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Comparative study of the mechanical behaviour of a cyanoacrylate and a bioadhesive

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We compared the mechanical resistance of 18 samples of calf pericardium bonded with a 100 mm^2 overlap, by two types of glues: a cyanoacrylate (Loctite 4011) and a bioadhesive (BioGlue). Comparative tensile testing was also carried out in 40 paired samples, 20 bonded with the cyanoacrylate and 20 unbonded controls. The findings at rupture showed a greater resistance of the calf pericardium glued with cyanoacrylate, with a mean tensile strength of 0.15 MPa *vs.* 0.04 MPa for the biological glue ($p = 0.000$). They also demonstrated a loss of resistance of the samples bonded with cyanoacrylate when compared with that of the unbonded other halves of the pairs: 0.20 MPa and 0.27 MPa *vs.* 19.47 MPa and 24.44 MPa ($p < 0.001$). The method of selection by means of paired samples made it possible to establish the equations that relate the stress and strain, or deformation, with excellent coefficients of determination (R^2).

These equations demonstrate the marked elastic behaviour of the bonded samples. Moreover, these findings show the cyanoacrylate to be superior to the biological glue, leading to the examination of the compatibility, inalterability over time and mechanical behaviour of the cyanoacrylate in sutured samples, as well as the study of the anisotropy of the biomaterial when bonded with a bioadhesive.

Introduction

The role of bioadhesives for medical use may prove to be highly interesting in the future if they fulfil two conditions: stability over time and sufficient mechanical resistance to guarantee their safety. These two conditions are indispensable when the glues are utilized to bond biological replacement structures such as cardiac bioprostheses or vascular grafts.

Our current experience in the use of bioadhesives in the medical field is limited, although they have been employed in a number of fields with reported advances. For example, it has been shown that the utilization of a cyanoacrylate glue contains bleeding from gastric varices with a higher rate of success and a lower mortality rate than the administration of ethanolamine, a sclerosing agent [1]. In blepharoplasty, a technique employed in ocular surgery, the Food and Drug Administration-approved cyanoacrylate, octyl-2-cyanoacrylate, has been employed with excellent results in comparison with suturing [2].

Three types of adhesives have been utilized in cardiovascular surgery: fibrin glues, which are resorbable but do not provide strong adhesion; enbucrilate, which has been used successfully for left ventricular free wall rupture [3] but produces a marked exothermic reaction and is unstable; and biological glues. The latter have been employed to bond pericardial patches and reinforce sutures.

In aortic dissection, a very serious clinical situation, a bioadhesive is used to bond the proximal and distal edges of the dissected aorta, which are then sutured. The mechanical behaviour of the bioadhesive in this clinical situation has yet to be characterized [4]. The use of glues as a substitute for sutures in the placement of bioprostheses or implants has not been authorized. There is a lack of experience in this approach and the

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fact that the devices are made of inert tissues raises essential questions with respect to their stability over the course of time and their initial and long-term resistance. The case is different when an adhesive is employed in living tissue since the latter soon recovers its function [5].

In order for glues to be used to secure an inert structure, together with sutures or perhaps someday alone, they must be shown to possess an adequate mechanical behaviour and stability over time.

When several materials work together as parts of a functional structure, they must absorb the loads to which said structure is subjected. Each material absorbs these loads according to a different mechanical or elastic behaviour, resulting in different internal stresses and strains, or deformations, in each [6, 7]. This type of stress–strain must be absorbed internally by the entire functional structure.

A bioprosthesis works repetitively at a rate of approximately one cycle per second. The sutures utilized to secure porcine bioprostheses or to shape the valve leaflets of those made of calf pericardium [8] generate stresses in the structure because they are practically inelastic when compared with the elasticity of the tissues employed [9, 10]. This inelastic behaviour results in a cutting or shearing effect, which is produced in every cycle, and can ultimately ruin the valve leaflet, an event that occasionally occurs early [11, 12]. Could bioadhesives improve this situation? It is still too soon to answer this question.

In this report, we study the mechanical behaviour of two types of glue: Loctite 4011, an instantaneous, biocompatible cyanoacrylate that has been used in the health field to secure multilumen angioplasty balloons and Foley or high-pressure catheters; and BioGlue, a surgical adhesive composed of glutaraldehyde cross-linked bovine serum albumin, used as a bond in aortic dissection.

