28]. To explain this phenomenon, we propose two theories that may not be mutually exclusive. One hypothesis would assume differences among the elastic behaviors of the different regions of a sample, with load concentrated in the area proximal to the suture or in the glued portion. The collagen fibers of said regions would absorb the load with permanent elongation and fibrillar tearing, while the collagen fibers of more distant regions would be subjected to lower loads that did not surpass the elastic limit and, after the load was removed, would recover their initial length [22]. Valve failure would result from the concentration of the load [28]. The valve should be designed in such a way as to prevent this. Using small-angle light scattering (SALS), a technique for quantitative light microscopic analysis, to observe the collagen fibers, it would be possible to confirm or refute this theory [29].

The second hypothesis would focus on the behavior of the calf pericardium as an amorphous material [30]. The work produced by the tensile stress during the elongation of the tissue would be stored and converted into elastic energy. If the theoretical value referred to as Young's modulus is not surpassed, it would remain within the elastic limit. The variation of Young's modulus in amorphous solid materials is complex and, when these materials are subjected to stress and heat, elastic, or reversible components can be generated together with plastic or irreversible components. If the tissue behaves as an amorphous material, the effect of a body temperature of 37°C on its mechanical behavior should be taken into account. Both theories are possible and they are not mutually exclusive.

The results of this study suggest the following conclusions:

- A bonding method involving the use of an adhesive confers considerable resistance, although probably too low to be effective in the bonding of valve leaflets, but also maintains a high degree of elasticity in tissue samples subjected to tensile testing.
- The application of an adhesive to the points at which the suture perforates the calf pericardium being secured enhances its resistance to tearing, while maintaining an excellent degree of elasticity and perhaps contributing to the anisotropic behavior of the tissue.
- The analysis of the elongation, or strain, produced in glued and in sutured and glued series tested 120 days after preparation reveals an elastic behavior, with no evidence of hardening or rigidity of the pericardium.

This same analysis reveals the elastic behavior of some of the samples, with reversible elongation once the elastic limit has been surpassed at rupture. This phenomenon could be the result of a concentration of load that is damaging to the pericardium, to the behavior of the tissue as an amorphous material, or perhaps to both circumstances.

These findings, should they be confirmed in future trials, should be taken into account in the design of prosthetic heart valve leaflets made of calf pericardium because of their possible effects on the durability of these structures.

Although the objective of this report was not to study the compatibility or cytotoxicity of cyanoacrylates, we are aware of the fact that, for their clinical use, they not only have to offer good mechanical resistance, a property demonstrated by the results of this study, but must also be stable, biologically inert, and nontoxic. The hydrolytic degradation of cyanoacrylates is accompanied by the release of formaldehyde, a potent irritant and sensitizer that can produce cell toxicity. *In vivo* contact and dilution tests should be performed in laboratory animals to rule out their having a subtle toxicity that may not be detected in routine biological tests [31,32,33].

The increasingly widespread use of these adhesives (ophthalmology, dentistry, osteosynthesis, tissue bonding, vascular anastomosis, pulmonary resection, etc.) makes it essential to establish the strictest possible toxicological controls.

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