Biomaterials Research (1998) 2(3) : 95-99

Biomaterials Research

Mechanical Properties of the UV Irradiated Porcine Valves

Hwal Suh[1*], Jong Chul Park[1], Kyung Tae Kim[1], Won Kyu Lee[1] and Bum Koo Cho[2]

[1]Department of Medical Engineering, Yonsei University College of Medicine, 134, Shinchon-dong, Seodaemun-ku, Seoul 120-752, Korea.
[2]Department of Thoracic and Cardiovascular Surgery, Yonsei University College of Medicine, 134, Shinchon-dong, Seodaemun-ku, Seoul 120-752, Korea.
(Received July 13, 1998 / Accepted August 21, 1998)

The effect of UV (ultraviolet ray) irradiation treatment to natural porcine aortic valves on their mechanical properties were investigated. The mechanical properties of natural porcine aortic valves cross-linked by UV irradiation were measured by uniaxial tension tests. Negative control group was a non-treated fresh aortic valve. As a positive control group, an aortic valve was treated with low concentration glutaraldehyde (GA) solution (0.625 wt.%) for more than 24 hours. Natural aortic valves were exposed to ultraviolet lay (UV) lamp (12 W) for 30 minutes, 1 and 2 hours at 4°C. The mechanical behavior of GA treated group and UV treated group was investigated by measuring ultimate tensile strength (UTS), and stiffness. The ultimate tensile strength of GA treated group (1.67±0.48 MPa), UV treated group for 30 minutes (1.35±0.29 MPa), UV treated group for 1 hours (1.29±0.45 MPa) and UV treated group for 2 hours (1.14±0.33 MPa) increased than that of fresh group (0.84±0.45 MPa). The stiffness 1 as secant lines up to a stress of 300 KPa were 0.61±0.24 MPa (fresh), 1.30±0.57 MPa (GA), 1.02±0.69 MPa (UV, 30 min), 1.07±0.26 MPa (UV, 1 hr) and 1.35±0.62 MPa (UV, 2 hrs), respectively. The stiffness 2 from 300 KPa to yielding point were 0.91±0.31 MPa (fresh), 1.54±0.81 MPa (GA), 1.34±0.72 MPa (UV, 30 min), 1.45±0.40 MPa (UV, 1 hr) and 1.56±0.75 MPa (UV, 2 hrs), respectively. The stiffness 1 and 2 of the UV treated groups were significantly different from those of negative control group ($P < 0.05$), but showed no significant difference from those of positive control group ($P > 0.05$). This study demonstrated that physically cross-linked aortic valve by UV irradiation has enhanced mechanical behavior for implantation and is useful for improving durability.

Key words : Leaflet, UV irradiation, Cross-linking, Mechanical property, Tension test

INTRODUCTION

Heart valve prostheses have been used since 1960, and approximately 75000 prosthetic valve replacements are performed in each year throughout the world.[1] The types of prosthetic valves are generally defined as mechanical valve, polymer valve and tissue valve according to the materials of which the valve is made. The adventage of mechanical valve, made of metal on which pyrolytic carbon is coated, comparing to the other valve is the long-term durability, but continuous anticoaguration therapy is necessary.[2] The tissue valve and polymer valve have a lower incidence of thromboembolic complications than the mechanical valve but have less longivity due to their weak resistance against the continuously pulsative blood flow, directly produced by heart. The prosthetic valves made of natural tissue have been also used in practice to decrease the adverse reactions that commonly occurs in the other types of prosthetic heart devices.[3] The ideal prosthesis for surgical replacement of defective heart valve should have appropriate histologic, biochemical, biomaterial, and biomechanical charicteristics. This study focuses on the mechanical evaluation of tissue valve among these aspects. The leaflet of an aortic valve is a composite material consists of dense connective tissue with abundant elastin that provides acceptable valve movement, and the mechanical properties of which are primarily due to the strong collagen bundles which consist of a highly anisotropic network.[4] A bioprosthetic cardiac valve must be primarily designed to perform and maintain the mechanical function which is ultimately confirm its long-term success as a replacement.[5] The bioprostheses, which is the cross-linked xenograft valve obtained by treating the natural tissue chemically and physically, have been used.[6] The chemical treatment by glutaraldehyde (GA) has been known as the efficient and popular method for replacement,