The trials consisted of tensile testing in samples of calf pericardium fractured transversely in the centre and subsequently glued, by overlapping 100 mm^2 of the tissue, with one of the two glues described above. The main objective of the study was to compare the mechanical resistance to tensile stress of samples repaired with these adhesives.

Materials 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 ages of 9 and 12 months. The tissue was transported to the laboratory in saline solution (0.9% sodium chloride), where it was carefully cleaned.

The sacs obtained corresponded to the parietal pericardium that covers the anterior portion of the heart between right and left ventricles. Once cut open, in such as way as to leave the diaphragmatic ligament in the centre and the sternopericardial ligaments on the circumference, the pericardial sacs measured ~ 150 mm long, from root to apex, and 100 mm wide. All specimens were procured in such a way as to guarantee that they would present a similar morphology.

Longitudinal (root-to-apex) strips measuring 120 mm in length and 20 mm in width were cut from each sac, and a transverse cut was made in the centre of all but those to be used as control samples.

Prior to gluing, the strips 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.

Glues

Loctite 4011, an instantaneous, transparent, low-viscosity bioadhesive, is an ethyl cyanoacrylate that is authorized for medical use and can be sterilized with ethylene oxide or gamma rays. It is a monocomponent cyanoacrylate associated with rapid healing and, according to the manufacturer, a high resistance to tensile and shearing stress. Moreover, it can be employed to bond different substrates.

BioGlue is a surgical adhesive composed of glutaraldehyde crosslinked bovine serum albumin. Once the two components of this glue are combined, its polymerization begins immediately, exhibiting bonding capability 2 min later.

Assay method

The initial experimental design included 64 trials, but the final total was 58. Eighteen assays were performed to compare the two types of glue: series PL_1 glued with Loctite 4011 ($n = 12$) and series PB glued with BioGlue ($n = 6$). In both cases, rough surface was bonded to smooth surface.

The objective of the remaining 40 assays was to assess two different gluing methods using Loctite 4011, and compare the results with the findings in two unbonded control series. One method of gluing involved the bonding of rough surface and smooth surface (A/A), while in the other, rough surface was bonded to rough surface (A/B) (Fig. 1). These samples were grouped as follows: series PL_2(A/B) glued with Loctite 4011 ($n = 10$); control series A/B ($n = 10$); series PL_3(A/A) glued with Loctite 4011 ($n = 10$); and control series A/A ($n = 10$).

With the exception of the controls, one of the two glues was spread over an area of 100 mm^2 that included the transverse incision that had been made in the centre of each specimen, that portion of the tissue was made to overlap by 5 mm and the two glued surfaces were pressed together. The surfaces brought into contact were: series PL_1, PB and PL_3, rough surface and smooth surface (A/A); series PL_2, rough surface with rough surface (A/B) (Fig. 1).

The first six strips in series PL_1 and the six strips of series PB had been cut contiguously from the pericardial sac, i.e., they were employed as paired samples. The samples in series PL_2A/B and PL_3A/A were also either contiguous within the sac or paired with the corresponding unbonded or control samples, A/B control and A/A control.

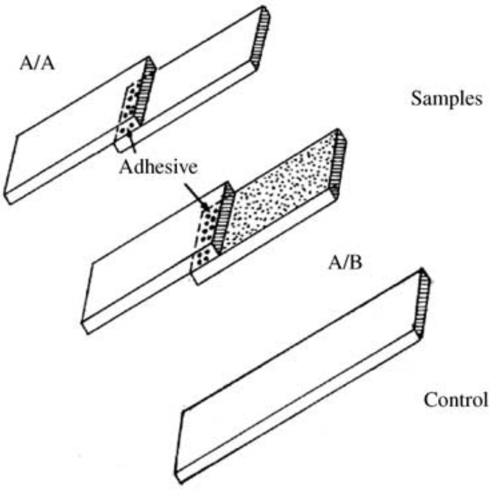

Figure 1 Drawing showing the gluing of the samples. A/A: bonding of the rough, or visceral, aspect to the smooth, or parietal, aspect of the pericardium. A/B: bonding of two rough, or visceral, aspects. A drawing of a control sample also appears.

The last six samples in series PB had to be excluded due to technical difficulties.

All the samples were maintained at 4 °C.

The thickness of each sample was determined by a series of measurements at 10 different points using a Mitutoyo digital micrometer (Elecount series E/A33/8), which has a precision at 20 °C of ± 3 microns.

