Laboratory Investigation

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Is Autologous or Heterologous Pericardium Better for Valvuloplasty?

A Comparative Study of Calcification Propensity

Pericardial calcification is detrimental to the long-term durability of valvuloplasty. However, whether calcification susceptibility differs between heterologous and autologous pericardium is unclear. In this study, we compared the progression of calcification in vivo between autologous and heterologous pericardium.

We randomly divided 28 rabbits into 4 equal groups. Resected rabbit pericardium served as autologous pericardium, and commercial bovine pericardium served as heterologous pericardium. We subcutaneously embedded one of each pericardial patch in the abdominal walls of 21 of the rabbits. The 7 control rabbits (group A) received no implants. The embedded samples were removed at 2 months in group B, at 4 months in group C, and at 6 months in group D. Each collected sample was divided into 2 parts, one for calcium-content measurement by means of atomic-absorption spectroscopy, and one for morphologic and histopathologic examinations.

*When compared with the autologous pericardium, calcium levels in the heterologous pericardium were higher in groups B, C, and D (*P *<0.0001,* P *<0.0002, and* P *<0.0006, respectively). As embedding time increased, calcium levels in the heterologous pericardium increased faster than those in the autologous, especially in group D. Disorganized arrangements of collagenous fibers, marked calculus, and ossification were seen in the heterologous pericardium. Inflammatory cells—mainly lymphocytes and small numbers of macrophages—infiltrated the heterologous pericardium.*

The autologous pericardium showed a stronger ability to resist calcification. Our results indicate that autologous pericardium might be a relatively better choice for valvuloplasty. ***(Tex Heart Inst J 2015;42(3):202-8)***

For several decades, pericardial tissue has been widely applied as a good material for valvuloplasty.[1] Two kinds of pericardium are available for valvuloplasty: autologous and heterologous. Most aortic regurgitation that is caused by defects in valve leaflets can be successfully stopped by replacing the defective leaflets with autologous or heterologous pericardium.[1,2] This procedure can provide a satisfactory hemodynamic effect upon long-term follow-up evaluation, and the results are comparable to those in valve-sparing aortic valvuloplasty.[3,4] However, calcification, fibrous changes, and contracture of pericardial tissue decrease the durability of either heterologous or autologous pericardial tissue after surgery. Among these detrimental factors, calcification is considered to be a trigger of pericardial degeneration.[5] To an extent, degeneration from calcification might restrict wider application of valvuloplasty and adversely affect the long-term surgical results.[6,7] It has been unclear whether calcification progression differs in the 2 kinds of pericardium. By means of subcutaneous embedding, we compared calcification progression in vivo between autologous pericardium and heterologous bovine pericardium in a rabbit model.

Materials and Methods

Our study was approved under the Guidelines for Animal Experimentation, Capital Medical University, Beijing. We obtained 28 male New Zealand white rabbits (mean weight, 2 ± 0.8 kg) from the Beijing Xingwang animal-breeding center. The rabbits

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were randomly divided into 4 groups of 7. Group A was the control group; groups B, C, and D were the experimental groups. They were housed in a clean room, with one rabbit per cage, at a temperature of 23 °C in a 12-hour light-dark cycle and a humidity-controlled (60%) environment.

Preparation of Pericardium. After undergoing intravenous anesthesia, the rabbits were placed in the supine position. Their limbs and heads were fixed on the operating table, and their tracheas were intubated. Incisions were made from the suprasternal fossa to 2 cm under the xiphoid process, and the pericardium was exposed. The pericardium was harvested for 2 mm inside the pericardial–pleural cross, and fat tissue was removed from the pericardial surface as completely as possible. The thoracic cavity was closed. Attention was paid to aseptic procedures, and the vital signs of the rabbits were kept stable during the surgery. The autologous pericardium was fixed in 0.625% glutaraldehyde for 15 minutes, rinsed with saline solution for 10 minutes, and cut into 1 × 1-cm^2 pieces. Commercial Balmedic bovine pericardium (Beijing Balmedic Biological Engineering Co. Ltd.; Beijing, PRC), the heterologous pericardium, was cut into 1 × 1-cm^2 pieces and flushed with saline solution for 10 minutes.