*To whom correspondence should be addressed.

otherwise the physical cross-linking is produced with drying method, heating method, UV irradiation, gamma ray irradiation and is reported that UV induced cross-linking method was more convinient and effective among the above methods.[6,7] Changes in molecular structure and cross-linking of collagen could also influence the response of the leaflet tissue toward a cyclic load,[8] for the mechanical behavior of the biological valve is sensitive to molecular structure of collagen. Mechanical parameters, such as stress-strain behavior, ultimate tensile strength (UTS) and stiffness, have been used to investigate the effects of various cross-linking treatments on the aortic valve tissue before implantation.[9,10] When a natural aortic valve cyclically opens and closes, the valve leaflets undergo cyclic loading and unloading, and such movement is the main reason that leads to cause leaflet fatigue which could be minimized by improving mechanical properties of the leaflet.[11] The long-term performance of tissue valves is highly dependent on the mechanical properties of leaflet materials.

In this study, The mechanical properties of cross-linked porcine aortic valves cross-linked by UV irradiation are evaluated by uniaxial tension test. Positive control group is treated by a low concentration GA solution (0.625 wt.%) for 24 hours to ensure optimum cross-linking, and negative control group was the fresh valve.

MATERIALS AND METHODS

Porcine valves were procured at a local slaughterhouse and stored in Hanks' solution (GIBCO) at 4°C immediately after excision. The leaflets were derived from porcine aortic valves within 24 hours and were prepared by UV treatment (wave length of 253 nm) with varying irradiation time (30 minutes, UV30; 1 hours, UV1; 2 hours, UV2) at 4°C. During the UV irradiation, valves was placed in an acrylic box to prevent specimen from the scattering UV rays.[6,7] The fresh leaflets were used as the negative control, while the positive control (GA) was the leaflets which were treated with 0.625% glutaraldehyde in phosphate buffered saline solution (pH 7.4) for 24 hours.[6,7]

Mechanical test

The specimen was prepared by cutting it into 4 mm × 20 mm pieces (Figure 1A), and stored in hank's solution at 4°C. After thawing at room temperature for 1 hrs, the effective cross-sectional area and length of leaflet were measured with micrometer (Mitutoyo co.). The uniaxial tension test was performed with a material testing machine (Micro Bionix, Tytron). The load cell capacity for detecting forces was 100 N (MTS, 661.09B-21). Both extremities of the specimen were