The trial consisted of subjecting each sample to increasing uniaxial (root-to-apex) tensile testing, along the major axis, until rupture, confirmed by the loss of load and the morphological confirmation of tears in the unbonded control samples or the detachment of the glued samples. These trials were performed on an Instron TTG4 tensile tester (Instron Ltd., High Wycombe, Bucks. England) which determines tensile stress under varying rates of strain or elongation.

The samples were clamped in such a way as to leave a free lumen of 50 mm. 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 unbonded control samples was calculated taking into account the minimum cross-sectional area. In the glued samples, this calculation was based on the area of contact ($20 \times 5\,\text{mm}^2 = 100\,\text{mm}^2$).

Selection criteria

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 zone to which it was assigned in the initial selection. Thus, those membranes with a minimum thickness greater than the mean value for the corresponding series plus one standard deviation or less than the mean value minus one standard deviation were excluded, as were those membranes in which the difference between the mean thickness of the series to which it belonged and the minimum thickness of the tissue sample was greater than the mean value for these differences in the corresponding set plus one standard deviation, indicating a lack of homogeneity.

In addition, in all the series (PL_1 and PB; PL_2(A/B) and A/B control; PL_3(A/A) and A/A control), a selection was made according to pairs, resulting in the exclusion of the two samples of any pair in which one or both fulfilled the aforementioned exclusion criteria.

On the basis of these criteria, 32 samples were selected (55% of the total), distributed as follows: series PL_1/PB, 10 samples; series PL_2(A/B)/A/B control, 10 samples; and series PL_3(A/A)/A/A control, 12 samples.

Statistical study

Statistical methods for the comparison of the means at rupture

The mean values at rupture, in MPa, of the following series were compared: series PL_1, glued with Loctite 4011, *vs.* series PB, glued with BioGlue; series PL_2(A/B) glued with Loctite 4011, *vs.* series PL_3(A/A), also glued with Loctite 4011; series PL_2(A/A) *vs.* its unbonded control series A/B; and series PL_3(A/A) *vs.* its unbonded control series A/A. The statistical tests employed were analysis of variance (ANOVA) and the Newman–Keuls test for multiple comparisons. The Kolmogorov–Smirnov test was used to confirm the normal distribution, and the homogeneity of variance was evaluated by the Levene test. All the tests were two-tailed and the level of significance was $p < 0.05$. The results are expressed as the mean plus or minus the standard deviation and the 95% confidence interval (95% CI). The statistical analysis was performed with the SPSS v.10.0 software package.

Mathematical fit of the tensile strength/elongation ratio

The tensile strength (MPa/strain) per unit elongation ratio was studied using the ''line of best fit'' with the least squares method. The best fit corresponded to a third-order polynomial, the shape of which is expressed as $y = a_1x + a_2x^2 + b_3x^3$, where y is the tensile strength and x the per unit elongation.

The same fit was determined after sample selection according to the criteria described in the preceding section.

Predictive study

Using regression analysis, the tensile stress values (MPa) obtained in series PL_1 glued with Loctite 4011, the independent variable (x), were correlated with those found in series PB glued with BioGlue, the dependent variable (y). The same method was employed to predict the tensile stresses in the two series glued with Loctite 4011, PL_2 and PL_3, on the basis of the corresponding control data. The mathematical model also corresponded to a straight line in which the tensile stress for the series glued with Loctite 4011 (PL_2 and PL_3) was estimated on the basis of the stresses found in the corresponding control series, where the former were dependent variables (y) and the latter the independent variables (x).

The correlation coefficients (r), the 95% CI and the statistical significance (p) for the fit in each case were also determined.

Results

The mean tensile stresses at rupture in series PL_1 (glued with Loctite 4011) and PB (glued with BioGlue), expressed in MPa, appear in Table I. The comparison of the two series resulted in statistically significant differences (0.15 MPa for PL_1 *vs.* 0.04 MPa for PB; $p = 0.000$).

Table II shows the mean tensile stresses at rupture for series PL_2 and PL_3 and their corresponding control series (A/B control and A/A control). The mean values in series PL_2 and PL_3 were 0.20 MPa and 0.27 MPa, respectively, there being no statistically significant differences between these findings. However, statistical significance was observed $(p < 0.001)$ when the mean values at rupture for series PL_2 and PL_3 were compared with the means in their corresponding control series (19.47 MPa and 24.44 MPa, respectively, as displayed in Table II).