Rabbit Subcutaneous Embedding Model. The pretreated autologous pericardium (Aa) and Balmedic pericardium (Ah) was not implanted into the group A rabbits; those samples were directly prepared for measurement of calcium content and for histologic evaluation. The results were compared with those of the other groups after the respective samples were harvested. In the 3 experimental groups, each sample was embedded subcutaneously with autologous pericardium into the left side of the abdominal wall and Balmedic bovine pericardium into the right side. Each rabbit was kept and observed in its own cage after surgery. Routine antibiotics, nutrition, and incision dressings were given to the rabbits during the next 5 days.

The embedded pericardial samples were removed at 2 months (group B), 4 months (group C), and 6 months after surgery (group D). The corresponding samples of autologous pericardium were called Ba, Ca, and Da, and the heterologous samples were called Bh, Ch, and Dh. Each sample was divided into 2 parts: one part for calcium-content measurement by means of atomic-absorption spectroscopy, and the other for morphologic and histopathologic examinations. After removal of the specimens, the rabbits were humanely killed.

Measurement of Calcium Content. The specimens were placed in deionized water-cleaned dishes, oven-dried at a temperature of 80 °C for 2 hours, and weighed (accurate to 0.0001 g) after natural cooling. Each sample and 10 mL of pure nitric acid were added to a 100-mL beaker. After the sample was completely dissolved, the beaker was heated until the solution was nearly evaporated. The specimens were equalized with deionized water and were transferred to a 10-mL volumetric flask. The blank reagent was prepared at the same time.

Calcium content was measured with use of a Z-8000 atomic-absorption spectrophotometer (Hitachi; Tokyo, Japan) with the following instrument values: calcium wavelength, 422.7 nm; lamp current, 7.5 mA; spectral passband, 1.3 nm; flame height, 12.5 mm; and flow of air/acetylene: 1.6/2.5 kg/cm^2.

For the standard work curve, standard liquids were prepared: calcium standard stock solution was serially diluted to 10 μg/mL with use of 5% superior-grade nitric acid. The concentrations of calcium in the standard-curve solutions were 0, 0.5, 1, 1.5, 2, and 2.5 μg/mL. The calcium content of the specimens was calculated by using the standard work curve. The calcium content was normalized to the tissue weight of each sample and was expressed in μg/g. All assays were performed in triplicate, and the mean was calculated.

Histopathologic Examination. After being fixed in formalin and embedded in paraffin, 4 consecutive 5-μm sections were collected from each specimen. Even-numbered sections were kept for hematoxylin and eosin (H & E) staining and were examined under a light microscope by a pathologist who was blinded to the details of this study.

Statistical Analysis

Data were analyzed with use of SPSS for Windows XP (IBM Corporation; Armonk, NY). Multiple comparisons among different times were made by means of analysis of variance. A paired-comparison *t* test was used to detect pairwise differences between experimental groups. Differences were considered statistically significant at $P < 0.05$. All data were expressed as mean ± SEM.

Results

Gross Appearance and Histologic Observations. Before embedding, the fresh autologous pericardium was soft, thin, transparent, and easy to curl or flatten (Fig. 1A). After pretreatment with glutaraldehyde, it became pale and slightly harder. The nonembedded heterologous pericardium was thick, soft, and easy to flatten (Fig. 1B). To the unaided eye, the autologous pericardium had a clear boundary with the peripheral tissue. It was still soft, even after extended embedding time. In contrast, the heterologous pericardium had a coated layer between it and the peripheral tissue, and the pericardial tissue gradually became hard and brittle as embedding time elapsed.