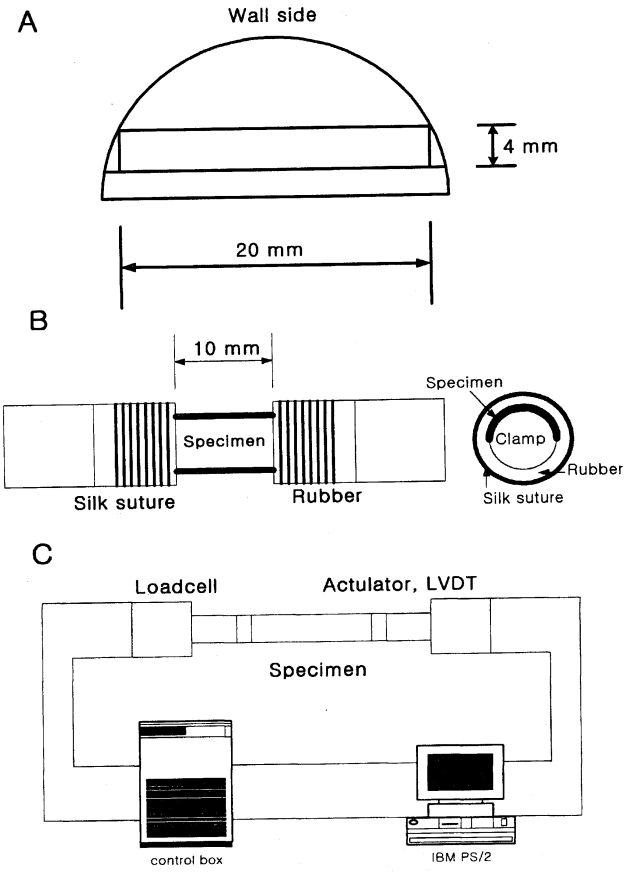

Figure 1. Schematic diagram of uniaxial tension test; A) Porcine aortic valve leaflet : the size of specimen was 4 mm(width), 20 mm(length), B) Gripping of the specimen : the ends of specimen were wrapped with rubber and tightened with silk suture onto the both clamps, C) Diagram of tension test : Micro Bionix, Tytron.

placed onto clamps, wrapped with rubber, then tightened by a silk thread (Figure 1B). The distance between the edge of one clamp to the opposite, so called as an initial length, was measured with the tauted specimens. Strain was expressed as the change in length as a percentage of the initial length. Preconditioning of the specimen was performed with five-times repeated loading (100 g) and unloading at the cross head speed of 10 mm/min,[12] and then it was stretched up to the failure point at the same rate.[13] All tests were carried out at room temperature. The data from the load cell and LVDT (linear variable differential transformer) were stored by the data acquisition software of Teststar 4.0 C (Figure 1C). The sampling rate was 5 Hz. Stress-strain curve was extracted. From the load-displacement relationship obtained by above system Ultimate tensile strength (UTS) and tangent stiffness were determined from the stress-strain relation. The tangent stiffness was defined by the slope of the stress-strain curve, which was determined by the least-square linear regression at a

stress of 300 KPa (physiologic range) [Stiffness 1] and from 300 KPa to yielding points [Stiffness 2].[14] The measured values were statistically analyzed using a paired Student's t-test.

RESULTS

Figure two shows cross-sectional area of the fresh leaflet, GA and UV induced leaflets. The cross-sectional area of the GA treated leaflet increased in comparision with the non-treated group, while the cross-sectional area of the UV irradiated leaflet decreased. There were no significant differences between the fresh group and treated group ($p>0.05$). Table 1 shows the failure modes in accordance with loads. Failure occurred mostly near to or at the edge of the clamp in all groups. Figure 3 and 4 shows the comparisons of the stress-strain curves for the groups. The ultimate tensile strength of GA (1.67±0.48 MPa), UV30 (1.35±0.29 MPa), UV1 (1.29±0.45 MPa) and UV2 (1.14±0.33 MPa) treated leaflets increased than that of the fresh leaflet (0.84±0.45 MPa), and the UTS of UV treated leaflets demonstrated a tendency of decrease in accordance with time (Figure 5). Figure 6 and 7 shows the value of stiffness 1 and stiffness 2. The

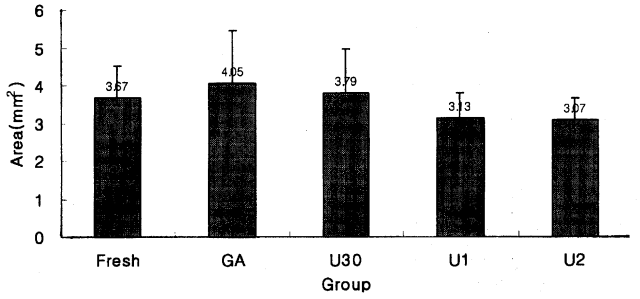

Figure 2. Cross-sectional area of the whole groups (corss-sectional area : width × length, Fresh : non-treated group, negative control, GA : 0.625% glutaraldehyde in saline solution for 24 hrs, positive control, UV30 : UV(254 nm) induced group, 30 mins, UV1 : UV(254 nm) induced group, 1 hr, UV2 : UV(254 nm) induced group, 2 hrs).

