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Bioprosthetic Heart Valves—Replacing Order with Chaos: Electron Microscopic Study

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Abstract: Rheumatic heart disease is a significant clinical entity in young children, especially in the developing world. One of the major long-term effects of ill managed rheumatic fever is irreversible damage to the cardiac valve leaflets, primarily on the left side. With the limited success of currently available mechanical and bioprosthetic valves, there is an urgent need for new directions in bioprosthetic valves, both in material, including source, degree of fixation, surface, bulk modifications, etc., and design. In the present paper, new proposals in the material selection and fabrication of bioprosthetic valves are proposed based on electron microscopic studies of natural valve leaflets and the pericardial surface. Current approaches for bioprosthetic valve fabrications include the wide use of the pericardium as a leaflet material. The present study indicates a need for nondestructive surface examination of pericardial sheets for the elimination of areas of surface voids resulting from gross fiber disorientation. Also, there seems to be a need for incorporation of an in situ fiber renewal mechanism in bioprosthetic leaflets to emulate the natural valve more closely. Apparently natural leaflets have built-in fiber renewal mechanism(s). **Key Words:** Natural heart valve—Rheumatic heart disease—Cardiac valve leaflet damage.

Cardiac valve leaflet damage is a central theme in ill managed cases of rheumatic fever (1,2). Being closely related to poor socioeconomic conditions, the rate of incidence is still very high, especially in the developing countries (1.8–11.0 cases/1,000 school children with an average of 6.5 in India) (3). Lesions

of rheumatic fever are disseminated widely throughout the body, especially in connective tissues, along with cardiac involvement, which constitutes the most significant consequential event with the definite need for the replacement of valves, primarily on the left side.

Currently, there are 2 types of valves available, mechanical and bioprosthetic. Of the various mechanical varieties, e.g., the Starr-Edwards silastic ball valve, Björk-Shiley valve, Medtronic-Hall disc valve, Omniscience heart valve prosthesis, and St. Jude medical hinged bileaflet prosthesis, the bileaflet variety probably holds the best long-term prospect for replacement of both aortic and mitral valves for patients below 60 years of age simply because of its modestly more favorable hemodynamic characteristics and lower thrombogenicity. Whereas in most of the mechanical varieties of valve prostheses, the reported incidences of thromboembolic complications vary between 1.5–5.8/100 patient years (percent year) depending on the duration of study, the double disk tilting variety has a reported incidence of embolization of 0.7% and 2.2%/patient year for aortic and mitral valve replacements, respectively (4–8). The incidence of anticoagulant related bleeding complications in mechanical valves ranges between 0.8 and 3.1%/patient year, depending on the type of agent used (4,5,8,9). In fact, with the use of antiplatelet agents, the observed thromboembolic rates for aortic and mitral valve replacements were much higher (6.2 and 16.7%/patient year, respectively) (10). Other complications include transient ischemic episodes, valve failure, reoperation, etc.

In contrast, bioprosthetic valves, e.g. the Hancock porcine xenograft, Carpentier-Edwards porcine valve, and Ionescu-Shiley bovine pericardial xenograft, show a relatively low incidence of thromboembolism, varying between 0.7 and 1.9%/patient year, depending on the type of valve (aortic or mitral) and the model (11–13). Other complications include anticoagulant related hemorrhage (0.3–0.5%/patient year) and primary tissue failure (0.2–1.1%/patient year) (14,15). By 10 years, one-third of the bioprosthetic valves require replacement, and by 15 years more than two-thirds need it (16).

Overall, whereas mechanical valves cause much of the blood cellular damage leading to coagulation and bleeding processes in addition to causing stress induced damages in the aortic walls at their roots, bioprosthetic valves, although in demand, do not meet the requirements for failing histories, with morbidity in the form of stiffening of leaflets leading ultimately to leaflet fracture and calcification which in turn constitute a vicious cycle leading to valve failure.

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In summary, there is a critical need for a new generation of substitute heart valves (SHV) of the bioprosthesis variety in which new materials, new designs, and new cellular and molecular biological approaches will be needed to make a significant impact on the clinical problems that occur with the currently available valves which, in effect, replace one disease with another (17).