The pericardial samples from the control group were examined histologically and for calcium content. Upon histologic observation after H & E staining (Fig. 2), the autologous pericardium consisted of mesothelial cells

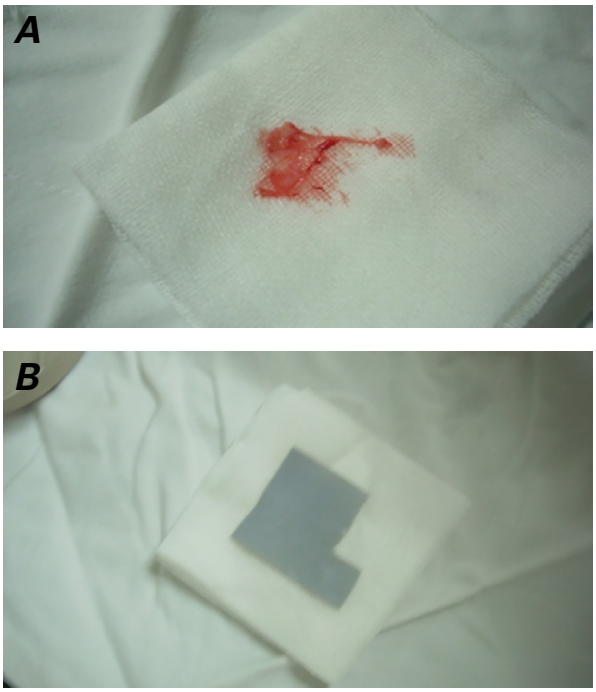


Fig. 1 *Photographs before embedding into the rabbits.* ***A****) The autologous rabbit pericardium was soft, thin, transparent, and easy to flatten.* ***B****) The heterologous bovine pericardium was thick, soft, and easy to flatten.*

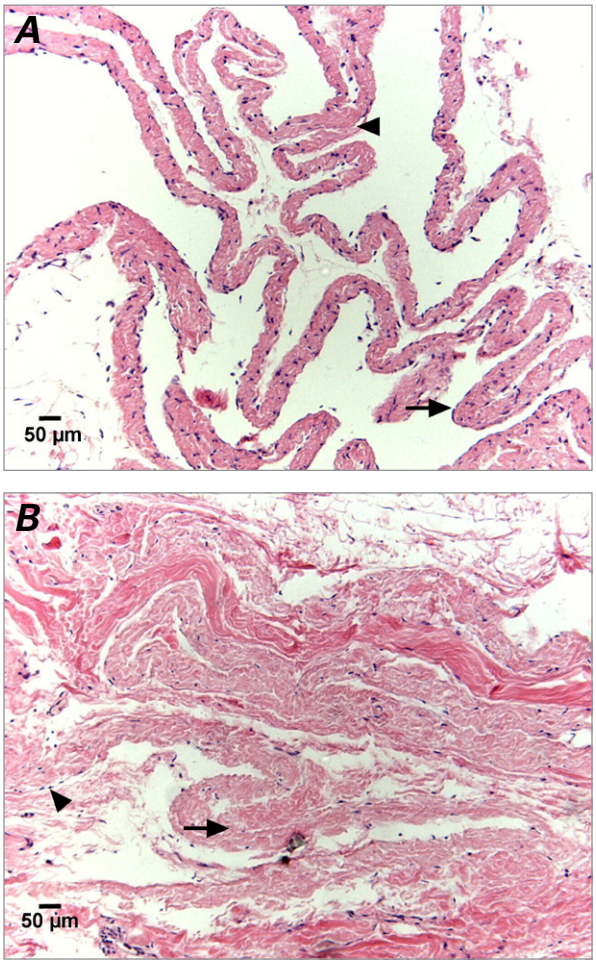


Fig. 2 *Photomicrographs of specimens from the control group.* ***A****) The autologous pericardium shows mesothelial cells (arrow) and fibrous tissue (arrowhead);* ***B****) the heterologous pericardium shows parallel-aligned fibrous tissue (arrow) and scattered nuclei (arrowhead) (H & E, orig. ×100).*

and fibrous tissue, whereas the Balmedic bovine pericardium consisted of parallel-aligned fibrous tissue with scattered nuclei. At other time points, the morphology of the autologous pericardium displayed no significant change, whereas the heterologous pericardium exhibited a more disordered structure as the embedding time elapsed.

