

SCVP Morning Session Companion Meeting  
Sunday, February 12<sup>th</sup> 2006  
Atlanta, Georgia

**Title: Infectious & Inflammatory Diseases of Native and Prosthetic Heart valves and Devices**

**Co-Chairs: Dr. Bruce McManus**, University of British Columbia and  
**Dr. Jagdish Butany**, University of Toronto

8:30 am **Dr. John Veinot**, University of Ottawa Heart Institute:  
“Pathology of Inflammatory Native Valvular Heart Disease”

9:00 am **Dr. Gaetano Thiene**, University of Padua Medical School:  
“Pathology and Pathogenesis of Infective Endocarditis in Native Heart Valves

9:30 am **Dr. Jagdish Butany**, University of Toronto, Toronto General Hospital: “Pathology of Infectious and Inflammatory Diseases of Prosthetic Heart Valves”

10:00 – 10:30 am Coffee Break

10:30 am **Dr. Robert Padera**, Brigham and Women’s Hospital, Harvard Medical School: “Pathology of Ventricular Assist Devices and the Role of Biofilms”

11:00 am **Dr. Frederick J. Schoen** (Awardee), Brigham and Women’s Hospital, Harvard Medical School: “New Frontiers in the Pathology and Therapy of Heart Valve Disease”

# PATHOLOGY OF INFLAMMATORY NATIVE VALVULAR HEART DISEASE

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Before considering some common causes of post inflammatory valve disease it is useful to review general concepts of valve related dysfunction and common causes of valve pathology. Native cardiac valves may be involved by dysfunctional states – stenosis and regurgitation – or by vegetations – infective or non-infective. Inflammatory non - thrombotic disorders will be the focus of the presentation.

## **Valves that are surgically excised are studied for a number of reasons:**

1. To document the surgical indication;
2. To correlate pathology with pre-operative diagnosis, hemodynamics, ECHO, angiography and suspected complications;
3. To document infective endocarditis or rule this out;
4. To determine the etiology of the valvular lesion – its natural history, surgical risk, post-operative prognosis and association with systemic disease;
5. To validate new diagnostic imaging techniques;
6. To assess operative technique and whether it could be done on a similar patient;
7. To determine the etiology and pathobiology of the valvular disease.

Dysfunction of a cardiac valve results from either structural abnormalities of the valve or abnormal function of a valve. Valves that are **stenotic** almost always have some anatomic abnormality - usually fibrosis or calcification. In contrast purely **regurgitant** valves do not always have anatomic abnormalities present with the excised specimen. In regurgitation the abnormality may be related to the valve or to the surrounding supporting structures. Stenotic lesions usually take years to develop, whereas regurgitant lesions may be chronic or acute in nature.

## **AORTIC STENOSIS**

Stenosis of the aortic valve is usually due to pathology of the valve cusps, commonly fibrosis, calcification and commissural fusion. Due to the aging population, age-related degenerative aortic valve changes will have important implications for future health care costs. The most common causes of aortic valve stenosis include:

- 1) age related degenerative (senile) changes
- 2) post-inflammatory changes – mostly rheumatic
- 3) congenitally bicuspid valve

## **AORTIC INSUFFICIENCY**

The most common mechanisms causing valve regurgitation are annular dilatation, cusp prolapse, scar retraction of the cusps, and cusp perforation. Aortic regurgitation may be due to diseases of the aortic valve cusps or the aortic root. Diseases that produce annular dilatation include cystic medial necrosis (medial degenerative changes), forms of congenital heart disease, and aortitis. Medial degenerative changes may be an age-related change, or may be related to connective tissue diseases including Marfan's syndrome. The most common causes of aortic regurgitation include:

- 1) **cuspid pathology**
  - a) post-inflammatory changes – mostly rheumatic
  - b) infective endocarditis
  - c) congenitally bicuspid valve
  - d) iatrogenic causes such as valvuloplasty
  - e) cusp prolapse – VSD related
  - f) medications – anorexogenic medications
- 2) **aortic root pathology**
  - a) age related medial degeneration
  - b) Marfan's syndrome or other connective tissue diseases
  - c) Aortic dissection
  - d) Aortitis, such as giant cell aortitis and syphilis

## **MITRAL STENOSIS**

Mitral stenosis is usually due to leaflet fibrosis and calcification. Almost all cases are post-inflammatory and rheumatic in etiology. Stenosis may be associated with valve regurgitation. The mechanism of stenosis is due to leaflet fibrosis, calcification, commissural fusion, chordal fusion and shortening. The subvalvular apparatus pathology can be assessed by echocardiography and the results used to determine whether valve repair is possible or whether replacement will be necessary. Other rare causes include storage diseases and medication related pathology including some ergot and migraine medications.

## **MITRAL INSUFFICIENCY**

The mitral valve apparatus is a complicated structure with numerous components, all of which must function to ensure valve competence. Leaflet pathology is but one component. The important components and their pathology include:

- 1) **leaflets**
  - a) perforation - infective endocarditis
  - b) scar retraction - post-inflammatory causes – rheumatic
  - c) medications – anorexogenic medications
  - d) floppy mitral valve – myxomatous degeneration
- 2) **chordae**
  - a) elongation – floppy valve
  - b) rupture – floppy valve or infective endocarditis
- 3) **mitral annulus**
  - a) left ventricular dilatation related, numerous causes
  - b) mitral annular calcification (MAC)
- 4) **left ventricle - dilatation**
  - a) ischemia
  - b) cardiomyopathy
- 5) **papillary muscles**
  - a) ischemia related dysfunction (stunned myocardium)
  - b) infarct related necrosis or fibrosis
  - c) infarct related rupture of papillary muscle

## INFLAMMATORY VALVE DISEASES

Rheumatic valve disease, a chronic result of rheumatic fever, is the valve disease most think of when one refers to “post-inflammatory” valve disease. This may be short sighted, as will be discussed. Many disorders that we considered to be passive and degenerative are now recognized as being active and inflammation related. This is exciting as it raises the possibility of active intervention and prevention. This handout discusses rheumatic, serotonin associated (carcinoid, migraine, anorexogenic), senile degenerative, dialysis and floppy valves. Other inflammatory valve diseases including those associated with collagen vascular disease or vasculitis are not covered.

## RHEUMATIC FEVER AND VALVE DISEASE

Rheumatic fever is a late inflammatory non-suppurative complication of pharyngitis caused by Group A beta-hemolytic Streptococcus. This multi-system disease is characterized by involvement of the heart, joints, central nervous system, subcutaneous tissues and skin.<sup>1</sup> Except for the heart, most of these organs are only mildly and transiently affected. Clinical diagnosis with the Jones criteria is recommended. No symptom, sign or laboratory test is pathognomic of the disease. The Jones criteria, proposed in the 1940s, have stood the test of time and are intermittently revised. Major and minor categories of clinical and laboratory findings may fulfill the criteria for diagnosis.<sup>2</sup> Most pathologists have seen chronic rheumatic valve disease. Many of us have not recognized an acute case, and this is also true for our clinical colleagues.

Rheumatic carditis is an important and frequent acquired cardiovascular disease in children and adolescents and an important cause of death from cardiac disease in young people in developing countries. In India there are an estimated one million new cases of rheumatic fever each year. It has been estimated that rheumatic fever related disease is responsible for 30 to 40 % of cardiovascular disease related hospital admissions in that country, and is a common indication for cardiac surgery in that country.<sup>3</sup> The pathoetiology of the disease is complex and the incidence and prevalence vary among countries. Environmental conditions may play a factor with some climates having an increased frequency of rheumatic fever. In addition, low socioeconomic status, malnutrition, poor hygiene, and poor access to health care have all been associated with increased prevalence and incidence.<sup>1,4,5</sup> Rheumatic fever is most frequently observed in children and adolescents, rare before age 5 and after age 25. The highest incidence is observed in children age 5 to 15.<sup>6</sup>

Genetic studies suggest that there is a vulnerable population with increased risk. Related family members of patients with rheumatic fever have a higher probability of developing disease. Relationships between the development of rheumatic fever and HLA-DR subtypes have been found. These associations are variable between countries and populations.<sup>1,7,8</sup>

Important antigenic structures of the Streptococcus include proteins M, R and T.<sup>1</sup> Streptococcal M-protein, which determines the serotype, extends from the cell surface as an alpha-helix with structural homology to myosin and other alpha-helix coiled molecules.<sup>9</sup> The M-protein is a virulence factor with potent anti-phagocytic activity.<sup>6</sup> In outbreaks, bacterial colonies isolated from those with rheumatic fever tend to have a mucoid morphology with thick capsules and certain M-proteins are more common.<sup>5,10</sup>