While designing the closer to natural bioprosthesis valve, the major points of consideration at present are that the transvalvular pressure gradient should be minimum, the regurgitant flow percentage (PCR) be minimum, and the central parabolic flow characteristics, apart from the question of the biocompatibility of the material in use. In terms of materials, the current considerations include the following. First, how to increase the durability of the material (used for fabrication of valve leaflet) is a consideration. The present problem of bioprosthesis variety is one of limited durability. This is currently being addressed by the processes of chemical crosslinking with various agents, e.g., formaldehyde and glycer-aldehyde (18), carbodiimides (19), polyethylene glycol (20), etc., under different treatment conditions to enhance the mechanical properties of the collagen sheet, either from the pericardium or duramater. These efforts have met with limited success as is evident from the low cyclic loading durability of the fabricated valve leaflets even from treated pericardium. A second consideration is how to minimize the cyclic events of dystrophic calcification and leaflet fracture, the mechanisms of which are not evident at present.

In the present study, the objectives were to examine through scanning electron microscopy (SEM) the normal leaflet surfaces, both left and right sided (mitral and tricuspid), and to examine the pericardial surface, which is widely used in fabricating bioprosthesis valves. This was done with the following few questions in mind. Why does calcification not take place significantly (except in post-bacterial endocarditis) in the normal valve leaflets compared to the fixed bioprosthesis valves? What is the incidence of surface damage (tear, crack, abrasions, etc.) in normal valve leaflets? Is there any obvious fiber renewal mechanism involved in valve leaflet repair? What is the disposition of the collagen bundle/sheet in the normal pericardium, vis-a-vis the right and left sided valve leaflets?

Methods

A whole goat heart was collected immediately on sacrifice and the tissues dissected. Isolated tissues were fixed overnight at room temperature in

5% formaldehyde and subsequently prepared and mounted for SEM (Jeol, JSM-5400, Japan) at various magnifications (Fig. 1a-e).

Observations

The following 5 structural features were observed. First, valve leaflets, both left and right sided, are composed of bundles of parallel collagen fibers. There is a definite periodicity of the bundles. Second, left sided leaflet bundles (aortic) are much thicker than the tricuspid leaflet bundles. This could be due to the extra pressure effect experienced by the systemic side (left) of the heart as is reflected in the thickness of the ventricular wall musculature. Third, crosslinking between the bundles may follow an order as well. Fourth, a 200 μm thick aortic valve leaflet has around 15-20 definitely stacked collagen bundles, which are very well organized without any area of surface void. Conversely, areas of surface voids on the pericardial surface may act as stress concentrators leading to cracks when loaded cyclically even after extensive physicochemical fixation in bioprosthesis valves and subsequently act as foci of calcification or vice versa. In contrast, the surfaces of valve leaflets (aortic) show regular smooth features with much protein adhesion. Finally, resident fibroblast cells are evident buried inside the layers as well as on the surface. This probably hints at a continuous natural renewal mechanism whereby new fibers are laid in response to stress induced damage (similar to dermal renewal of the epidermis).

Conclusions and suggestions

Based on the observations, the following 2 suggestions are put forward. First, between the 2 varieties, bioprosthesis heart valves probably have better long-term prospects. One of the current approaches for the fabrication of bioprosthesis valves involves pericardial collagen membrane, primarily after different physicochemical treatments. These treatments incorporated in the fabrication protocol are to improve the in vivo life span of the bioprosthesis valves as well as to minimize/eliminate the inherent cyclic problems of calcification, enhanced rigidity, load induced leaflet fracture, and further calcification. Extensive study has been completed, as mentioned, with limited success. The problems of in situ calcification and fracture still remain a major block. In the present study, the electron microscopic approach was adopted to understand the surface textures of normal valve leaflets vis-a-vis the pericardial surface which is taken widely as the starting material for fabrication of these leaflets.

The random selection of pericardium, as is probably the practice at present, may not be appropriate.

