Original Article / *Klinik Çalışma - Araştırma*

Glutaraldehyde Fixation of Autologous Pericardial Patches[*]

Otolog Perikard Yamalarının Gluteraldehid ile Fiksasyonu

Nazmiye SELÇUK KAPISIZ[1], Hasan Fahri KAPISIZ[1], Orhan Veli DOĞAN[2], Alp DOLGUN[2], Ertan YÜCEL[2]

[1]*Department of Cardiovascular Surgery, Ankara Etlik Specialty Hospital, Ankara*
[2]*Department of Cardiovascular Surgery, Ankara Yıdırım Beyazıt Training and Research Hospital, Ankara;*

Submitted / Başvuru tarihi: *05.05.2008* ***Accepted / Kabul tarihi:*** *18.06.2008*

Objectives: The aim of our study is to measure ultimate tensile strength and elongation of pericardial patches prepared by a fixation method that can be applied just in the operating room before its implantation.

Patients and Methods: Pericardial patches taken from 60 patients of 50-70 years of age who underwent surgery for coronary artery bypass were studied. Pericardial patches cut in horizontal and vertical directions were treated with 0.5%, 1%, 2% concentrations of glutaraldehyde, each for 5, 10, 20-minute durations. Uniaxial tensile test was applied on pericardial samples. Thickness, tensile strength at yield, elongation at yield, tensile strength at break, elongation at break were compared between different fixation groups.

Results: Ultimate tensile strength at yield and at break, elongation at yield and at break were found to be highest in pericardial patches treated with 2% glutaraldehyde concentration for five-minute duration.

Conclusion: Fixation of pericardial samples by 2% glutaraldehyde concentration for five-minute duration resulted in higher tensile strength and elongation with uniaxial stretching. Further similar studies with biaxial stretching is suggested before application in clinical practice.

Key Words: Glutaraldehyde; fixation; pericardial patch; tensile test.

Amaç: Çalışmamızın amacı implantasyondan hemen önce ameliyathanede uygulanabilen bir fiksasyon metodu ile perikardial yamaların gerilme kuvveti ve elastikiyetlerini ölçmektir.

Hastalar ve Yöntemler: Koroner bypass ameliyatı geçiren 50-70 yaşları arasındaki 60 hastadan alınan perikard yamaları çalışılmıştır. Yatay ve dikey yönde kesilen perikard yamaları %0.5, %1, %2 konsantrasyonlarındaki gluteraldehid solüsyonu ile 5, 10 20 dakika süreyle muamele edilmiştir. Perikardial kesitler uniaksial germe testine tabi tutulmuştur. Kalınlık, maksimum germe kuvveti, maksimum uzama, kopma kuvveti, kopma uzaması farklı fiksasyon grupları arasında karşılaştırılmıştır.

Bulgular: Maksimum germe kuvveti, maksimum kopma kuvveti, maksimum uzama ve kopma uzaması %2 konsantrasyonundaki gluteraldehid solüsyonu ile beş dakika muamele edilen perikard yamalarında en yüksek bulunmuştur.

Sonuç: Yüzde ikilik gluteraldehid solüsyonunda beş dakika süreyle muamele edilen perikardial kesitlerde uniaksial germe testi ile daha yüksek germe kuvveti ve uzaması elde edilmiştir. Klinik uygulamaya konmadan önce benzer çalışmaların biaksial germe testi ile yapılması önerilmektedir.

Anahtar Sözcükler: Glutaraldehid; fiksasyon; perikardial yama; germe testi.