The pathogenesis of rheumatic fever is related to humoral and cellular mediated immune responses with development of autoimmunity.<sup>7</sup> The clinical manifestations of rheumatic fever occur 1 to 3 weeks after the onset of Streptococcal infection. After an apparent convalescence of the pharyngitis, products of the Streptococcus have **molecular mimicry** to human tissue and are recognized by the immune system thus initiating an autoimmune response. Cross-reactivity between the M-protein and cardiac proteins is important.<sup>9</sup>

The acute involvement of the heart in rheumatic fever is pancarditis with inflammation of the myocardium, pericardium and endocardium. Carditis is the most severe clinical manifestation of rheumatic fever and can lead to valvular heart disease, heart failure or death. Carditis occurs in approximately 40 to 50 % of patients at the first attack.<sup>6</sup> Pericarditis occurs in 5 to 10 % of patients and is characterized by chest pain, decreased heart sounds and a pericardial rub.<sup>6</sup> Rarely there may be tamponade. Pericarditis rarely occurs as a sole manifestation and if encountered alone, other causes should be suspected. Myocarditis occurs in 10 % of patients and may present with heart failure, arrhythmias, pulmonary edema and cardiomegaly. Isolated myocarditis is also rare.<sup>6</sup>

Endocarditis and acute valve disease may be asymptomatic or present with a new murmur. In the acute phase, murmurs do not indicate a permanent valve defect and are mostly transient. The valves most affected are the mitral, aortic, tricuspid and pulmonary, in that order. Acutely, there is development of thrombi along the lines of closure of the valve (atrial side of the atrioventricular valves and the ventricular side of the semilunar valves). These small thrombi have been termed "verrucous" endocarditis and do not produce valve destruction. The leaflets may have associated edema and cellular infiltration of the leaflets. CD4 and CD8 T cell subsets are present within acute rheumatic fever valves and the major histocompatibility complex class 2 antigens are expressed on vessel endothelium and valve fibroblasts.<sup>6,11</sup>

The pathogenesis of valve disease involves humoral and cell mediated immunity and molecular mimicry.<sup>7</sup> Individuals develop antibodies to the carbohydrate of the Streptococcal organism and antibodies to the M-protein. The anti-carbohydrate antibodies cross react with the valvular endothelium. This produces valve injury or dysfunction with up regulation of cell adhesion molecules including VCAM. This serves as an infiltration site for activated lymphocytes into the valve. The M-protein antibodies contribute to the valve disease via molecular mimicry with myosin. Cardiac myosin is not present in the valve, but it is suggested that laminin links myosin with the valve. The cytotoxic anti-myosin antibody recognizes laminin, an extracellular matrix alpha helix coiled protein, part of the valve basement membrane structure.<sup>7,9</sup>

T-cells responsive to the Streptococcal M-protein infiltrate the valve through the valvular endothelium activated by the binding of anti-Streptococcal carbohydrate antibodies cross reactive to the endothelium. Within the valve tissue the inflammatory cells are responsible for local cytokine release, and interstitial cell damage with neovascularization and chronic inflammation.<sup>7</sup> Local production of tumor necrosis factor is thought to have an important role. Destruction of the valve tissue may expose more antigens and thus the process may be progressive.<sup>4</sup> Patients with rheumatic fever also have increased serum cytokines, including interleukin 6, interleukin 8 and tumour necrosis factor, and increased CD4 and CD8 lymphocytes in the peripheral blood.<sup>12</sup>

The inflammatory component of the valve disease results from the infiltration of valves with T-cells and macrophages leading to scarring of the valve and alteration in cardiac function. Within the valve, the T-cells produce cytokines including tumour necrosis factor and interleukins. Macrophages are activated and attract T cells.<sup>13</sup>

In support of this mechanism, structural and immunological mimicry between the Streptococcal M-protein and cardiac myosin has been shown in the Lewis rat model.<sup>9</sup> T cells isolated directly from these cardiac valves react with Streptococcal M-protein peptides.<sup>9</sup> In addition, T-cells have been cloned from human heart valves and have been found to recognize M-protein peptides and heart tissue derived proteins.<sup>8,9,13</sup>

The chronically scarred, inflamed and neovascularized valve is most commonly encountered by the pathologist. Chronically, rheumatic fever leads to neovascularization, chronic inflammation, commissural fusion, valve thickening and calcification. Scarring, important in the progression of valvular disease, is accompanied by neovascularization of the otherwise avascular valve. For initial entry into the valve, transendothelial migration of the lymphocytes through the endothelial surface plays an important role. Once the valve is inflamed and there is neovascularization, lymphocytes can infiltrate the valve both through the valve surface as well as

through the neovascularization channels. Even in old calcified rheumatic valves, lymphocytes and neovascularization are still present, indicating progression or persistence of disease in the valve.<sup>11</sup>

By gross examination these rheumatic valves have fibrosis, with or without calcification. The commissures are often fused. Valve tissues may be thickened and show scar retraction. The chordae are often thick and shortened. The sub-valvular chordal space may seem to disappear with short thick chords attached almost directly to the papillary muscles. At the commissures of mitral valves there is often loss of surface endothelium and erosion with overlying thrombus material. This does not seem to be as common in the aortic position. Sections show neovascularization, chronic inflammation and fibrosis with alteration of the underlying valve architecture. Large fibrous endocardial onlays are present.

Other systemic manifestations of rheumatic fever are more self limited. Arthritis is frequent and least specific manifestations of rheumatic fever. The arthritis is transient, migratory and self-limiting, usually involving large joints.<sup>1,6</sup> Sydenham's chorea (Saint Vitus dance) occurs in approximately 10 % of patients with rheumatic fever. It presents with hypo and hyperkinetic manifestations with brief, random and voluntary non-rhythmic movements, dysarthria and speech abnormalities. Emotional changes are also noted. This may be a late manifestation of rheumatic fever and is usually self-limited.<sup>6</sup> Other features of rheumatic fever include erythema marginatum and subcutaneous nodules.<sup>1</sup>

## **CARCINOID VALVE DISEASE AND SEROTONIN - RELATED VALVE DISEASE**

Serotonin related valve disorders include carcinoid valve disease and disease associated with serotonin agonists such as migraine and diet medications. The valvulopathy associated with these agents is hyperplastic in nature with hyperplastic and endocardial lesions.<sup>14</sup>

An injured valve may respond by the accumulation of extracellular matrix, increased valve interstitial cells, chronic inflammation and calcification. The myofibroblast form of the interstitial cell is a major participant in valve repair. This cell expresses alpha smooth muscle actin and can proliferate, migrate, make and remodel matrix.<sup>15,16</sup> Many adult valve diseases have in common proliferation and accumulation of myofibroblasts and interstitial cells.<sup>15-17</sup>

Carcinoid syndrome and the resultant valve disease are thought to relate to increased serum levels of serotonin. Serotonin has been found to induce TGF beta expression. TGF beta can induce valvular endothelial cells and interstitial cells to trans-differentiate into myofibroblasts.<sup>17,18</sup> Serotonin up-regulates TGF beta expression and increases erk signaling via MAP kinase.<sup>19</sup>

The pathogenesis of carcinoid valve disease is thought to involve valve serotonin receptors (5HT-2A and 5HT-2B receptors).<sup>14,19,20</sup> The addition of serotonin to cultured valve interstitial cells increases TGF beta expression and increased extracellular matrix probably through this serotonin receptor mechanism.<sup>17,19</sup> Addition of serotonin to sheep aortic valve interstitial cells increases TGF beta mRNA and TGF beta activity with an increase in collagen synthesis.<sup>14</sup> Excised carcinoid valves show increased amounts of latent TGF beta associated peptide and latent TGF beta binding protein present in the interstitial cells and extracellular matrix.<sup>14</sup>

Carcinoid heart disease is seen in approximately 50 % of patients with carcinoid syndrome.<sup>21</sup> The affected valves commonly are the pulmonary and the tricuspid valves. Left-sided disease may occur rarely if there is a patent foramen ovale. The plaques involve the cusps of the semilunar valves and the leaflets, chords and papillary muscles of the atrioventricular valves. Carcinoid heart disease usually produces regurgitant valves, most commonly the pulmonary valve, with mixed pulmonary stenosis and pulmonary insufficiency. The endocardial plaques cause valve thickening and retraction leading to regurgitation and stenosis.

The valve thickening is due to cellular proliferation of myofibroblast like cells and accumulation of extracellular matrix in the endocardial onlay plaque lesions. These onlays or plaques tend to occur on the arterial surface of the pulmonary valve and both surfaces of the tricuspid valve, but predominantly the ventricular side. Carcinoid plaques do not destroy the

underlying valve architecture. The matrix is rich in collagen and ground substances and some studies have found small amounts of elastin. In a study of surgically excised carcinoid valves from the Mayo Clinic, 94 % had neovascularization, 94 % had chronic inflammation including lymphocytes, plasma cells and macrophages and 64 % of valves had mast cell inflammation associated with neovascularization.<sup>21</sup> Prior autopsy studies also noted a high degree of neovascularization and the presence of chronic inflammation. An autopsy study of 18 patients with carcinoid syndrome by Thorson found 94 % valves were neovascularized, and 67 % had inflammation.<sup>22</sup> Mast cells are variable in numbers in the reported studies, some finding abundant cells, others none. Mast cells tend to be in areas of neovascularization and they may also be in adjacent valve tissue and not actually in the endocardial plaque.