Table I shows the higher calcium content in the heterologous pericardium relative to the autologous pericardium in vivo. In the autologous pericardium, calcium levels differed among the 4 time points of 0 (baseline), 2, 4, and 6 months (Fig. 3A). The calcium level in group Ba was higher than that in group Aa by more than 100% (0 vs 2 mo, P <0.0001). However, there were no significant differences among the tissues embedded for 2, 4, and 6 months (2 vs 4 mo, P=0.23; and 4 vs 6 mo, P=0.32). In comparison with the control samples, the calcium level in the autologous pericardial tissue increased significantly at 2 months (P <0.0001) and then reached a plateau, during which the content increased only slightly (Fig. 3B).

In the heterologous tissue, calcium levels differed among the 4 time points (Fig. 3C). The differences between group Ah and groups Bh, Ch, and Dh were statistically significant (0 vs 2 mo, P <0.0001; and 4 vs 6 mo, P <0.0005). There was no significant difference between group Bh and group Ch (2 vs 4 mo, P=0.39). Calcium levels increased significantly at 2 months, then rose more slowly from 2 to 4 months (Fig. 3B). At 6 months, the calcium level in group D rose sharply, more than 30 times that of group Ch (P <0.0005).

When calcium levels were compared between the 2 pericardial tissues at the same time point (Fig. 3D), no significant difference was observed in group A (0 mo, P=0.19). The calcium levels in the heterologous pericardium were higher than those in the autologous pericardium in groups B, C, and D (2 mo, P <0.0001; 4 mo, P <0.0002; and 6 mo, P <0.0006, respectively). As embedding time elapsed, calcium levels in the heterologous pericardium increased faster than those in the autologous pericardium, especially at 6 months.

Differences in Inflammatory Reaction. In comparison with the autologous pericardium, heterologous collagen fibers at 2 months were disordered and showed signs of inflammation, chiefly caused by lymphocytes (Fig. 4). As embedding time elapsed, inflammatory cells, mainly lymphocytes and a small number of macrophages, accumulated in the heterologous pericardium. Infiltra-

TABLE I. Calcium Content (μg/g) of Pericardial Tissue at Each Time Point in vivo*

Rabbit No.	Group A (Control)		Group B (2 mo)		Group C (4 mo)		Group D (6 mo)	
	Auto-logous	Hetero-logous	Auto-logous	Hetero-logous	Auto-logous	Hetero-logous	Auto-logous	Hetero-logous
1	0.67	0.88	1.07	1.81	0.92	1.09	0.87	36.5
2	0.35	0.43	1.11	1.82	1.2	1.32	1.03	65
3	0.15	0.38	1.12	1.86	1.34	1.47	1.17	67.2
4	0.77	0.71	1.26	1.86	1.44	1.68	2.11	68.7
5	0.56	0.49	1.38	1.96	1.65	2.59	2.11	71.6
6	0.67	0.65	1.43	1.99	1.69	3.18	3.05	72.7
7	0.23	0.96	1.59	2.11	2.5	6.39	3.65	135.2
Mean	0.49	0.64	1.28	1.92	1.53	2.53	2	73.84

*There were 7 rabbits in each group.