**Presented at the XVI. Annual Meeting of Mediterranean Association of Cardiology and Cardiac Surgery, September 26-29, 2004, Bodrum, Muğla, Turkey (XVI. Akdeniz Kardiyoloji ve Kalp Damar Cerrahisi Derneği Yıllık Toplantısı'nda sunulmuştur 26-29 Eylül 2004, Bodrum, Muğla).*

Correspondence (İletişim adresi): Dr. Nazmiye Selçuk Kapısız. Ankara Etlik İhtisas Hastanesi, Kalp ve Damar Cerrahisi Kliniği, 06018 Etlik, Ankara. Tel: 0312 - 251 93 19 Fax (Faks): 0312 - 323 42 70 e-mail (e-posta): hkapisiz@superonline.com

A variety of biologic and synthetic materials are needed for the corrective and reconstructive procedures in cardiac surgery.[1,2] With new developments, the use of patches has been increasing day by day. In comparison to synthetic materials, pericardial patches are cheap, biocompatible, nonporous, easy to use, resistant to infection and easily available. Reconstructive procedures that require use of patches are enlargement of right ventricular outflow tract in Fallot's Tetralogy or isolated pulmonary stenosis, left ventricular outflow tract enlargement, atrial septal defect closure, reconstruction of valvular annulus and aortic root abscess in infective endocarditis, left ventricular aneurysm reconstruction, atrial switch operations in transposition and so on.[3-6]

Pericardial patches could be used in these operations fresh or fixed with glutaraldehyde. Fibrous retraction develops when pericardial patch is used fresh, whereas fixed pericardium is protected from retraction. Also the surgical manipulation of the tissue is easier when it is fixed with glutaraldehyde. There is another disadvantage of using fresh pericardium which is the possibility of aneurysm development with the growth of the patient.[7] In this study, we tried to find a fixation method for the pericardial patch in terms of glutaraldehyde concentration and fixation duration to add more strength and elasticity to the patch.

PATIENTS AND METHODS

We studied pericardial patches taken from 60 patients (42 males, 18 females; mean age 60.6±6.1 years; range 50 to 70 years) who underwent surgery for coronary artery bypass between December 2002 and April 2003, in SB Training Hospital, Ankara. In each case, the pericardium was routinely partially excised and left unclosed after surgery. The collection and use of pericardium in this study was approved by Ethical Committee of SB Training Hospital, Ankara (Date: 8.8.2002, No: 168), and was covered under the surgical consent form assigned by each patient upon admission. This study conforms with the Declaration of Helsinki.

Human pericardium often had loosely adherent fat; it was removed prior to cutting. Pericardial samples were taken at the size of approximately 5 cmx5 cm at most and the right upper sites were marked with a clip. Each sample was immediately immersed in glutaraldehyde solution at room temperature. The amount of solution used in each fixation was approximately 100 ml for a pericardial sample size of at most 5x5 cm. The concentrations of glutaraldehyde solutions we used were 0.5%, 1% and 2% that were buffered with disodium hydrogen phosphate dihydrate and potassium dihydrogen phosphate. Samples were treated with each glutaraldehyde concentration (0.5%, 1%, 2%) for three distinct elapsed fixation durations (at 5, 10, 20 minutes after the initiation of tissue fixation respectively). So we had three different glutaraldehyde concentrations and three different fixation times, a total of nine different fixation methods. Since pericardium is an anisotropic material, we applied these nine different fixation methods to both horizontal and vertical pericardium. Therefore, we had 18 different fixation groups for comparison of pericardial samples.

Pericardial samples taken from patients in the operating room were transported to the Turkish Standards Institute (TSI) in normal saline at room temperature where we applied tensile test. The time period between fixation and the measurement was <6 hours. Vertical and horizontal strips were cut in standard frames with standard size shown in Figure 1. Before the application of tensile test, the thickness of each strip was measured using a Mitutoyo thickness gauge (No: 7301) and taken as the sample thickness. Mechanical testing was performed using a Zwick Model -1445 tensile testing machine, by attaching the samples with a piece of paper to prevent slippage of the sample.

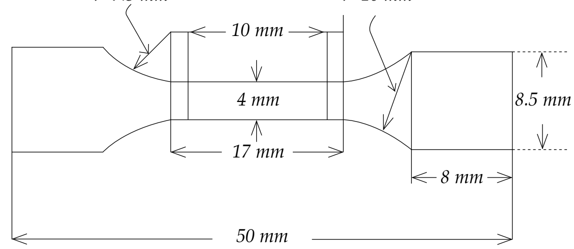

Fig. 1- Standart size and shape of the pericardial sample.