Mathematical fit

The coefficients for the stress/strain curves resulting from the mathematical fit of the findings in series PL_1 and PB, when the tissue selection criteria were not applied and when they were, appear in Tables III and IV, respectively, which also show the coefficients of determination (R^2).

Tables V and VI present the coefficients for the stress/strain curves, without and with tissue selection, for series PL_2A/B, A/B control, PL_3A/A and A/A control. Again, the coefficients of determination (R^2) are provided.

Fig. 2 illustrates the stress/strain ratio for series PL_1 and PB, while Fig. 3 shows those for series PL_2A/B, A/B control, PL_3A/A and A/A control.

Regression analysis

Table VII displays the coefficients of the regression curves $y = ax + b$ for the samples selected for series PL_1, where the tensile stresses (MPa) are the independent variables (x) and those with which each was paired in series PB are the dependent variables (y). The 95% CI, p values and correlation coefficients (r) for these values are also presented.

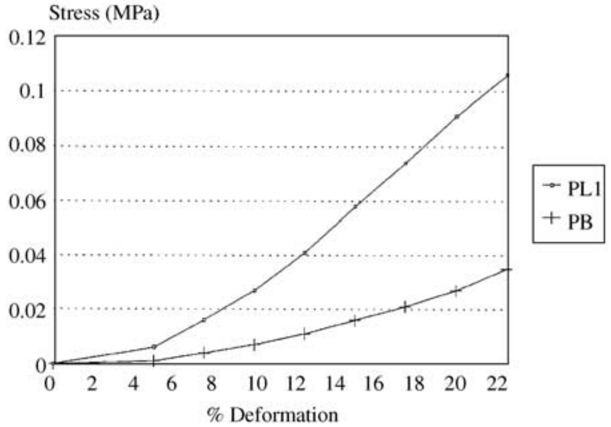

Figure 2 Stress/strain (deformation) curves of the series glued with cyanoacrylate (PL_1) or with the biological glue, BioGlue (PB).

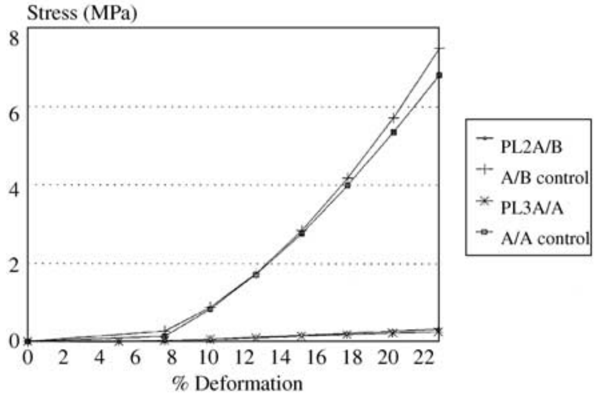

Figure 3 Stress/strain (deformation) curves of the series glued with cyanoacrylate and their corresponding controls. PL_2 A/B and PL_3 A/A (cyanoacrylate); A/B control and A/A control (A/A: rough aspect glued to smooth aspect; A/B: gluing of two rough aspects).

TABLE I Results of tensile testing until tearing point in the series bonded with Loctite 4011 (PL_1) or with BioGlue (PB)

Series	No.	MPa at tearing point	Standard deviation	95% Confidence interval
PL_1	12	0.15*	0.05	0.12, 0.18
PB	6	0.04*	0.01	0.02, 0.05

*$p = 0.000$.

TABLE II Results of tensile testing to tearing point in two series glued with Loctite 4011 (PL_2 and PL_3) and a control series

Series	No.	MPa at tearing point	Standard deviation	95% Confidence interval
PL_2A/B	10	0.20	0.04	0.39, 0.46
A/B control	10	19.47	6.31	14.9, 23.98
PL_3A/A	10	0.27	0.09	0.31, 0.44
A/A control	10	24.44	6.94	7.36, 14.99

cont.: control series tested without gluing.
PL_2A/B *vs.* A/B control $p < 0.001$ for MPa at tearing point.
PL_3A/A *vs.* A/A control $p < 0.001$ for MPa at tearing point.
A/A; A/B: see text and Fig. 1.