## **ANOREXOGENIC MEDICATIONS AND OTHER DRUGS**

Medications, including anorexogenic drugs, and ergotamine and methysergide (migraine medications) have been found to produce carcinoid like valve disease. The mechanism is thought to be via activation or agonist activity of 5HT-2B receptors. Medications have been screened for activity at serotonin receptor subtypes by ligand binding studies and functional assays.<sup>23</sup> Fenfluramine metabolites, ergotamine, and methylsergide have high affinity for these receptors and these drugs have been associated with serotonin related valve disease.

Ergot alkaloid drugs include methysergide and ergotamine, both used for treatment of migraine headaches. Ergotamine associated valve disease chiefly affects the mitral valve and produces a carcinoid like gross appearance that may be severe. Mitral stenosis and regurgitation have been seen. Valve leaflets are typically very thick with chordal fusion and shortening and commissural fusion. Large “myxoid collagenous” myofibroblast rich plaques are stuck on the underlying valve proper without underlying valve leaflet destruction.<sup>24,25</sup> Methysergide, a migraine medication, has also had morphologically similar mitral valve disease described, chiefly causing mitral regurgitation. This medication has been associated with retroperitoneal and pleuropulmonary fibrosis. With these drugs inflammatory cell infiltration has not been reported to be prominent.<sup>24,25</sup>

The anorectic diet medications have previously been used as monotherapy for short duration therapy for many years. The combination of fenfluramine and phentermine (Fen-Phen) was introduced in North America in 1996. Over a million prescriptions were written, and not all were for severe obesity. No information concerning effectiveness or chronic effects was known.<sup>26</sup> Connolly et al, from the Mayo Clinic, reported heart valve shortly thereafter in 1997.<sup>27</sup> There is still some debate as to the risk and incidence of the valvulopathy associated with anorexogenic agents, but it is probably low.<sup>28</sup> Susceptibility or risk of valvulopathy may depend upon the dose, the duration of treatment and individual risk factors including the presence of pre-existing valve disease and concomitant medication use.<sup>29</sup>

Grossly and microscopically the valve disease or valvulopathy associated with anorexogenic drugs has been reported to be similar morphologically to that of carcinoid valve disease.<sup>27,30</sup> The left sided valves are affected more often with aortic insufficiency being the most common clinical manifestation.<sup>27</sup> White plaques are noted grossly. There may be chordal encasement and fusion, but not rupture. The commissures are not fused, in contrast to rheumatic disease. Doming or hooding of the mitral leaflets is not seen, in contrast to floppy valve disease. By microscopic examination, the valves have myofibroblast and glycosaminoglycan rich onlay endocardial lesions with preservation of the underlying valve architecture. These onlay lesions are “neo-tissue” of glycosaminoglycan, collagen, and myofibroblasts that are superficial to the valve elastic membrane but deep to the surface endothelium. The “downstream” side of the valve (ventricular side of mitral valve and the aortic side of the aortic valve) are most commonly involved. The valve proper may have myxoid degeneration with accumulation of glycosaminoglycans. The onlays also may contain chronic inflammatory cells (CD3 positive lymphocytes and CD68 positive

macrophages) and there is neovascularization within the onlay lesions and in the valve proper.<sup>31</sup> Mast cells have also been noted.<sup>30</sup>

A careful and detailed digital imaging assisted study of geometry and composition reported interesting findings to aid in the distinction of anorexogenic disease from floppy mitral valves, rheumatic and carcinoid disease.<sup>32</sup> The size and number of the onlay lesions, the amount of glycosaminoglycans in the onlay lesions and the valve proper, as well as the location of the inflammation and neovascularization are important in this distinction.

Carcinoid valves have large onlays with preservation of the underlying valve proper architecture. The valve proper and the onlays are glycosaminoglycan rich and there may be chronic inflammation throughout the valve. Floppy mitral valves have medium numbers of onlays, but these have no significant neovascularization. The valve proper also usually has little neovascularization but there is accumulation of glycosaminoglycan in the spongiosa layer. Rheumatic valves have large fibrous onlays that are few in number. Vascularization of the valve proper is prominent. The valve proper and the onlays show fibrosis.<sup>32</sup> Anorexogenic valves had the most variable findings perhaps reflecting variability in dose, duration of exposure, and individual susceptibility. These valves have the largest amount of glycosaminoglycan of all the valve groups. The onlays were small, numerous and glycosaminoglycan rich. Chronic inflammation may be present. Interestingly, both the valve proper and the onlays are neovascularized. There appears to be some similarities between the valve groups, but there are significant morphological trends and differences to assist in the separation of the categories.<sup>32</sup>

There remains much unknown and poorly understood about these disorders. Why fenfluramine produces aortic regurgitation, whereas ergotamine and methylsergide produce mitral valve disease is unknown. Why methylsergide associated valve disease is more symptomatic is unknown. Why methylsergide can produce extracardiac fibrosis with pleural fibrosis and pericarditis in addition to valvular disease is unknown.<sup>23</sup>

Similar carcinoid like valve disease has also been noted recently with pergolide.<sup>33,34</sup> This is an ergot derived dopamine receptor agonist used for Parkinson's disease. Pericardial, retroperitoneal and pleural fibrosis have been reported. Valve disease in these patients has been reported as being fibroproliferative with preserved underlying valve architecture. Tricuspid and mitral valve regurgitation have been reported. The valve disease was described in 2002, even though the drug has been marketed for 20 years.<sup>33,34</sup> Pergolide may activate serotonin 5HT-2B receptors. It also influences potassium channels in pulmonary artery smooth muscle cells causing vasoconstriction. Thus monitoring of individuals taking this drug for pulmonary arterial hypertension, similar to the anorexic drugs, has been suggested.<sup>34</sup>

The saga of serotonin related valve disease continues. "Ecstasy", MDMA (3,4-methylenedioxymethamphetamine), an illicit street drug, has been found to induce proliferative actions of cardiac valve interstitial cells, again via 5HT-2B receptors.<sup>35</sup>

## **DEGENERATIVE AORTIC VALVE DISEASE – AN OLD PROBLEM REVISITED**

Age related (senile) degenerative change of aortic valves is the most common cause of adult aortic valve stenosis encountered in North America. With an aging population this will not likely change, and the disease will be commonly encountered at autopsy or as a surgical specimen by many pathologists.

Traditionally the valve calcification process was thought to be passive in nature representing dystrophic calcification of degenerative material in the valve. Wear and tear was postulated. Increasingly, this theory has been shown to be incomplete. An early event appears to be endothelial dysfunction from wear and tear, flow and low shear stresses. After this, numerous active mechanisms ensue including lipid accumulation, inflammation, alteration of cytokines, growth factors and valve matrix metalloproteinases.<sup>36</sup>

The process of valve calcification in prostheses and native valves is an active process with much in common with atherosclerosis and bone formation.<sup>37-39</sup> Progression of aortic valvular disease in patients from the general population has been associated with many of the traditional risk factors for atherosclerotic disease including systemic arterial hypertension, hyperlipidemia and diabetes mellitus.<sup>40-42</sup>

The process of vascular and valvular calcification is a highly coordinated and active with a mechanism similar to physiologic bone formation.<sup>37,39</sup> This process involves the expression of non-collagenous matrix proteins such as osteopontin, osteonectin, osteocalcin, bone sialoprotein and bone morphogenic protein. The expression of these collagenous and non-collagenous matrix proteins has been demonstrated in native cardiac valves and arteries.<sup>37,39,43,44</sup> Calcification of bone is a tightly regulated process that starts with osteoblasts producing a matrix of predominately type 1 collagen. The collagen is subsequently mineralized by hydroxyapatite, which is thought to initially form within the osteoblasts and move to the matrix via vesicles. The question remains as to how bone cells gain entry into the endothelium and cardiac valves. The myofibroblasts of the valve have been shown to trans-differentiate into osteoblast like cells.<sup>36</sup>

If one histologically sections calcified valves, rather than only examining them grossly, the calcified valves may have a variable degree of inflammatory infiltrate including macrophages, plasma cells, and lymphocytes.<sup>39,43</sup> These cells are capable of synthesizing osteopontin, which may act to hold surrounding cells to the calcified deposits.<sup>45</sup> Osteopontin has effects on chemotaxis, cellular proliferation, inflammation and mineralization. It is strongly associated with macrophages and has been reported to influence macrophage invasion, migration and phagocytosis.<sup>46</sup> The accumulation of T cells within the cardiac valves is a likely source of cytokines important for the recruitment of more inflammatory cells.<sup>47</sup> Expression of cytokines such as tumor necrosis factor (TNF) alpha has been noted in stenotic valves.<sup>47</sup> Metalloproteinases are also altered in the valve matrix.<sup>48</sup> The inflammatory cells may activate growth factors such as TNF, that in turn stimulate the valve myofibroblasts to proliferate and express matrix metalloproteinases which remodel the valve matrix.<sup>47</sup> Mast cells may influence matrix metalloproteinases, release proangiogenic peptides and activate cytokines and growth factors.<sup>39</sup> Lymphocytes are found in congenitally bicuspid aortic valves, as well as in degenerative tricuspid valves.<sup>38</sup>

Diseased valves may progressively accumulate lipid and some have postulated a common link between degenerative valve disease and atherosclerosis.<sup>40</sup> Stenotic aortic valves have a larger amount of lipid compared to non-stenotic valves.<sup>49</sup> The lipid may oxidize and attract inflammatory cells. These cells may release cytokines and contribute to neovascularization and the bone morphogenic protein mechanism.