Prior to testing, the strips were preconditioned by cyclic loading at 5 mm/min extension rate between zero load and a maximum load of 90 g until reproducible load/elongation curves were attained. The load/elongation curves were transformed to engineering stress versus strain curves by normalizing with the initial, unstrained dimensions of the specimens. The strips were extended to fracture at a constant extension rate of 5 mm/min.

In order to obtain more reliable results and because of anisotropicity of pericardium, we carried out 10 different tests for each fixation group. These 10 tests were carried out on the samples taken from three patients. As a control group we took the fresh pericardium used vertically and horizontally.

Tensile strength at yield: the maximum strength of pericardial sample.

Tensile strength at break: the strength of pericardial sample at break.

Elongation at yield: elongation of pericardial sample at maximum strength.

Elongation at break: elongation of pericardial sample at break.

These measurements were obtained automatically by the tensile testing machine which draws graphics of each sample showing maximum strength, strength at break, elongation at maximum strength and elongation at break.

Statistical analysis

Tensile testing was carried out on fresh pericardial samples and the pericardial samples treated with concentrations of 0.5%, 1%, and 2% buffered glutaraldehyde solutions for 5, 10, 20 minutes of duration (For example, Group 0.5%-5′: group of pericardial samples treated with a glutaraldehyde concentration of 0.5% for 5-minute duration and others so on...). Tensile testing was carried out on vertical and horizontal strips separately. Groups were compared for thickness, strength and elongation (elasticity). T-test for independent samples is used in comparison for thicknesses, tensile strengths at yield, elongations at yield, tensile strengths at break, elongations at break of horizontal and vertical pericardial samples. The variances of all variables were homogeneous according to Levene's test ($p>0.05$). The data were scattered normally.

Also one-way ANOVA test was used for comparison between durations in vertical and horizontal groups. The differences in terms of durations between all groups were found to be important for both horizontal and vertical groups. So, differences in terms of durations were compared two by two with the use of “Tukey HSD”, a form of post-Hoc tests.

RESULTS

Comparison of vertical and horizontal groups (according to T-test for independent samples) is presented in Table 1.

Table 1. Comparison of vertical and horizontal groups

	Thickness	F_{max}	F_{break}	E_{max}	E_{break}
Fresh (%)	S (v >h)	ns	S (v >h)	ns	S (v <h)
0.5-5′	ns	ns	S (v >h)	ns	ns
0.5-10′	S (v >h)	ns	ns	S (v <h)	S (v <h)
0.5-20′	ns	ns	ns	S (v <h)	S (v <h)
1-5′	ns	S (v<h)	S (v <h)	S (v <h)	S (v <h)
1-10′	S (v >h)	ns	ns	S (v <h)	ns
1-20′	ns	ns	ns	ns	ns
2-5′	S (v <h)	ns	ns	ns	S (v <h)
2-10′	S (v <h)	ns	ns	S (v >h)	S (v >h)
2-20′	ns	S (v <h)	S (v <h)	ns	S (v >h)

S: Significant; ns: Nonsignificant; v: Vertical; h: Horizontal.