TABLE III Series glued with Loctite 4011 (PL_1) or with BioGlue (PB) coefficients of the mechanical behaviour curves (stress/strain). Fit without tissue selection

Series	a_1	a_2	a_3	R^2
PL_1	− 0.05	3.81	− 7.08	0.869
PB	− 0.04	1.53	− 2.96	0.892

$y = a_1 + a_2x^2 + a_3x^3$, where y is the stress in MPa and x the per unit strain, or deformation.
R^2: coefficient of determination.

TABLE IV Coefficients of the mechanical behaviour curves (stress/strain) for series glued with Loctite 4011 (PL_1) or with BioGlue (PB). Fit after tissue selection

Series	a_1	a_2	a_3	R^2
PL_1	− 0.07	4.22	− 8.03	0.861
PB	− 0.02	0.98	− 1.09	0.919

$y = a_1 + a_2x^2 + a_3x^3$, where y is the stress in MPa and x the per unit strain, or deformation.
R^2: coefficient of determination.

TABLE V Coefficients of the mechanical behaviour curves (stress/strain) for series glued with Loctite 4011 (PL_2 *vs.* PL_3) and control series. Fit without tissue selection

Series	a_1	a_2	a_3	R^2
PL_2 A/B	− 0.88	18.49	− 37.23	0.910
A/B control	− 8.46	164.21	76.71	0.865
PL_3 A/A	− 0.60	15.01	− 31.52	0.929
A/A control	− 15.74	293.13	− 435.58	0.829

$y = a_1x + a_2x^2 + a_3x^3$, where y is the stress in MPa and x the per unit strain, or deformation.
R^2: coefficient of determination.
A/A, A/B: see text.

TABLE VI Coefficients of the mechanical behaviour curves (stress/strain) for series glued with Loctite 4011 (PL_2 *vs.* PL_3) and control series. Fit after tissue selection using paired samples

Series	a_1	a_2	a_3	R^2
A/B	− 0.88	20.07	− 42.78	0.920
A/B control	− 3.84	150.63	73.17	0.924
A/A	− 0.61	16.16	− 34.51	0.920
A/A control	− 25.73	403.00	− 631.58	0.813

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

Table VIII shows the coefficients of the regression curves $y = ax + b$ for series A/B control and A/A control, the independent variables (x) and the glued series $PL_2(A/B)$ and $PL_3(A/A)$ as dependent variables (y). The table also displays the 95% CI, p values and correlation coefficients (r) for these values.

Discussion

The function of valve leaflets of implanted cardiac bioprostheses subjects them to a mechanical stress of less than 0.25 MPa [13, 14]. At this level of stress, the deformation of the suture that maintains the shape of the bioprosthetic valve leaflet made of calf pericardium is barely perceptible, while the pericardium itself may have suffered an elongation of up to 8% with respect to its original length [9, 15]. This situation generates internal stresses in the valve leaflets that can lead to leaflet failure, sometimes occurring early, due to tearing of the tissue [7, 12]. The search for materials to join tissues that are more elastic than sutures but, at the same time, exhibit adequate resistance and stability, may lead to the design of bioprostheses more durable than those presently available [16–18].

We initiated this study by comparing the mechanical resistance to tensile stress of two bioadhesives: a cyanoacrylate that is not employed or approved for use in cardiovascular surgery and a biological glue utilized to reinforce the suture in the repair of aortic dissection. In terms of mechanical resistance, the results with the biological glue are discouraging.

Taking into account the surface area of the glued portion, tearing point was reached at much lower levels of stress $(p = 0.000)$ in samples bonded with the biological glue (BioGlue), as compared to those bonded with the cyanoacrylate: 0.04 MPa *vs.* 0.15 MPa. These findings are shown in Table I.

Perhaps the major problem associated with the biological glue is the fact that in order to ensure the necessary efficacy and resistance, it must be applied over a dry field, which is difficult to achieve in calf pericardium that has been treated with glutaraldehyde until the moment of bonding. Likewise, it appears difficult to achieve a dry surgical field in the repair of aortic dissection [4, 19].