Table 1. Failure modes and failure loads of the specimen used for tensile testing. (Fresh : non-treated group, GA : glutaraldehyde induced group, UV30 : UV induced group, 30 mins, UV1 : UV induced group, 1 hr, UV2 : UV induced group, 2hr, * n : number of tensile tested specimen)

Group		Substance	Near edge	Edge
Fresh	n	0	6	5
	Force(N)	-	4.16±1.8	1.71±0.32
GA	n	0	5	2
	Force(N)	-	4.97±1.26	3.51±0.43
U30	n	-	6	0
	Force(N)		5.43±1.74	-
U1	n	0	7	2
	Force(N)	-	5.89±1.56	4.52±1.37
U2	n	0	6	3
	Force(N)	-	3.83±0.6	3.83±0.07

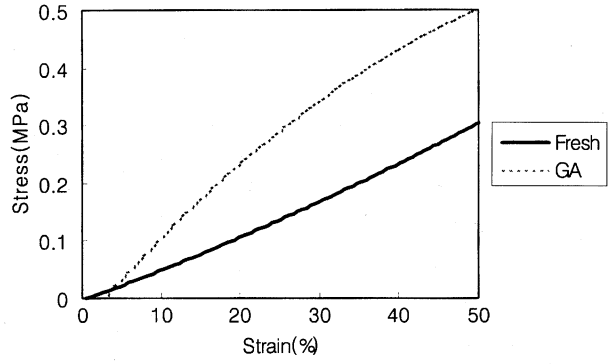

Figure 3. Comparison of stress-strain curves between fresh group and GA treated group. (Fresh : regression of fresh group, negative control, GA : regression of glutaraldehyde induced group, positive group).

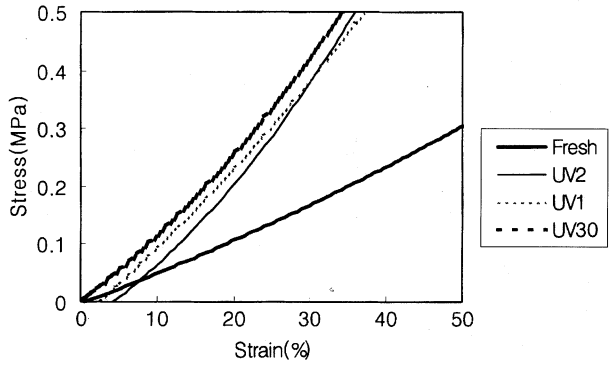

Figure 4. Comparison of stress-strain curves between fresh group and UV treated groups. (Fresh : regression of fresh group, negative control. UV30 : regression of UV ray induced group for 30 mins, UV1 : regression of UV ray induced group for 1 hr, UV2 : regression of UV ray induced group for 2 hrs).

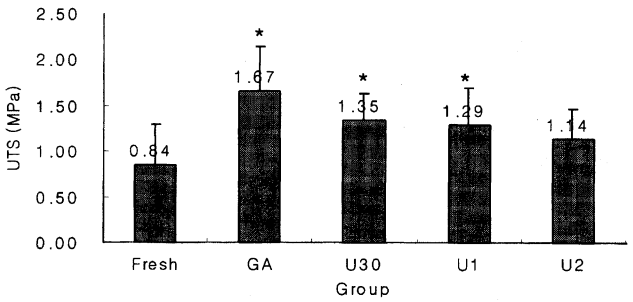

Figure 5. Ultimate tensile strength of whole groups. (* : significantly different compared to the fresh group ($P<0.05$). Fresh: non-treated group, negative control, GA : 0.625% glutaraldehyde in saline solution for 24 hrs, positive control, UV 30 : UV(254 nm) induced group, 30 mins, UV1 : UV(254 nm) induced group, 1 hr, UV2 : UV(254 nm) induced group, 2 hrs).