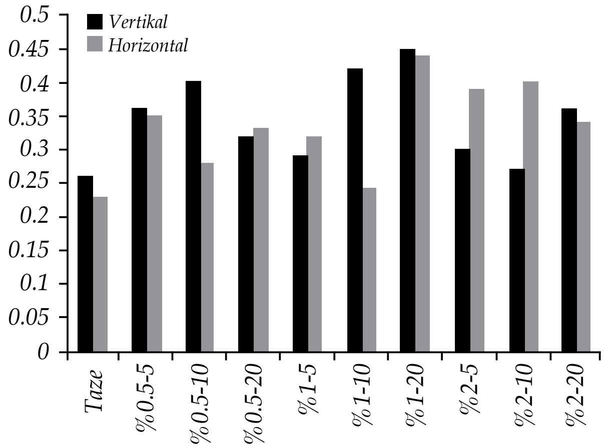

Fig. 2- *Thickness of pericardial samples (mm).*

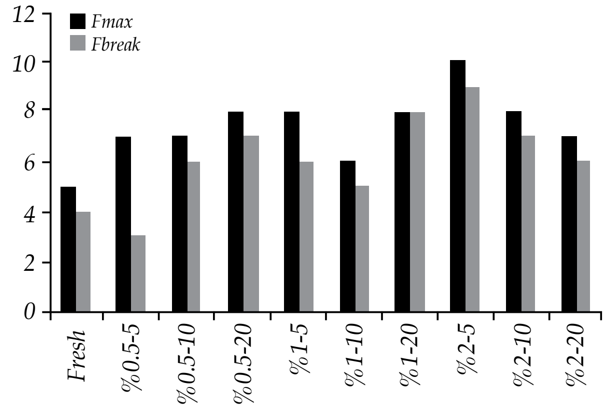

Fig. 3- *Maximum tensile strength (Fmax) and tensile strength at break (Fbreak) of vertical cut pericardial samples (N/mm²)*

The differences in terms of durations between all groups were found to be important for both horizontal and vertical groups (according to one way ANOVA test). So, differences in terms of durations were compared two by two with the use of "Tukey's HSD", a form of post-Hoc tests.

Thickness of vertical and horizontal samples were shown in Figure 2.

Maximum tensile strength (F_{max}) (Newton/ mm^2=N/mm^2), and tensile strength at break (F_{break}), (N/mm^2) were shown for vertical samples in Figure 3, and for horizontal samples in Figure 4.

Elongation at maximum tensile strength (E_{max}) (mm) and elongation at F_{break} (E_{break}) (mm) were shown for vertical samples in Figure 5, and for horizontal samples in Figure 6.

DISCUSSION

Pericardial patches have an extensive use in cardiac surgery. The most important problem related with their use is to increase their strength and elasticity, both in short- and long-term duration. The patches implanted in the heart should stand against the forces caused by the pressures in cardiac chambers generated during the contraction and relaxation of myocardium. The implanted pericardial patch, when faced with a force should extend for an enough level, that it should not disturb the regularity of the contraction of heart and that the heart should not do an extra work to extend the patch either. When the force is removed, pericardial patch should be so elastic that it should return back to its original position rather than forming an aneurysmatic structure instead. In a normal

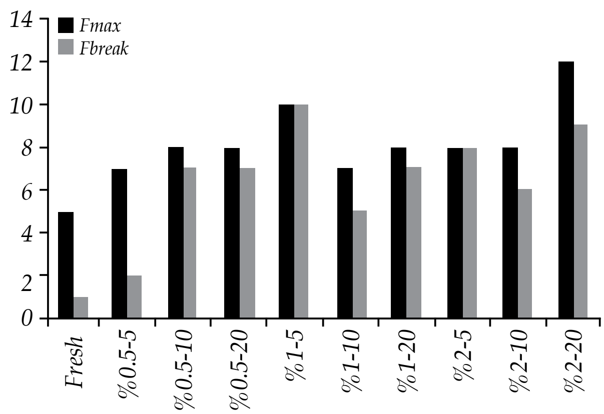

Fig. 4- *Maximum tensile strength (Fmax) and tensile strength at break (Fbreak) of horizontal cut pericardial samples (N/mm²).*

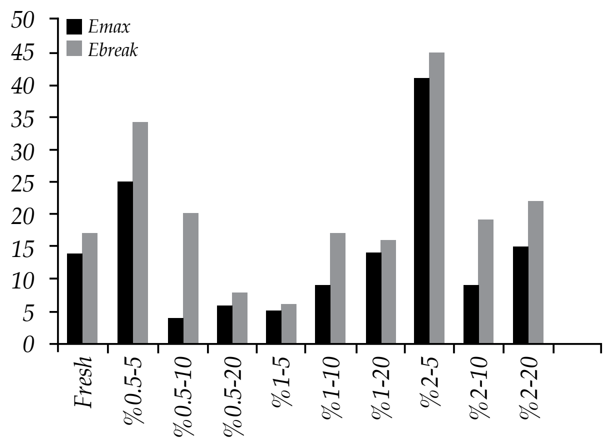

Fig. 5- *Elongation at maximum tensile strength (Emax) and Elongation at break (Ebreak) of vertical cut pericardial samples (mm).*