As illustrated in Fig. 2, both adhesives allow marked deformation of the samples at very low stress levels. We observed deformations of $\sim 20\%$ at stresses of less than 0.1 MPa in samples bonded with the cyanoacrylate and less than 0.04 MPa in those joined with the biological

TABLE VII Series glued with Loctite 4011 (PL_1) or with BioGlue (PB). Coefficients of the regression curves. PL_1: independent variable (x); PB: dependent variable (y)

Series PL_1/PB Pairs	a	(95% CI)	p	b	95% CI	p	r
no. 2	1.5×10^{-3}	(− 0.002, 0.005)	0.302	0.424	(0.329, 0.519)	0.000	0.976
no. 3	-3.4×10^{-3}	(− 0.008, 0.001)	0.108	1.035	(0.743, 1.327)	0.000	0.971
no. 4	-1.2×10^{3}	(− 0.002, 0.00)	0.045	0.480	(0.435, 0.524)	0.000	0.994
no. 5	-2.8×10^{-3}	(− 0.007, 0.001)	0.141	0.470	(0.302, 0.638)	0.001	0.955
no. 6	-2.6×10^{-3}	(− 0.005, 0.00)	0.057	0.376	(0.328, 0.423)	0.000	0.977

Regression curve: $y = a + bx$; a: constant; b: slope: $(pb_y) = a + b(pl_x)$.
95% CI: 95% confidence interval.
p: statistical significance; r: correlation coefficient.

TABLE VIII Series glued with Loctite 4011 (PL_2 and PL_3). Coefficients of the regression curves of control series (independent variable x) and the glued series (dependent variable y)

Series	*a*	95% CI	*p*	*b*	95% CI	*p*	*r*
PL_2 A/B	0.006	(– 0.09, 0.022)	0.386	0.047	(0.043, 0.052)	0.000	0.992
PL_3 A/A	0.022	(0.09, 0.035)	0.004	0.038	(0.035, 0.042)	0.000	0.991

Regression curve: $y = a + bx$; $pl_y = a + b(\text{control}_x)$.
a: constant; *b*: slope; 95% CI: 95% confidence interval.
p: statistical significance; *r*: correlation coefficient.

glue. Do these adhesives provide the structure with the necessary elasticity?

In the second phase of the study, we compared the resistance to tensile stress of series of samples bonded with the cyanoacrylate, according to the bonding method, with that of unbonded control samples. The bonding method, either the rough or visceral aspect with the smooth or parietal aspect (A/A), or two rough aspects (A/B), did not influence the results (0.27 MPa at tearing point *vs.* 0.20 MPa, respectively). The comparison of these mean stresses with those recorded in the corresponding unbonded controls demonstrated the loss of resistance of the former. The controls showed a much higher resistance to tearing, with mean values of 24.44 MPa and 19.47 MPa, respectively. Fig. 3 illustrates the elastic behaviour of these series, demonstrating the low resistances to tearing of the bonded samples.

In this study, we also applied a method for tissue selection according to morphological criteria, using paired samples, to achieve the mathematical fit of the stress/strain (deformation) curves [20]. Using this method, the coefficients of determination (R^2) ranged between 0.813 and 0.929.

The purpose of using pairs of samples to improve tissue selection is to guarantee that a given sample was cut from the region or zone of the pericardial sac from which it was supposed to be obtained, ensuring the homogeneity of the biological specimens and, thus, making the comparison of the results possible [21, 22]. This method enables the prediction of the results on the basis of regression curves. The determination of the elastic behaviour of a one sample of the pair enables the prediction of that of the other. Table VII shows the equations applied, where the results in series bonded with the cyanoacrylate (PL_1) represent the independent values (x) and those for the pairs bonded with BioGlue (PB) the dependent values (y). The correlation coefficients (r) obtained ranged between 0.955 and 0.994.

To conclude the study, we confirm the superior mechanical resistance to tensile stress of the cyanoacrylate adhesive in comparison to that of the biological glue. If its use in cardiovascular surgery were to be approved, it should replace the latter in aortic dissection repair.

Another important aspect that should be emphasized is the elastic behaviour of the glued samples. Perhaps their resistance is not sufficient enough that they be trusted to bond the valve leaflet of a cardiac bioprosthesis, despite their compatibility and unalterability over time, but this elastic behaviour encourages further research in two directions. One effort would focus on the mechanical behaviour of pericardium samples glued and sutured in the attempt to provide the structure with greater elasticity without compromising resistance. The other would strive to maintain the anisotropic behaviour of the biomaterial, imitating that of a native valve leaflet, a feature that is lost in sutured tissue [23].

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