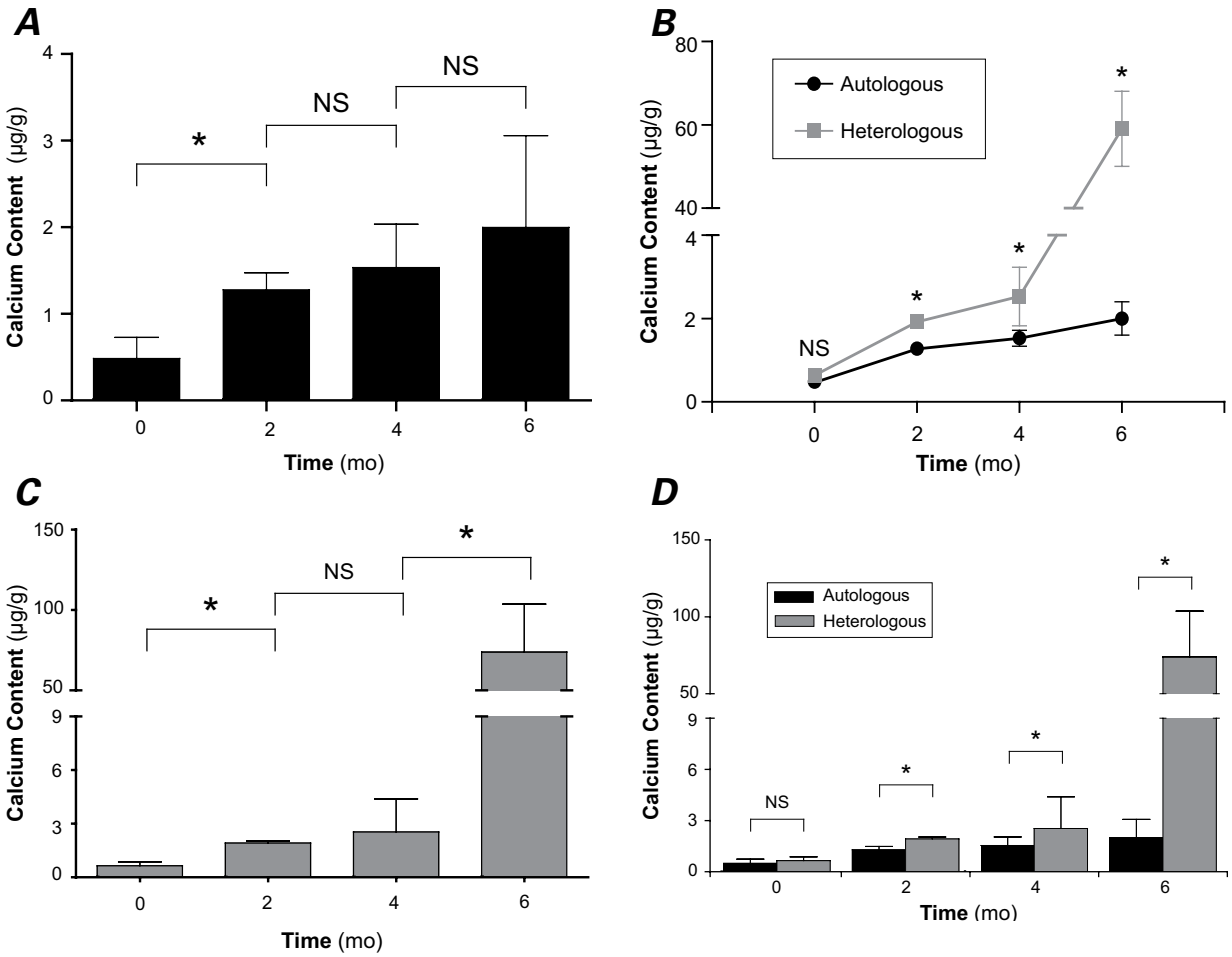


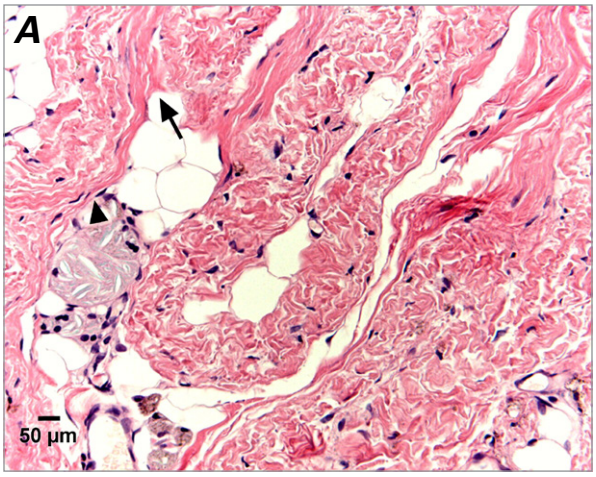
Fig. 3 *Graphs show changes in calcium content** in the pericardial tissue after embedding. **A**) Calcium levels in the autologous pericardium increased significantly at 2 months, then reached a plateau (0 vs 2 mo, P <0.0001; 2 vs 4 mo, P=0.23; and 4 vs 6 mo, P=0.32. **B**) Calcium levels were higher in the heterologous than those in the autologous pericardium (P <0.0001, P <0.0002, and P <0.0006 at 2, 4, and 6 mo, respectively). **C**) Calcium in the heterologous pericardium increased significantly at 2 months, increased slowly from 2 to 4 months, and rose sharply at 6 months (0 vs 2 mo, P <0.0001; 2 vs 4 mo, P=0.39; and 4 vs 6 mo, P <0.0005). **D**) Calcium levels were compared between the tissues at the same time point (0 mo, P=0.19; and P <0.0001, P <0.0002, and P <0.0006 at 2, 4, and 6 mo, respectively).*