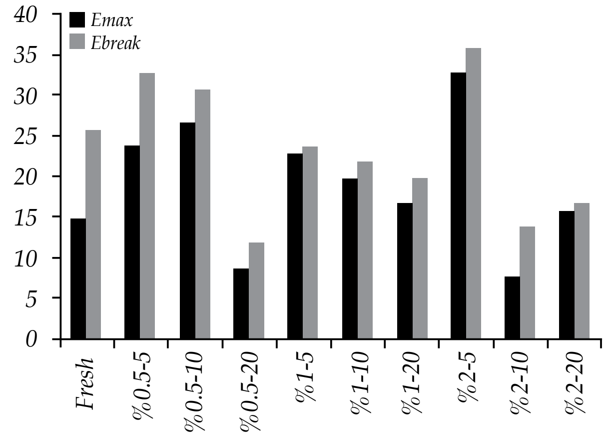

Fig. 6- *Elongation at maximum tensile strength (Emax) and Elongation at break (Ebreak) of horizontal cut pericardial samples (mm).*

healthy individual, this contraction and relaxation occurs again and again in every cardiac cycle. Depending on the rate of heart and developing pressures in cardiac chambers, the forces that pericardial patches face would change. Therefore, obtaining a pericardial patch with more elasticity and strength becomes very important.

Glutaraldehyde fixation (crosslinking) of connective tissue decreases biodegradation, protects the anatomical structure, provides strength and endurance to collagen fibers and also makes the tissue biocompatible and nonthrombogenic. Tissues fixed with glutaraldehyde retain most of the viscoelastic characteristics of collagen fibrillary network and this property makes them appropriate to be used as a bioprosthesis.[8]

Unfortunately, until recently, little attention was paid to mechanical properties of chemically modified pericardium and its suitability for cardiac surgery. It is well known that mechanical properties may prove a valuable means for quantitating the structural changes in tissue. It is difficult, however to determine which is more appropriate for estimating the mechanical properties for example elastic modulus, ultimate strength, maximum force, viscoelasticity, fatigue property, creep relaxation and so on.[9]

In this study, we used glutaraldehyde concentrations of 0.5%, 1%, 2%, approximately similar to previous studies. Since autologous pericardium would be prepared in the operating room until preparations for cardiopulmonary bypass (CPB) were started, maximum possible durations of 20, 10 and 5 minutes were chosen for fixation of pericardial samples.

In the evaluation of samples in terms of thickness, maximum tensile strength, tensile strength at break (F_{break}), elongation at maximum tensile strength and elongation at F_{break}, we saw that there was no remarkable difference between vertical and horizontal groups, except in group 1%-5′ in which all parameters were better in horizontal samples.

Elongation at maximum tensile strength and elongation at F_{break} and tensile strength at break were found to be higher in only one group that is the group 2%-5′ in both vertical and horizontal groups. Maximum tensile strength in vertical samples was again higher in the group 2%-5′, but the difference was not statistically significant.

Elongation at maximum strength and elongation at F_{break} would be higher only in group 2%-5′ for horizontal samples.

In this study, we had a large patient group between ages of 50-70 years and that we had 10 measures for each fixation group with correlated results. In other studies, pericardium of adults and children was said to behave differently as a pericardial patch.[3,10] Hjelms, Gavin and their group reported on an experiment with autologous pericardium in the right ventricular outflow tract of adult dogs and puppies. These investigators made the right ventricle hypertensive in some animals by banding the pulmonary artery. Examination of the patches 7 to 14 months later revealed no necrosis or aneurysm in any of the adult dogs. Among the puppies, aneurysm developed in one of six normotensive and two of six hypertensive animals.[3] Also, Holt (1970) points out that the waviness of pericardial collagen is absent at birth, increases to a maximum in the young adult, and disappears again in old age. The increase in waviness at adolescence correlates with the appearance of elastin in the sac; the amount reaches a maximum in the young adult and remains constant until death. They found no systematic differences in responses when the 26-

and 34-year-old individuals were compared with the 67- and 73-year-old individuals.[10]