With coronary atherosclerosis, lipid lowering therapy with statin medications (3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors) have effects thought to stabilize arterial plaques. These include reduction in the proteolytic activity of macrophages, increased plaque collagen content, reduction of the size of the lipid core, reduction in the degree of inflammation, restoration of endothelial function, and a decrease in tissue factor expression thus decreasing propensity for thrombosis.<sup>50,51</sup> Repression of Major Histocompatibility Complex Class II expression, blockage of LFA-1 (leukocyte function antigen -1) / ICAM-1 (intercellular adhesion molecule 1) interaction and inhibition of CD40-CD40L signaling are all molecular mechanisms that have been described to explain the pleiotropic effects of the statins.<sup>52</sup> In clinical trials designed for lipid lowering, one of the unexpected observations was the occurrence of fewer coronary events in the treated patients.<sup>49,53-55</sup> Statins may cause slower progression or regression of atheroma.

Animal studies have demonstrated that hypercholesterolemic rabbits develop valve disease similar to human aortic valve disease.<sup>46</sup> Treatment with statin medications was found to decrease the extent of the lipid deposits in the animal valves.<sup>56,57</sup> One could speculate that statins might be useful for prevention and treatment of valvular heart disease. Clinical trials are underway and those reported so far are mostly positive, but others have conflicting results.<sup>58-62</sup>

## **VALVE DISEASE IN END - STAGE RENAL DISEASE PATIENTS**

There is an increased incidence of native valve calcification and stenosis in patients with end - stage renal disease (ESRD) as compared to the general population.<sup>63,64</sup> Native valve calcification/stenosis seems to progress more rapidly in renal failure patients.<sup>65,66</sup> The presence of multiple risk factors for atherosclerosis may explain the more rapid progression of aortic stenosis; however, the uremic environment, hyperphosphatemia, hypercalcemia and a high calcium phosphate product have also been implicated in the increased risk of cardiac and vascular calcification.<sup>63,64,67</sup>

In a recent study to determine if valvular calcification in patients with ESRD is similar to patients without ESRD, we examined surgically excised native cardiac valves of hemodialysis patients and while under blind, valves were compared pathologically to age, gender and valve type controls without renal failure. We found inflammation, neovascularization and bone morphogenic proteins in degenerative valves from both the normal and the dialysis populations. The demonstration of both osteopontin and bone morphogenic protein in the valves of patients with ESRD and from the general population supports the notion that native valve calcification is an active process analogous to bone formation. Interestingly, when the results were un-blinded, the patients with dialysis seemed to have more neovascularization and chronic inflammation as compared to the non-dialysis patients.<sup>68</sup> Enhanced inflammation in patients with ESRD is consistent with the marked elevation of acute phase reactants in patients with ESRD compared to the general population. The elevation of a reactant such as C-reactive protein (CRP) has been associated with vascular calcification scores and with an increased risk of death from all cause and cardiovascular specific mortality.<sup>69,70</sup>

## **MITRAL VALVE PROLAPSE – DEGENERATIVE, INFLAMMATORY OR BOTH ?**

Mitral valve prolapse may be seen with myxomatous valve degeneration as a degenerative age related change or in association with syndromes such as Marfans, Ehlers Danlos or osteogenesis imperfecta individuals. These are the classic large redundant, thickened “floppy” valves with endocardial fibrous thickening and accumulation of ground substances, glycosaminoglycans, in the valve spongiosa layer. It should be recognized that this is not the only cause of mitral valve prolapse. Mitral prolapse may also be seen with Turners syndrome, hypertrophic cardiomyopathy, atrial septal defect, ischemic heart disease and chest trauma. Aortic valve prolapse may be noted in many disorders including ventricular septal defect, aortoannular ectasia, Marfans, aortic dissection and infective endocarditis. Tomaru et al. described the entity of “post-inflammatory” valve prolapse.<sup>71,72</sup> They studied 42 aortic and mitral valves that were removed for prolapse and found fibrosis, neovascularization and chronic inflammation in over 50 %. These valves were scarred.

There may be some overlap between rheumatic/ post-inflammatory changes and floppy myxomatous valves if one takes the effort to examine these valves by histology. By gross examination one is commonly called upon to distinguish between rheumatic valves and floppy mitral valves. The rheumatic valve is fibrotic and firm with thickened and fused leaflets and commissures. The ostium is elongated and funnel shaped due to chordal fusion. Calcification and rigidity are seen. Chordal ruptures are not common. By contrast the floppy myxomatous valve is thickened and redundant. The valve remains soft and pliable and tends to dome above the valve insertion. Chordal rupture and attenuation are common. The ruptured chords may fuse in a random irregular fashion on the underside of the valve leaflets. The commissures are not fused.<sup>73</sup>

This seems simple. Unfortunately, it is not always simple if one examines the valves carefully and especially if one histologically examines the valves. In classic rheumatic valves one sometimes finds some ground substance accumulation. More commonly, in classic myxomatous

valves one sometimes sees some chronic inflammation and neovascularization. With changes in chordal tension the valve endothelium may be disrupted and the valve interstitial cells may alter their expression of cytokines and growth factors.<sup>48</sup> It may not be as clean cut as we would like.

## **SUMMARY**

Inflammatory cardiac valve disease encompasses many disorders and should not be solely equated with rheumatic valve disease. Granted, rheumatic disease remains an important cause, especially if one considers a global perspective, but with increased understanding of the pathoetiology of valve disease and valve injury it is evident that inflammation may play a role in many valve disorders. Cellular immunity, humoral immunity and direct cell activation via pharmacological agents may all contribute. We are only beginning to understand these complex processes. Pathologists have made many contributions to this field. With increasing knowledge that many of these processes are active, there may be opportunity for intervention or even prevention.

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# **PATHOLOGY AND PATHOGENESIS OF INFECTIVE ENDOCARDITIS IN NATIVE HEART VALVES**

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## **Definition**

Endocarditis is an endovascular microbial infection of intracardiac structures facing the blood including infections of the large intrathoracic vessels and of intracardiac foreign bodies (1). Formerly known as bacterial endocarditis, endocardial infections are currently named infective endocarditis, in order to include both bacterial and fungal microorganisms. As a consequence, sterile thrombotic lesions (thrombotic non-bacterial endocarditis) should be termed non-infective endocarditis (2).

## **Pathogenesis and predisposition**

Sterile thrombotic vegetations are considered the crucial lesions underlying the development of infective endocarditis, since they serve as a suitable milieu for bacterial sticking on valve surfaces (3). Endothelial injury is the most plausible factor leading to platelet deposition. The rarity of endocarditis, despite frequent transient bacteriemia, indicates that the intact endothelium is resistant to infection.

Haemodynamic and mechanical stress seem to play an important role in the development of initial lesions and location of the infection (4). The predilection site of infective endocarditis is the area of valve's line closure, due to the impact of the pressure, a matter which accounts for the prevalent involvement of the left side valves. Altered hemodynamics, due to preexisting valve damage, may predispose to endothelial damage and platelet deposition, thus increasing the likelihood of endocarditis during bacteriemia. Entry of microorganisms into circulation, due to focal infection or trauma, ultimately converts thrombotic non bacterial endocarditis into infective endocarditis. Events that traumatize the oral mucosa, particularly the gingiva, the genitourinary and gastrointestinal tracts are associated with an increased risk of bacteriemia. The adherence propensity of some microorganisms to non bacterial thrombotic deposits plays a major role, and fibronectin, a glycoprotein that is the main surface constituent of mammalian cells, has been identified as an important factor in this process (5). Decrease of host defenses mechanisms most probably plays a major role as well. The local blood flow pattern changes, as a result of alteration of the valve's geometry, concur to thrombus formation (non infective thrombotic endocarditis), microorganisms adhesion during bacteremia and onset of infective endocarditis (injury-thrombus-

infection theory. The microorganisms then can grow and induce further thrombus formation and neutrophils chemotaxis. Thus, underlying disease with deformed valve is the main risk factor of infective endocarditis. Likewise, jet or friction lesions of the endocardium, as seen on the left ventricular outflow tract in aortic incompetence and hypertrophic cardiomyopathy, are a well known site of infective colonization.