n=7 per group

*P <0.05; NS = not significant

***All calcium values were normalized to the tissue weight of each sample and are expressed as μg/g.*

P <0.05 was considered statistically significant.



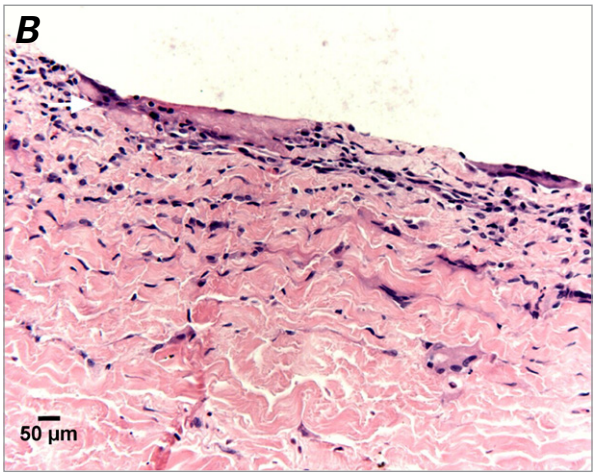
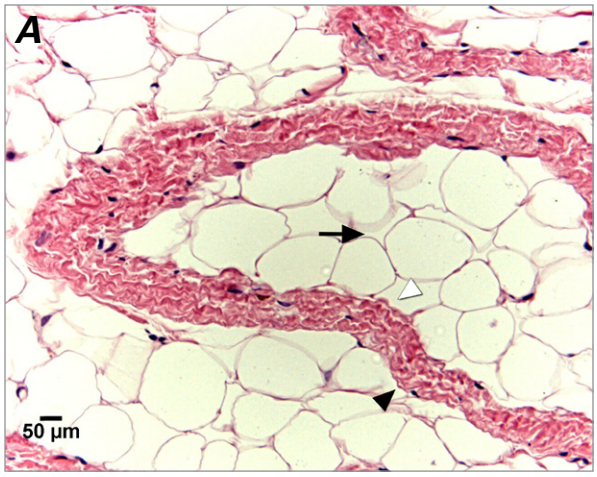


Fig. 4 *Photomicrographs show differences in inflammatory reaction at 2 months.* ***A****) The autologous pericardium was made of fibrous connective tissue (arrow) and mesothelial cells (arrowhead), with no observed inflammatory cells.* ***B****) Inflammatory cells, mainly lymphocytes (arrow), infiltrated the heterologous pericardium. Representative images from 3 independent experiments (n=7) are shown (H & E, orig. ×200).*