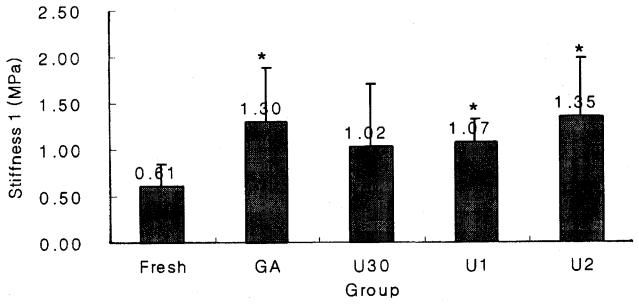

Figure 6. Stiffness of groups at 300 KPa. (* : significantly different compared to the fresh group (P<0.05), Stiffness 1 : the secant line up to a stress of 300 KPa, Fresh : non -treated group, negative control, GA : 0.625% glutaraldehyde in saline solution for 24 hrs, positive control, UV30 : UV(254 nm) induced group, 30 mins, UV1 : UV(254 nm) induced group, 1 hr, UV2 : UV(254 nm) induced group, 2 hrs).

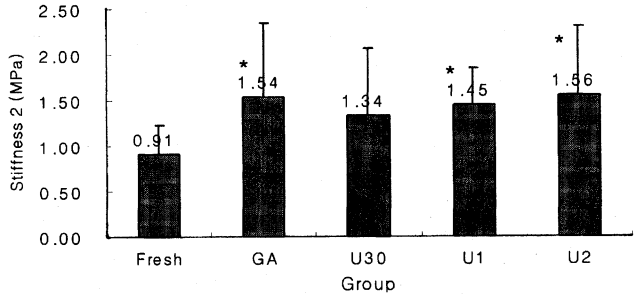

Figure 7. Stiffness of groups from the stress of 300 KPa to the yielding point. (* : significantly different compared to the fresh group (P<0.05), Stiffness 2 : the secant line from the stress of 300 KPa to yield stress, Fresh : non-treated group, negative control, GA : 0.625% glutaraldehyde in saline solution for 24 hrs, positive control, UV30 : UV(254 nm) induced group, 30 mins, UV1 : UV(254 nm) induced group, 1 hr, UV2 : UV(254 nm) induced group, 2 hrs).

values of stiffness 1 were 0.61±0.24 MPa (fresh), 1.30±0.57 MPa (GA), 1.02±0.69 MPa (U30), 1.07±0.26 MPa (U1), and 1.35±0.62 MPa (U2), while the values of stiffness 2 were 0.91±0.31 MPa (fresh), 1.54±0.81 MPa (GA), 1.34±0.72 MPa (U30), 1.45±0.40 MPa (U1), and 1.56±0.75 MPa (U2).

DISCUSSION

The cross-sectional areas of the GA treated group were slightly larger than those of the fresh group, for the liquid may induce swelling to the specimen. However, the cross-sectional areas of the UV treated groups were less than those of the fresh group, and it is assumed that the collagen fiber may experience shrinkage due to the cross-linking with the treatment. Every specimen tends to fail near the edge of the clamp. It was due to the stress concentration near the edge by the gripping. Generally, the ultimate tensile strength of soft tissue is affected by gripping method. The gripping itself for soft tissues is very difficult because a slippage must be prevented without causing any structural damage and premature failure at the clamp. In this study, the GA and UV treatments induced cross-linking of the collagen fiber, making the UTS of the treated groups greater than that of the fresh group. And the UTS of UV treated groups decreased with time. However, determination of the UTS is of little value because UTS gives no information about the material behavior in physiological ranges. Therefore, we measured the stiffness of the material. The stiffness 1 of porcine aortic valve was obtained at a stress of 300 KPa, which is the stress acted on a working valve in the closed position caused by a blood pressure of 150 mmHg. The stiffness 2 of porcine aortic valve was calculated by the slope from the stress of 300 Kpa to the yield stress. In the values of stiffness 1 and 2, UV treated groups showed higher stiffness than the fresh group. Statistical analysis showed that the stiffness 1 and 2 of GA, U1 and U2 treated groups were significantly different from the fresh group ($p<0.05$). But there was no significant difference between the fresh group and the U30 group ($p>0.05$) in the stiffness 1 and 2. The GA treated group was stiffer than the UV treated groups, but statistically there were no significant difference ($p>0.05$). UV treated groups showed slightly higher stiffness in accordance with the treatment time ($p>0.05$).