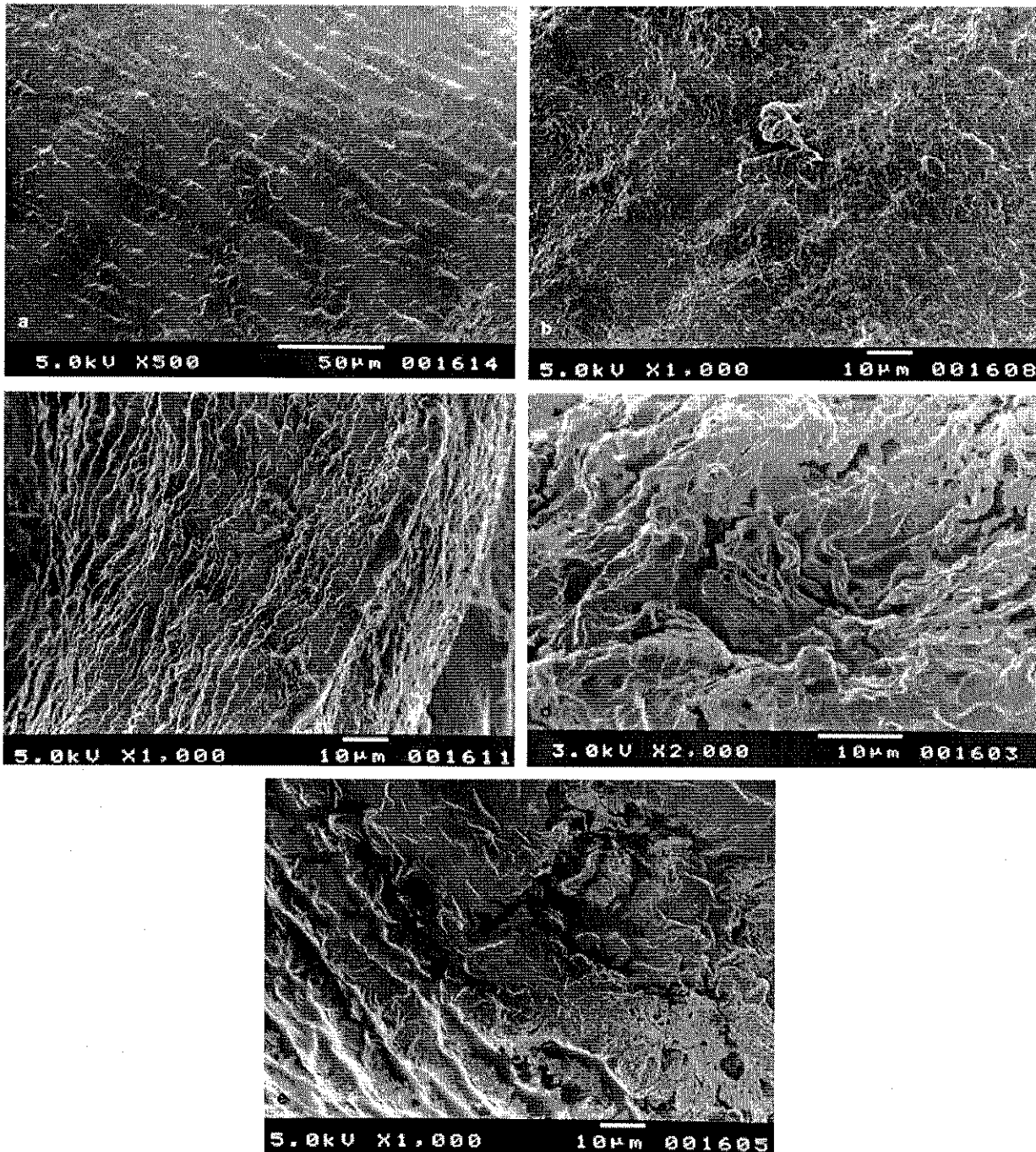


FIG. 1. The scanning electron micrographs are of the tricuspid (a), aortic valve (b) and (c), and pericardium (d) and (e) at various magnifications. Note the collagen bundle periodicity (a), which is obviously of a different size than the aortic valve collagen bundle, probably because of the difference in the pressure gradient between the 2 ventricles. Also note the resident cells attached to the leaflet surface (b). The orderly stacking of about 15–20 layers of collagen in the aortic valve leaflet section is obvious (aortic valve edge [c]). Interestingly, the pericardium, although consisting of stacked collagen layers, shows areas of surface failure (d) and (e).

The maturation of fibers and orientation and degree of crosslinking may have to be taken into consideration more carefully to eliminate the areas of surface failures. More precisely, a noninvasive and nondestructive protocol of surface examination needs to be developed for detecting these areas of fiber disori-

entation and chaos. Chemical crosslinking probably does not improve the areas of fault, and calcium deposits have been described in bioprosthetic valves in association with sites of collagen disorganization and/or disintegration (21,22). The calcium deposit patterns, both in terms of time and disposition along