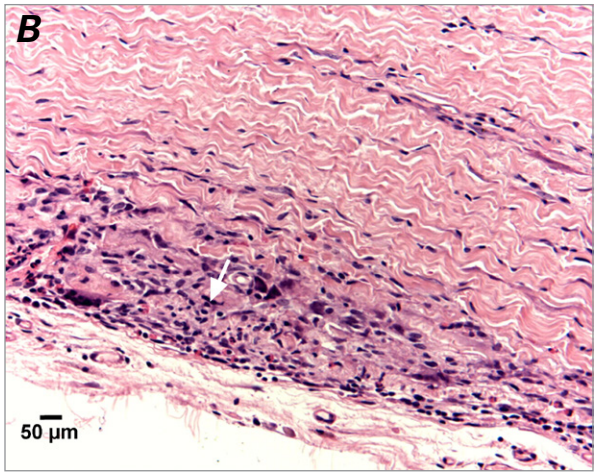


Fig. 5 *Photomicrographs show differences in inflammatory reaction at 4 months.* ***A****) The autologous pericardium was made of fibrous connective tissue (white arrowhead) and mesothelial cells (black arrowhead). Many adipocytes (arrow) surrounded the pericardium, and no inflammatory cells were observed.* ***B****) Many inflammatory cells, mainly lymphocytes (arrow), were found in the heterologous pericardium; the infiltration peaked at this time. Representative images from 3 independent experiments (n=7) are shown (H & E, orig. ×200).*

tion of inflammatory cells reached a peak at 4 months in the heterologous group (Fig. 5). At 6 months, the heterologous pericardium still had many inflammatory cells, and damage in local fiber structures was obvious. Larger calcified plaques accompanied by numerous inflammatory cells were found in the heterologous pericardium (Fig. 6).

Discussion

Valvuloplasty can result in central blood flow, low transvalvular pressure, and a low occurrence of embolism, with no need for lifelong anticoagulation.[8,9] An aortic valve-expanding repair technique with use of autologous pericardium was first reported by Durán and colleagues.[10] Isovaleraldehyde pretreatment might improve the anticontractive and antidegenerative properties of autologous pericardium and maintain the inherent flexibility of the tissue. Results of clinical studies have indicated that aortic valvuloplasty with use of an autologous pericardial patch can yield excellent hemodynamic results and a satisfactory quality of life. However, the long-term durability of autologous pericardial patches in aortic valvuloplasty is unknown.[11,12] Heterologous pericardium has also been applied in aortic valve repair. After 11 to 14 months, no or only trace aortic regurgitation was detected in patients whose right coronary aortic valve leaflet was replaced with bovine pericardium.[13]

In comparison with the control specimens in our study, the calcium levels in the autologous pericardial tissue increased significantly at 2 months after embed-

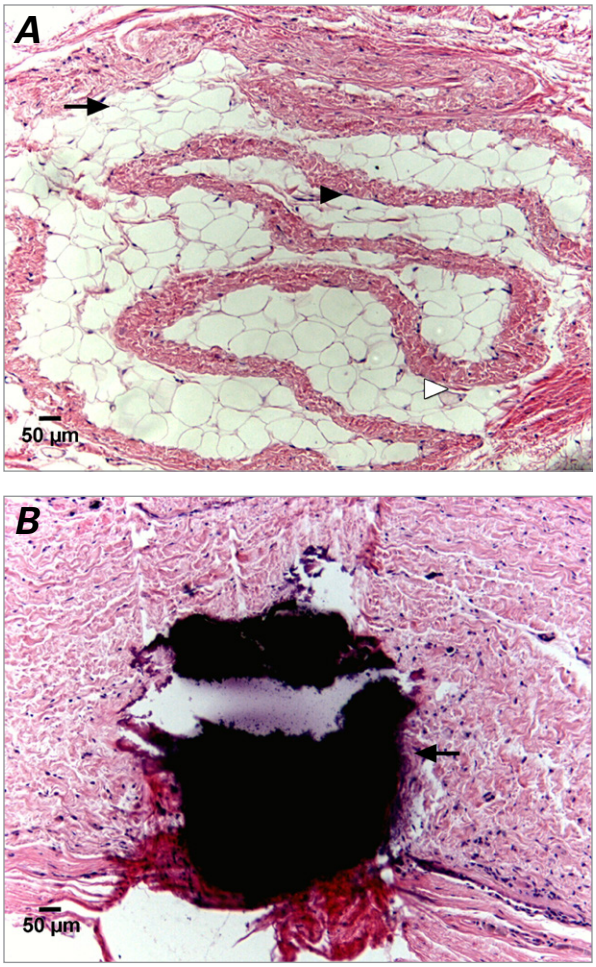


Fig. 6 *Photomicrographs show histologic changes at 6 months. **A**) No significant changes occurred in the autologous pericardium between 4 and 6 months. The autologous tissue was still composed of fibrous connective tissue (white arrowhead), mesothelial cells (black arrowhead), and adipocytes (arrow) around the pericardium; no inflammatory cells were observed. **B**) Calcified plaques (arrow) were observed in the heterologous pericardium, and fewer inflammatory cells than before were detected. Representative images from 3 independent experiments (n=7) are shown (H & E, orig. ×100).*