However, studies of experimental endocarditis showed that injury to the endocardium and vascular endothelium may serve as a focus of infection even before the development of sterile thrombotic vegetations (6).

Most of gram + bacteria are resistant to the bactericidal activity of the serum, whereas gram - are not. This explain why gram + bacteria are more likely to be a cause of infective endocarditis.

### **Pathology and complications of native valve endocarditis.**

The pathology of infective endocarditis may be local, including valvular and perivalvular destruction (7), and distal, due to detachment of septic vegetations with embolism, metastatic infection and septicemia (2).

As far as the *distal complications*, they differ whether endocarditis is right-sided or left-sided, and whether emboli from vegetations are septic or bland. Right-sided endocarditis may be complicated with pulmonary artery embolism and infarcts, pneumonia and lung abscesses. Left-sided endocarditis may be complicated with systemic embolism and cerebral, myocardial, kidney, splenic, intestinal infarcts and/or abscesses. Embolic events are the most common extracardiac complication associated with infective endocarditis, with the incidence ranging from 22 to 43% (8). Valvular vegetations seem to be a significant risk factor for embolism only in case of infection with *Streptococcus viridans* (8). Cerebrovascular accidents occur in nearly 10% of infective endocarditis of the left-sided heart and a similar incidence was found in patients receiving anticoagulants and in patients who did not (9). Paradoxical emboli may occur in congenital heart disease with intracardiac shunt.

Metastatic infection may lead to apostematous meningitis, myocarditis and pyelonephritis. Splenic abscesses are at risk of rupture, so abdominal computed tomography is indicated for monitoring splenic involvement (10). Septicemia may stimulate disseminated intravascular coagulation. Deposition of circulating complexes may account for diffuse or focal glomerulonephritis. Mycotic aneurysms may involve both the large-medium size arteries and the small vessels (Osler's nodes) (11).

*Local complications* of infective endocarditis occur in the valve itself or in the perivalvular region, and they also vary, whether atrioventricular or semilunar valves are affected. Vegetations

are usually attached to atrial aspect of atrioventricular valves and to ventricular aspects of semilunar valves, at the valve line closure. Infective endocarditis of the aortic valve may present with vegetations of various size, which in the acute phase consist of septic thrombus entrapping microorganisms and neutrophil infiltrates. Sometime, they are so small as to be overlooked by the pathologist. Echocardiography can demonstrate only lesions 2-3 mm or more in size. In subacute-chronic phase, microorganisms may disappear, granulomatous inflammation including giant cells occurs and vegetations may transform into coarse calcific deposits. Cusp disruption with loss of substance account for tearing, fraying, perforation and bulging, especially when the microorganism is staphylococcus. Valve incompetence with left ventricular decompensation and congestive heart failure is the usual hemodynamic complication. It may be associated with some degree of functional stenosis, if vegetations are remarkable. Local spread of infection includes extension to the aortic wall that may lead to development of sinus of Valsalva aneurysms, ring abscess, tunnels and fistulae to the surrounding cardiac chambers (right and left atria, right and left ventricles) and pericardial cavity itself with cardiac rupture and tamponade. Transesophageal echocardiography is highly accurate in the detection of complications, such as paravalvular abscesses or mycotic aneurysms. Aortic root complications carry an increased operative mortality and a high incidence of postoperative regurgitation (12). Extension of the infective endocarditis from the aortic to the mitral valve occurs through mitro-aortic fibrous continuity. A marker of such complication is the development of a septic aneurysm in the anterior leaflet of the mitral valve (satellite infection or kiss lesion), with or without perforation. Involvement of the atrioventricular conduction system may account for atrioventricular block. Rupture of the membranous septum may induce acquired ventricular septal defect.

A part from cusp vegetations and perforations, which do not differ substantially from those occurring at semilunar valve level, infective endocarditis of atrioventricular valves are peculiar in so far as the subvalvular apparatus (chordae tendinae and papillary muscle) may be also affected. Chordal rupture may occur in the setting of infective disruption. Papillary muscle rupture may also occur, either due to septic localization on the tip or to myocardial necrosis because of coronary embolism. Perivalvular extension of the infection and ring abscesses are exceptional at atrioventricular valve level.

Healed endocarditis is marked by indentation of the free margin of a cusp, perforation of the body of the cusp with thick edges, cusp aneurysms, ruptured chordae tendinae and healed fistulae.

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# **PATHOLOGY OF INFECTIOUS AND INFLAMMATORY DISEASES OF PROSTHETIC HEART VALVES**

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## **Pathology Infectious and Inflammatory Diseases of Prosthetic Heart Valves**

Prosthetic heart valves are one of numerous cardiovascular prosthetic devices used for the management of valvular heart disease. Prosthetic heart valves, as well as most prosthetic cardiovascular devices, serve their "owner", well and for long periods of time. In the process, treating the serious underlying condition, as well as making the quality of life for the individual, much better. Prosthetic heart valves have been in use since the mid 1960's and came into their own, a few years later. Today, well over 2 million individuals receive a cardiovascular prosthetic device in the United States. Worldwide, the numbers are considerably larger.

Of these, prosthetic heart valves (PHV) account for over 250,000). Prosthetic heart valves may be mechanical heart valve prosthesis, when they are made totally of synthetic materials or they may be bioprosthetic valves when they are made, at least in part of biological materials (1,2). Clearly, the name bioprosthetic valves, is misleading, since they are only partly made of biological materials, which are in the majority of instances, porcine tissues. It is only in the autograft that the graft valve is a living tissue.

Prosthetic heart valves, as any other cardiovascular prosthesis can show post-implantation changes. These can be broadly categorized as:

1. Thrombi and thromboembolism
2. Anticoagulation related hemorrhage
3. Infection.
4. Pannus or an exaggerated response to the prosthetic heart valve
5. Tissue degeneration or other forms of biomaterials failure
6. Pannus or host tissue reaction including inflammation and / or toxicity
7. Adverse systemic effects: such as migration of entire device, of biomaterials or a hypersensitivity reaction to the materials.

### ***Underlying Basis of Reactions:***

Most of the reactions that are reported are

- A) the result of interaction of the device with the host's tissues or
- B) the effect of the host on the device and the results of this.

Device failure is usually a complex process with many potential and real causes some that are easily understood, while others that are still not clearly defined or understood. One must keep in mind that these PHV are constantly bathed in a rapidly flowing blood stream, in a living and often growing heart.

### ***Prosthetic heart valves may be mechanical or biological***

Mechanical heart valve prosthesis needs the individual to be on life-long anticoagulant therapy and that the patient's anticoagulant status (listed as the INR) be regularly measured (or checked), at least once every two weeks. Depending on the PHV, the INR is maintained at different recommended levels, ranging from 1.4 to over 2.2 international units.

Bioprosthetic heart valves, on the other hand, do not need anticoagulant therapy. Contemporary prosthetic heart valves include:

#### A Mechanical Valves:

- 1a. Starr-Edwards, Ball in cage, mechanical prosthesis (Edwards Life Sciences)
- 1b. Bileaflet valves
2. Tilting disc valves

#### B. Bioprosthetic valves:

1. Stented porcine valves
  - a) Unstented - St. Jude Medical T-SPV; Medtronic - Freestyle
2. Pericardial valves:
  - a) Carpentier-Edwards bovine pericardial
3. Homograft valves:
  - a) Cryopreserved
4. Autografts (Ross procedure)

As already listed, inflammation of some degree at least, occurs at every heart valve implantation site. The act of removing the native valve, either partially or completely, damages tissues, leads to the formation of a small amount of thrombus, and the migration of a variable number of inflammatory cells. Implantation of a PHV, leads to further trauma and the presence of a new "foreign material", leads to further inflammatory cell exudation. Immediately after the patient comes off the Heart-Lung bypass machine, a variable film of thrombus is deposited on the fabric of the prosthesis stent and to an even more variable degree, the surface of the prosthetic tissues (especially bioprosthesis). Depending on the site at which the prosthesis is implanted, the reaction may be more or somewhat less aggressive.