Differences in the stiffness among the groups would indicate the effects of treatment on the integrity of the collagen fibers, which are the main structural elements of the leaflet.

In an effort to obtain a better understanding of the engineering requirements for construction of a successful aortic valve prosthesis, considerable analysis has been done of the structure and deformations of human and porcine aortic valves. Clarke *et al.*[15,16] and Ghista *et al.*[17,18] had used silicon rubber moulds of human aortic valves and close-range stereology to examine the geometry of the leaflets under changing pressures, and finite element analysis to calculate the stress in the leaflets. Chong *et al.*[19] used the theory of thin membranes to analyze stress, and Missirlis *et al.*,[20] Chong *et al.*[21] also extended this analysis to include both the theories of thin shells and membranes. While each of these analysis required detailed geometric information to predict stress, they also required knowledge of the mechanical properties of the leaflet material to simplify the constant thickness and homogeneity. For this reason, Ghista *et al.* assumed their leaflet material to be a block polymer. Only Chong and Missirlis attempted a stress analysis incorporating both the inhomogeneity of the porcine leaflet material. And Lim and Boughner demonstrated the viscoelastic characteristics of the leaflet material.[22]

In this study, UV irradiated aortic leaflet demonstrated

improved mechanical properties through uniaxial tension test. But the leaflets revealed a viscoelasticity, a largey independent strain rate, and a significant dependence on the degree of preconditioning by cyclic loading. Therefore, the additional testing of viscoelastic properties is required.

CONCLUSION

In this study, we measured the mechanical properties of porcine aortic valve cross-linked by UV irradiation to obtain the stress-strain characteristics (UTS, stiffness). UV irradiated valves had better mechanical properties (high stiffness and high UTS) for the replacement of bioprostheses, in comparison with the fresh ones. The increase in tensile property by UV treatment would probably be resulted from the cross-linking of collagen, and collagenous tissue prepared by UV irradiation may be useful for improving durability.

ACKNOWLEDGEMENT

This research was supported by the Korea Ministry of Health and Welfare (Grant No. HMP-96-G-2-45). The authors thank Dr. Jeong Wook Shin (Inje University) for his kind advice for mechanical test.