ding and increased only slightly thereafter. In contrast, the calcium levels in the heterologous pericardium increased significantly at 2 months, increased less rapidly from 2 to 4 months, and rose sharply at 6 months, increasing more than 30 times than the rate at 4 months. Although the autologous pericardium calcified, the process was weaker and slower; obvious calculus was seldom observed by means of light microscopy, even at 6 months. The calcium levels in the heterologous pericardium were higher than those of the autologous pericardium, especially at 6 months. This suggests that heterologous pericardium calcifies over time, and that the calcification process persists and progresses. The autologous pericardium revealed its lower calcification propensity.

The calcification of autologous or heterologous pericardium is chiefly why patients undergo reoperation after aortic valve repair with the use of pericardium.[13,14] The structural and organizational compatibility of the allograft plays an important role in the calcification process.[14-16] Investigators found that fixing pericardium in glutaraldehyde could kill cells and eliminate their bioactivity; however, the dead cells were not cleared, and they often became the starting point of calcification.[17] As calcium deposition continued and the calcific area expanded, large plaques would appear. These made the heterologous tissue stiff and brittle, and eventually caused loss of function.[18] After we stained the nonembedded heterologous pericardium with H & E, we found deeply stained nuclei. These cellular components might trigger calcium deposition and cause faster, more severe calcification. Calcification of the heterologous pericardium progressed with time. Larger calcified plaques and even ossification were apparent in the heterologous pericardium upon our histologic examination. In contrast, we observed no obvious calcification of the autologous pericardium, and the damage to its organizational structure was slighter.

Inflammation and immune response are also important in the calcification of biological material.[19-21] In our study, lymphocytes and a small number of macrophages infiltrated the heterologous pericardium, and the infiltration of inflammatory cells persisted during the embedding period. This infiltration varied with embedding time: it was obvious from 2 months to 4 months, but had diminished by 6 months. However, calcium in the heterologous pericardium increased sharply during this period and peaked at 6 months. The results indicate immune rejection of heterologous pericardium throughout the embedding period and suggest that injury and repair caused by chronic rejection might be integral in progressive degenerative calcification of biological tissue. In contrast, we observed no significant structural damage in the autologous pericardium and no substantial inflammatory-cell infiltration. Calcium in the autologous pericardium increased 2 months after embedding but reached a plateau thereafter. This indicates that the autologous pericardium was more compatible and evoked no obvious immune rejection, and that its histologic compatibility was much better.

Limitations of the Study. This study has some limitations. First, the changes in subcutaneously implanted pericardium might differ slightly from changes in pericardium that has been implanted in a valve leaflet or annulus. Second, the subcutaneous rabbit model has been established as a guide for the estimation of the calcification of biological valves; however, the medical literature provides no hint of a standard implantation method.[22] Third, the subcutaneous implantation model can repeatedly produce a pathologic morphology of calcification, the same as that seen in clinical explants and

in circulatory models.[23] However, the limitation of the subcutaneous model should not be ignored: the implanted materials are not in the circulatory system, so they do not experience the dynamic stresses imposed by the host heart and blood; furthermore, these implants are not exposed to blood-borne proteins, lipids, and cells that might affect valve calcification.[24] Our results might offer new insight in regard to the calcification process that affects implanted heterologous and autologous pericardium in vivo; however, the materials should be studied further when they are implanted in the host's circulatory system. Finally, the commercial heterologous (Balmedic) bovine pericardium was treated with glutaraldehyde. Further details about preparation and fixation were unavailable because of the company's patent-protection policy.

In our study, the compatibility and calcification resistance of autologous pericardium were superior to those of heterologous pericardium. Therefore, autologous pericardium might be the better choice for valvuloplasty.

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