A florid acute inflammatory reaction or Infective endocarditis is rare but may be seen through the lifetime of the device implant duration. While usually seen in about 2% of cases, it can range from 1 - 6% of prosthetic valve replacements and is associated with a 50% mortality. The effects of this infection include embolization of vegetations to different sites including coronary arteries, resulting in microemboli into the myocardium with resultant acute myocarditis (bacterial), congestive heart failure secondary to the obstruction of the orifice and resultant stenosis and / or regurgitation due to the presence of large vegetations and possibly destruction of the valvular tissues by virulent microorganisms (3,4,5).

In the case of mechanical heart valve prosthesis, tissue destruction surrounding the annulus or the sewing cuff of the prosthesis, generally results in a "ring" abscess, since micro-organisms cannot grow on the synthetic surfaces (6)

Mechanical and bioprosthetic heart valves have a more or less similar infection rate. The incidence of infective of endocarditis is higher in patients who have been operated for infective endocarditis and in intravenous drug abusers (4,5).

### ***Incidence:***

Prosthetic valve endocarditis (early), is generally highest in the first few months after surgery. In fact, it used to be even higher in the early post-operative period. In this instance, the endocarditis

was likely related to microorganisms present on the device. Today, this is seldom seen, except in patients who undergo valve replacement for infective endocarditis. While common in the first few months, it can occur, through the life of the implant.

***Microorganisms:***

The large majority of cases of infective endocarditis are due to the common microorganisms seen in hospitals and on the patient's skin. Early infections are from the patient's own cutaneous flora suggesting possible contamination and bacteremia with resultant infective endocarditis.

Later infections are almost always due to an infection located at some other site and are generally associated with Staphylococcal bacteria, especially Staph. aureus and epidermidis. Less common microorganisms are gram-negative bacilli and fungi (5-9).

***Type of vegetation:***

The types of vegetations associated with Staphylococci infection are generally small, and highly tissue destructive, reflecting the virulence of the microorganism. Streptococcal infections tend to lead to larger vegetations with slower milder destruction of tissue.

Fungi: Fungi, being generally of low virulence, are associated with large bulky vegetations.

Due to the location of the infection to an avascular or virtually avascular milieu, host defense mechanisms, as well as antibiotics cannot really lead to a cure. Bioprosthetic materials allow the growth of thrombotic vegetations with the microorganisms in them and can result in the gradual destruction of the cuspal tissue.

Mechanical prosthesis, on the other hand, not having any biological components, do not support either bacterial or fungal growth. Infections, in these instances, are almost always located in the periprosthetic tissues that are at the interface of the sewing cuff and the native tissues. They often are associated with a ring abscess and the prosthesis itself may be nearly, totally dislodged, at least from its "anchorage" to the native vascular or myocardial tissues.

This tissue destruction leads to "valve dehiscence" giving rise to a fairly typical echocardiographic appearance of a "dancing" prosthesis. It also leads to a paravalvular leak, which gradually increases in size and significance, with resultant deterioration of cardiac function.

***Biological reaction to heart valve prosthesis:***

Inflammatory reactions (other than infective endocarditis) to biological heart valves are rare. A mild mononuclear cell reaction, invariably macrophages, is at times seen in biological materials. This is particularly common in pericardial valves where a single layer (or a multilayer) rim of macrophage-like cells is seen on the surface of the "cusps". This reaction is more pronounced on the sinus or non-flow surface. In time, this appears to increase and can be seen on both the flow and non-flow surfaces. The significance of these macrophages is uncertain, since they are seen in areas where there is relatively mild, or even no destruction of cuspal tissue, as well as in areas where there is significant damage to cuspal tissue. Whether this is the same mass of mononuclear cells, that was present soon after implantation or appeared later, is uncertain.

Macrophages contain lytic enzymes and it is difficult to imagine that these lytic enzymes do not release the enzymes with resultant destruction of the cuspal tissues. Whether the process is slower and longer in aldehyde fixed (cross linked collagen) tissue or not, is difficult to assess.

***Mechanical heart valves:***

Most of these prosthesis have "naked" synthetic fabric as the material of the sewing cuff. Some have sponge-like material forming the body of the sewing cuff. A reaction to this fabric material is usual and has been listed above.

***St. Jude Medical leaflet, Silzone® Coated:***

A few years ago, the St. Jude Medical bileaflet valve-sewing cuff was coated with elemental silver. This was an effort to eliminate infective endocarditis. In the early post implant period, there was clinical evidence of infective endocarditis / annular abscesses from some centers. At the time of surgery, these abscesses were huge, associated with a marked degree of native tissue destruction and at the time of surgery, some of these devices were found to be held in place by just one or two sutures and were found in a deep abscess-like cavity filled with grey necrotic material. This material was invariably culture negative. Examination of the tissues showed necrotic debris and again no morphologic evidence of microorganisms was seen. However, the tissues surrounding the "abscess" or "annular abscess" showed abundant tissue necrosis. No good explanation has been available for this. The tissue necrosis was possibly related to the effect of the elemental silver, which may have leached out of the fabric into the surrounding tissues and resulted in myocytolysis, as well as the destruction of the interstitium.

These early explants showed several other features, different from those seen with the old model of SJM valves.

**Biological valves** have seldom, if ever, been reported to induce a significant cellular reaction, in the absence of infective endocarditis. Whether this is due to the fact that this is dead tissue, aldehyde fixed tissue on a stented frame or some other reason, is not yet certain. There have been virtually no reports of cellular or humoral rejection of these xenografts or even of homografts.

The stentless valves, not having a prosthetic stent or sewing cuff, could be more likely to have a cellular reaction against them (10-14). However, in most instances, including homografts and the Toronto-Stentless Porcine Valve®, this has not been reported. The tissues have never shown a reaction, either the porcine aortic tissue, nor the porcine cuspal tissue. In the instance of the other stentless valve, the Freestyle valves, there is only a minimal cuff of fabric covering the proximal edge of the porcine aortic tissue. We have recently seen a mononuclear cell reaction to the tissue with resultant damage to the porcine aortic tissues. Clinically, this has resulted in prosthesis dysfunction, with resultant incompetence. This reaction is suggestive of graft cellular rejection

Infective endocarditis has also been reported with the stentless porcine valves, just as it has with the stented porcine valves. The role of biofilms in the infections seen with mechanical heart valves is still to be determined (15).

***Conclusion:***

Prosthetic heart valves, Mechanical or biological (Xenograft valves, stented or unstented, have been reported to show an inflammatory reaction (infective endocarditis). This is predominantly associated with bacterial / fungal infection. Somewhat surprisingly, no immune reaction has been reported thus far. This may, amongst other reasons, be related to the fact that the tissues "sit", virtually in a cocoon of synthetic material (the valve ring and the fabric covering it). The stentless valves, however, have no such "cocoon". While the T-SPV has such a "cocoon", albeit significantly thinner and narrower, the Freestyle valve has no such "cocoon". It is perhaps not so surprising therefore that at intermediate term (five to six years), we are beginning to see some occasional cases in which such a reaction is noted.

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# PATHOLOGY OF VENTRICULAR ASSIST DEVICES AND THE ROLE OF BIOFILMS

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## Introduction

Ventricular assist devices (VADs) have been used for more than a decade to improve hemodynamics and end-organ function as a bridge-to-transplant in patients with end-stage heart failure. Treatment with VADs as destination therapy has also been shown to increase survival over optimal medical management in patients with end-stage heart failure who are not transplant candidates. Infection in patients with VADs is common, difficult to treat and one of the most common causes of death in patients with VADs.

## Infection in Ventricular Assist Devices

Infection is unfortunately a common complication of mechanical circulatory support. The presence of infection may delay transplantation in bridge-to-transplant patients, and is a major cause of both pre-transplant mortality in bridge-to-transplant patients and overall mortality in patients with destination therapy VADs. In contrast to most permanent implantable cardiovascular medical devices such as coronary artery stents, heart valves, pacemakers and automated implanted cardioverter defibrillators, most currently available VADs maintain percutaneous connection with the epidermal surface and external environment via a driveline, which is required to provide power and controller functions.

The Randomized Evaluation of Mechanical Assistance for the Treatment of Congestive Heart Failure (REMATCH) trial demonstrated the superiority of mechanical circulatory support to optimal medical management in sustaining the lives of patients with severe symptoms of congestive heart failure that were not heart transplant candidates. However, infection was a common complication in the VAD arm of this trial. Freedom from sepsis was only 58% at 1 year and 48% at 2 years in the VAD arm, with the hazard for initial diagnosis of sepsis peaking within the first 30-60 days after implantation. The most common cause of death in the VAD arm was sepsis (20/52 patients). A total of 28 patients suffered a sepsis syndrome, 19 patients had a driveline infection and 11 patients had a pump infection. Many other studies have yielded similar rates of infection in bridge-to-transplant VAD patients.