REFERENCES

1. W. W. Angell, J. D. Angell, A. Sywak, "Selection of tissue or prosthetic valve," *J. Thorac. Cardiovasc. Surg.*, **73**, 43-53 (1977).
2. M. E. Lee, T. Murakami, B. Stanczewski, "Etiology of thrombus formation on prosthetic metal heart valves : the role of spontaneous *in vivo* interfacial potentials and measurements," *J. Thorac. Cardiovasc. Surg.*, **63**, 809-819 (1972).
3. P. N. Sawyer, B. Stanczewski, S. Srinivasan, "Electron microscopy and physical chemistry of healing in prosthetic heart valves, skirts, and struts : modification by electrochemically clean physiologic surfaces," *J. Thorac. Cardiovasc. Surg.*, **67**, 24-43 (1972).
4. J. Michael Lee, David W. Courtman, Derek R. Boughner, "The glutaraldehyde-stabilized porcine aortic valve xenograft, I. Tensile viscoelastic properties of the fresh leaflet material," *J. Biomed. Mater. Res.*, **18**, 61-77 (1984).
5. R. J. Brewer, R. M. Mentzer, J. D. Deck, R. C. Ritter, J. S. Trefil, S. P. Nolan, "An in vivo study of the dimensional changes of the aortic valve leaflets during the cardiac cycle," *J. Thorac. Cardiovasc. Surg.*, **74**, 645-650 (1977).
6. H. Suh, "Fundamental concepts for the tissue engineering," *Biomaterials Research*, **2**, 1-7 (1998).
7. W. K. Lee, J. C. Park, Y. H. Park, B. K. Cho, J. K. Kim, K. D. Park, Y. H. Kim, H. Suh, "Crosslinking of porcine valve by UV irradiation," *Trans. Soc. Biomaterials*, **24**, 293 (1998).
8. Mano Thubrikar, William C. Piepgrass, J. David Deck, Stanton P. Nolan, "Stresses of natural versus prosthetic aortic valve leaflets in vivo," *Ann Thorac Surg.*, **30**, 230-239 (1980).
9. A. J. K. Tan, D. L. Holt, "The effects of sterilization and storage treatments on the stress-strain behavior of aortic valve leaflets," *Ann. Thorac. Surg.*, **22**, 188-194 (1976).
10. R. E. Clark, S. Louis, "Stress-strain characteristics of fresh and frozen human aortic and mitral leaflets and chordae tendineae," *J. Thorac. Cardiovasc. Surg.*, **66**, 202-208 (1973).
11. S. L. Hilbert, L. C. Sword, K. F. Batchelder, M. K. Barrick, V. J. Ferrans, "Simultaneous assessment of bioprosthetic heart valve biomechanical properties and collagen crimp length," *J. Biomed. Mater. Res.*, **31**, 503-509 (1996).
12. I. Vesely, L. Gonzalez-Lavin, D. Graf, D. Boughner, "Mechanical testing of cryopreserved aortic allografts," *J. Thorac. Cardiovasc. Surg.*, **99**, 119-123 (1990).
13. J. Michael Lee, Derek R. Boughner, David W. Courtman, "The glutaraldehyde-stabilized porcine aortic valve xenograft, II. Effect of fixation with or without pressure on the tensile viscoelastic properties of the leaflet material," *J. Biomed. Mater. Res.*, **18**, 79-98 (1984).
14. P. E. Oyer, D. Craig Miller, B. Stinson, A. Reitz, J. Moreno-Cabral, E. Shumway, "Clinical durability of the hancock porcine bioprosthetic valve," *J. Thorac. Cardiovasc. Surg.*, **80**, 824-833 (1980).
15. R. E. Clark, H. M. Karara, A. Cataloglu, P. L. Gould, "Close-range stereophotogrammetry and coupled stress analysis as tools in the development of prosthetic devices," *Trans, Am. Soc. Artif. Intern. Organs*, **21**, 71-77 (1975).
16. A. Cataloglu, R. E. Clark, P. L. Gould, "Stress analysis of aortic valve leaflets with smoothed geometrical data," *J. Biomech.*, **10**, 153-158 (1977).
17. D. N. Ghista, H. Reul, "Optimal prosthetic aortic leaflet valve : Design parametric and longevity analyses : Development of the avchthane-51 leaflet valve based on the optimum design analysis," *J. Biomech.*, **10**, 313-324 (1977).
18. D. N. Ghista, H. Reul, G. Ray and K. B. Chandran, "Optimal design of aortic leaflet prosthesis," *J. Eng. Mech. Div. (ASCE)*, **104**, 97-117 (1978).
19. K. P. Chong, D. W. Wieting, N. H. C. Hwang and J. H. Kennedy, "Stress analysis of normal human aortic valve leaflets during diastole," *Biomater. Med. Devices Artif. Organs*, **1**, 307-321 (1973).
20. Y. R. Missirlis and C. D. Armeniades, "Stress analysis of the aortic valve during diastole : Important parameters," *J. Biomech.*, **9**, 477-480 (1976).
21. M. Chong and Y. F. Missirlis, "Aortic valve mechanics part II : A stress analysis of the porcine aortic valve leaflets in diastole," *Biomater. Med. Devices Artif. Organs*, **6**, 225-244 (1978).
22. K. O. Lim and D. R. Boughner, "The low frequency dynamic viscoelastic properties of human aortic valve tissue," *Circ. Res.*, **39**, 209-214 (1976).