The use of 2% glutaraldehyde concentration for five-minute fixation duration resulted in higher tensile strength at yield and at break and higher elongation at yield and at break. In this study, we chose uniaxial stretching. In fact, uniaxial stretching is not an appropriate method to determine the stiffness of an anisotropic material. This point has been made by Yin,[11] Abe et al.[12] and Moriarity,[13] who explain that two materials with significantly different behavior under biaxial loading can have identical uniaxial stress-strain relations. Since biological tissue is generally considered incompressible,[14] biaxial tissue testing is preferable for describing three-dimensional constitutive relations (stiffness).[15] However, we chose uniaxial over biaxial tissue testing, because the technique for determining gauge length and measuring the very high extensibility region of the stress/strain curve cannot be readily applied to the biaxial testing machine. Also, we could not find an appropriate biaxial testing machine. This is one of the drawbacks of our study. We recommend further similar studies to be done under biaxial stretching.

Acknowledgments

The authors wish to thank A. Sarıgül for his help in this study.

The authors also wish to thank A. Öztürk for her collaboration in permitting the study of the samples in Turkish Standards Institute (TSI) and also the authors wish to thank A. Kalkan, M. Özçepne, I. Uludağ for their technical assistance.

REFERENCES

1. Ghosh PK. On cardiac reconstructions. In: Ghosh PK, editor. Cardiac reconstructions. Berlin-Heidelberg: Springer-Verlag; 1989. p. 1-5.
2. Seybold-Epting W, Chiariello L, Hallman GL, Cooley DA. Aneurysm of pericardial right ventricular outflow tract patches. Ann Thorac Surg 1977; 24:237-40.
3. David TE, Feindel CM, Ropchan GV. Reconstruction of the left ventricle with autologous pericardium. J Thorac Cardiovasc Surg 1987;94:710-4.
4. Kirklin JK, Kirklin JW, Blackstone EH, Milano A, Pacifico AD. Effect of transannular patching on outcome after repair of tetralogy of Fallot. Ann Thorac Surg 1989;48:783-91.
5. Hjelms E, Pohlner P, Barratt-Boyes BG, Gavin JB. Study of autologous pericardial patch-grafts in the right ventricular outflow tracts in growing and adult dogs. J Thorac Cardiovasc Surg 1981;81:120-3.
6. Iguidbashian JP, Follette DM, Contino JP, Pollock ME, Lutrin C, Berkoff HA. Pericardial patch repair of left ventricular aneurysm. Ann Thorac Surg 1993;55:1022-4.
7. Haluck RS, Richenbacher WE, Myers JL, Miller CA, Wise RK, Waldhausen JA. Pericardium as a thoracic aortic patch: glutaraldehyde-fixed and fresh autologous pericardium. J Surg Res 1990;48:611-4.
8. Jayakrishnan A, Jameela SR. Glutaraldehyde as a fixative in bioprostheses and drug delivery matrices. Biomaterials 1996;17:471-84.
9. Sato M, Maeta H, Okamura K, Ohshima N. Relationship between mechanical and hydrodynamic properties of bioprosthesis produced from canine aortic valve. Artif Organs 1985;9:184-91.
10. Lee JM, Boughner DR. Mechanical properties of human pericardium. Differences in viscoelastic response when compared with canine pericardium. Circ Res 1985;57:475-81.
11. Yin FC. Ventricular wall stress Circ Res 1981; 49;829-42.
12. Abe H, Nakamura T, Motomiya M, Konno K, Aria S. Stresses in left ventricular wall biaxial stress-strain relations of the cardia muscle fiber in the potassium arrested heart. Trans ASME J Biomech Eng 1978;100:116-21.
13. Moriarty TF. The law of Laplace. Its limitations as a relation for diastolic pressure, volume, or wall stress of the left ventricle. Circ Res 1980;46:321-31.
14. Carew TE, Vaishnav RN, Patel DJ. Compressibility of the arterial wall. Circ Res 1968;23:61-8.
15. Yin FC, Chew PH, Zeger SL. An approach to quantification of biaxial tissue stress-strain data. J Biomech 1986;19:27-37.