The most common organisms responsible for VAD infection are biofilm-forming bacteria such as *Staphylococcus sp.*, *Pseudomonas sp.*, and *Enterococcus sp.*, as well as fungal infections such as *Candida sp.* Not surprisingly, the percutaneous driveline is the most common portal of entry for these organisms. Many VAD related infections occur early after implantation before the driveline site has fully healed and been integrated into the host tissue. Factors that retard healing, such as excessive movement or trauma at the exit site, poor nutritional or overall status of the patient at the time of implantation, and diabetes mellitus, may also increase the likelihood of driveline infections in these patients.

## Biofilms

It has long been known that a substantially smaller number (by several orders of magnitude) of organisms is required to form an infectious abscess in the presence of a foreign material than in the absence of one, largely due to the advantages afforded an organism by its development of a biofilm. A biofilm is a multicellular consortium of microbial cells that is irreversibly associated with a surface and enclosed in a self-produced extracellular matrix composed primarily of polysaccharides. Van Leeuwenhoek first described this phenomenon as he investigated organisms on tooth surfaces using his simple microscopes. Others later described that bacteria demonstrated enhanced growth and activity in the presence of a surface, and that the concentration of organisms on a submerged surface was significantly greater than that in the surrounding fluid. The advent of the electron microscope allowed detailed analysis of surface biofilms, first in environmental (e.g., rocks in a stream) and industrial (e.g., water pipes) arenas, and later on medical devices. Biofilms also grow on native tissue surfaces, examples being *Pseudomonas aeruginosa* biofilms forming in the airways of patients with cystic fibrosis and organisms forming biofilms on teeth resulting in dental plaque.

When a foreign material is placed into the body, a host of molecules interact with the surface, including fibronectin, vitronectin, fibrinogen and other proteins, glycoproteins, proteoglycans, polysaccharides, lipids, and ions, to form a conditioning film. The nature of this conditioning film depends on properties of the material, such as surface chemistry and charge, microarchitecture and degree of hydrophobicity or hydrophilicity, as well as the properties of the tissue/fluid, such as pH, protein concentration and hydrodynamic forces, to which it is exposed. These interactions occur over the time course of seconds to minutes upon exposure of a surface to biological fluid. Free-floating (or planktonic) organisms initially interact with the conditioned surface nonspecifically through electrostatic, Van der Waals and hydrophobic interactions to reversibly adsorb to the material. Properties of the organisms, such as their surface charge, the presence of fimbriae and flagella and the production of an extracellular polysaccharide coat, also influence the attachment of microbes to a surface. Some microorganisms such as *Staphylococcus epidermidis* have surface molecules through which they can directly attach to bare polymer surfaces (staphylococcal surface protein-1, autolysin E). These same molecules, as well as others such as the fibrinogen receptor ClfA (clumping factor) and the fibrinogen-binding protein FbpA in *Staphylococcus aureus*, allow organisms to attach to the components of the conditioning film. Once attached, the organisms begin to proliferate and accumulate in multicellular clusters, spread across the surface and secrete extracellular polysaccharides to further their adhesion to the surface. These activities require intercellular adhesion mediated by, in *Staphylococcal sp.* for example, polysaccharide intercellular adhesin (PIA – a glycosaminoglycan) and accumulation associated protein (AAP). The organisms also can communicate with each other through diffusible molecules in a process termed quorum sensing in order to coordinate their behavior. The biofilm develops into a three-dimensional structure with internal architecture including pillars and channels through which fluid can flow. The mature biofilm is composed of about 10-25% organisms and 75-90% extracellular material mostly in the form of polysaccharide matrix.

Once a microorganism has attached to a surface, there are changes in gene expression that allow the formation of the biofilm; the discovery of this phenomenon and the realization that surface-associated organisms are behaving in a fundamentally different manner than their planktonic counterparts is advancing the understanding of material-associated infections and providing insight into potential therapeutic targets. DNA microarray analysis and gene expression profiling, common research tools in the study of tumors and many other human diseases, are being used to study differences in gene expression between organisms in a biofilm and planktonic organisms under a variety of different environmental conditions and as a function of time over the development and growth of the biofilm. There is even evidence of differences in gene expression

within the biofilm, depending on where the organism is in relation to landmarks (e.g., deep within the polysaccharide matrix vs. at the outer surface of the biofilm) and the environmental conditions (e.g., pH, pO<sub>2</sub>) at that location.

Several mechanisms are employed by organisms in biofilm to evade the host defense system. The extracellular polysaccharide matrix impedes the penetration of opsonizing antibodies so they do not reach the underlying organisms, making uptake and killing by phagocytes less efficient. The presence of the matrix has been shown to reduce the phagocytic ability of macrophages and polymorphonuclear leukocytes both on material surfaces and even after organisms have been released from the biofilm, promoting sepsis. There is evidence that this may be at least partially due to a resistance to reactive oxygen species produced by the phagocytic cells.

The biofilm also hampers the efficacy of antibiotics in the treatment of material-associated infections. Organisms in a mature biofilm divide at a lesser rate, making them less susceptible to certain antibiotics. The organisms tend to be organized deep within the extracellular polysaccharide matrix, making them less accessible to therapeutic antibiotics. Antibiotic concentrations between 1,000 and 15,000 times greater are needed to kill biofilm-associated organisms than their planktonic counterparts as a result of the physicochemical properties of the matrix. The difficulty in eradicating organisms from a biofilm often necessitates prolonged periods of antibiotic use, furthering the development of resistant organisms within the biofilm. Bacteria within a biofilm can become resistant to certain antibiotics through the accelerated acquisition of resistance plasmids (extrachromosomal DNA) from other bacteria through the process of conjugation, which occurs with greater ease within the physically protective environment of the biofilm. Another change in biofilm-associated organisms is that of the proteins of the cell wall, often furthering resistance to antibiotics.

Organisms from the biofilm, or parts of the biofilm itself, routinely dislodge into the bloodstream. This dissemination of organisms can lead to sepsis and the metabolic alterations that arise therefrom, or embolic phenomenon resulting in ischemia and infarction in the affected organ. Gram-negative bacteria within biofilms may shed lipopolysaccharide into the bloodstream causing septic physiology. These mechanisms are common pathways resulting in mortality in patients with ventricular assist device related infections.

### **Implications for Patient Care**

The risk of a patient with a VAD developing infection is greatest around the time of implantation, so careful attention to infection prevention and control guidelines in the perioperative period is essential. Considerations include patient selection and preparation, correction of malnutrition, appropriate prophylactic antibiotics, proper maintenance of indwelling catheters and lines, and elimination of other sources of potential bacteremia (e.g., poor dentition) prior to implantation, meticulous attention to sterility and proper implantation technique in the operating room, and attention to immobilization, promotion of wound healing and sterility of the driveline in the postoperative setting.

It is extremely difficult to eradicate a device-related infection, especially in light of the protective properties of the biofilm. Identification of the offending organism and its susceptibility to antibiotics is an important first step in controlling the infection. Treatment with appropriate antibiotics is essential. In bridge-to-transplant VAD patients, infection tends to delay, but not preclude, eventual transplantation, and does not tend to negatively affect long-term survival. A likely explanation for this is that the device, and therefore source of the infection (biofilm), is removed at or around the time of transplantation. In destination VAD patients however, it is

possible to control the infection over prolonged periods of time, but recurrences of the infection are common and cause significant morbidity and mortality in this population.

### **Future Directions**

A decrease in the incidence of device-related infections would be expected to yield a further increase in survival and decrease in morbidity in VAD recipients. Several strategies are being employed to address the issue of infection, now with a better understanding of the mechanisms and physiology of biofilm and its role in VAD infections: 1) development of completely implantable systems with transcutaneous energy transmission to eliminate the need for a percutaneous driveline, 2) materials technology to promote tissue integration and prevent biofilm formation, to allow host tissue to win the “race to the surface”, 3) controlled release technology to allow for prolonged antibiotic release from component of the VAD itself, 4) improvements in patient selection, operative technique and postoperative management, 5) development of novel therapeutics that may interfere with the mechanisms of biofilm formation such as intercellular adhesion or quorum sensing, 6) device design to smaller and more durable pumps, and 7) creative placement of driveline exit sites (e.g., postauricular) to allow for better tissue integration. It should be noted that substantial progress has already been made, as evidenced by lower infection rates in the post-REMATCH era, and lower infection rates for many of the newer devices in clinical trial and use currently.

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# NEW FRONTIERS IN THE PATHOLOGY AND THERAPY OF HEART VALVE DISEASE

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This presentation summarizes several areas of ongoing evolution in our understanding and management of valvular heart disease, including the biology of the native valves, the pathology/pathobiology of common lesions, and novel insights about and innovative approaches to valve repair, replacement and regeneration.

## **Structure and Biology of the Normal Cardiac Valves**

New understanding of the structural basis of function and biology of the native heart valves has elucidated mechanisms of disease, fostered the development of improved tissue heart valve substitutes and informed innovative approaches to heart valve repair and regeneration.<sup>i</sup>

During cardiac development, the valve cusps and leaflets originate as outgrowths (*endocardial cushions*) from mesodermal connective tissue (*mesenchyme*).<sup>ii</sup> Endothelial cells lining the inside surface of the cushion forming area undergo an *epithelial-to-mesenchymal transdifferentiation* (EMT) and migrate from the blood-contacting internal heart surface deep into the connective tissue of the subendocardium (*cardiac jelly*) to become precursors of mature *valvular interstitial cells* (VIC). Using a complex molecular signaling sequence involving NFATc, VEGF and TGF- $\beta$ , these newly formed mesenchymal cells remodel the cushions into leaflets and cusps.<sup>iii</sup>

Valve function is enabled by a structure that consists of three well-defined tissue layers, each enriched in a specific extracellular matrix (ECM) component: this concept is well illustrated by the aortic valve.<sup>iv</sup> The principal determinant of valve durability is the valvular ECM; its quantity and quality depend on viability and function of VIC that synthesize and maintain it by ongoing physiologic remodeling, mediated by matrix metalloproteinases (MMPs). Basic investigation of the mechanisms of heart valve development and response to injury<sup>v</sup> have shown that VIC have remarkably plasticity, and that dramatic phenotypic modulation can occur in developing and fully mature valves. For example, fetal valves have a dynamic/adaptive structure and contain VIC with an activated/immature phenotype that mediates active ECM remodeling; during postnatal life, activated cells eventually become quiescent, suggesting a progressive, mechanically-mediated adaptation in-utero and after birth.<sup>vi</sup> Although interstitial cells are fibroblast-like in normal valves of children and adults, VIC become activated and mediate functional adaptation of valves exposed to environmental stimulation.<sup>vii,viii</sup> For example, when valves are subjected to changes in mechanical loading in disease (e.g., myxomatous mitral valves)<sup>ix</sup> or adaptation (pulmonary-to-aortic autograft), VIC become activated to a myofibroblast phenotype and mediate connective tissue remodeling to restore the normal stress profile in the tissue. When equilibrium is restored, the cells return to quiescence.

Additionally, recent investigation suggests that the endothelial cells (EC) covering the heart valves may have different phenotypes than EC elsewhere in the cardiovascular system.<sup>x,xi</sup> Moreover, EC on the two surfaces of the aortic valve cusps may be different; specifically, EC on the aortic side of the normal (pig) aortic valve express different genes than those on the ventricular side; aortic side EC express less inhibitors of calcification and proinflammatory molecules and enhanced antioxidative genes, which may contribute to differential disease susceptibility.<sup>xii</sup>

## **Valvular Pathology/Pathobiology**

*Emerging data are yielding key insights into the pathology and pathobiology of common forms of valvular heart disease. Some examples follow.*

### **Congenitally Bicuspid Aortic Valve (BAV)**

The most common congenital cardiac malformation overall, BAV occurs in up to 2% of the population. BAVs usually have normal hemodynamics at birth and in early life but are predisposed to progressive calcification causing stenosis.<sup>xiii</sup> BAV may also become incompetent as a result of aortic dilation, cusp prolapse or infective endocarditis. New data confirm that BAV has a high degree of heritability, often with other congenital malformations, suggesting that BAV is either related to a primary defect in valvulogenesis or is secondary to other elements of faulty cardiogenesis.<sup>xiv,xv</sup> BAV was related to specific mutations in the signaling and transcriptional regulator NOTCH1 in several kindred families with aortic valve and congenital heart disease.<sup>xvi</sup> Moreover, evidence is accumulating that BAV is accompanied by a fundamental defect in the connective tissue of the aorta, which may be responsible for the increased incidence of aortic dilation and dissection seen with BAV.<sup>xvii</sup>

### ***Calcific Aortic Stenosis***

*Acquired aortic stenosis* is usually the consequence of calcification intrinsic to the cuspal tissue owing to progressive and advanced age-associated "wear and tear" of either previously anatomically normal aortic valves or BAV.<sup>xviii</sup> With the rising average age of the population the prevalence of aortic stenosis, estimated at 2%, is increasing.<sup>xix</sup> The mechanisms of aortic valve calcification are traditionally believed to be due to degenerative, dystrophic and passive accumulation of hydroxyapatite mineral in the setting of sclerosis.<sup>xx</sup> However, recent studies suggest active regulation of calcification in aortic valves similar to that in atherosclerotic arteries, with inflammation, lipid infiltration, and phenotypic modulation of VIC to an osteoblastic phenotype.<sup>xxi,xxii,xxiii</sup> Although it is attractive to speculate that statin drugs may decrease the rate of aortic stenosis progression, studies to date have not supported this contention.<sup>xxiv,xxv</sup>

### ***Myxomatous Degeneration of the Mitral Valve (Mitral Valve Prolapse, MVP)***

MVP is currently the major indication for mitral valve surgery in the United States. With the echocardiographic definition of MVP recently revised (based on an enhanced understanding of mitral valve anatomy), the prevalence has been estimated at 2-3% of adults.<sup>xxvi</sup> Usually an incidental finding on physical examination, MVP is associated with serious complications such as bacterial endocarditis and sudden death in a small minority (probably less than 3% of all those affected by MVP).

There is general agreement that the final common pathway for the development of MVP is the weakening of valvular connective tissue that leads to leaflet stretching, but the molecular basis for the changes within the valve leaflets and associated structures remains largely unknown. MVP is associated with some heritable disorders of connective tissue including Marfan syndrome, in which it is usually associated with mutations in *fibrillin-1 (FBN-1)*.<sup>xxvii</sup> It is unlikely that more than 1-2% of patients with MVP have a well-defined connective tissue disorder and sporadic MVP is not generally associated with FBN-1 abnormalities. Nevertheless, specific genetic loci have recently been identified in 3 families in which MVP was inherited as an autosomal dominant trait and segregated to loci on 11p, 13q and 16p.<sup>xxviii</sup> The most recently identified locus on 13 has several important candidate genes related to extracellular matrix remodeling<sup>xxix</sup>, which is clearly abnormal in this condition. Moreover, a recently developed mouse model of MVP has suggested that TGF- $\beta$

dysregulation in connective tissue likely plays an important role in Marfan syndrome-related and possibly other forms of MVP.<sup>xxx</sup>

### ***Mitral Regurgitation (Secondary to Ischemic Injury or Heart Failure)***

Patients with heart failure and left ventricular systolic dysfunction frequently develop mitral regurgitation (MR) owing to alterations in LV geometry including ventricular dilation and deformation of the normal mitral valve apparatus that result in incomplete closure of the mitral valve leaflets. Those with severe MR present in the setting of dilated cardiomyopathy or ischemic heart disease have a significantly worse prognosis than patients without associated MR.<sup>xxxii</sup> Although studies have not yet indicated that survival of this patient group is improved by reduction of MR<sup>xxxiii</sup>, there is considerable interest in the development of minimally invasive procedures to alleviate MR in this setting (see below).

### **Heart Valve Replacement and Repair**

Replacement of damaged cardiac valves by prostheses and various types of open repair (particularly for MR) is common and often life-saving.<sup>xxxiii</sup> With contemporary valve replacements, patient prognosis is good at 15-20 years.<sup>xxxiv</sup> Nevertheless, the not inconsequential mortality and morbidity of open surgical procedures has stimulated considerable interest and progress in minimally invasive and catheter-based percutaneous valve procedures. Percutaneous catheter-based interventions already play a role in the management of valvular heart disease, but predominantly in relatively low-volume lesions. Technological advances may allow the growth of percutaneous treatment of common valvular lesions such as mitral regurgitation and calcific aortic stenosis, collectively the most frequent valve pathologies (estimated at 70% in Western countries). Thus endovascular procedures may in the next decade provide an alternative to many open-heart operations, with potential reduction of risk (and cost). This section summarizes the status of surgical cardiac valve replacement, open valve repair, and innovative percutaneous procedures, including valve repair and replacement.

### ***Cardiac Valve Replacement***

Studies begun in the 1980's show that approximately 60% of substitute valve recipients (with contemporary *mechanical prostheses*, such as tilting disk or hinged semicircular rigid flap valves, and *tissue valves* consisting of chemically treated animal tissue, either porcine aortic valve or bovine pericardium preserved in dilute glutaraldehyde and mounted on a prosthetic frame) develop a serious prosthesis-related complication within 10-15 years postoperatively.<sup>xxxv</sup> *Thromboembolic complications* comprise the major problem with mechanical valves, necessitating long-term anticoagulation in patients with these devices, which induces a risk of hemorrhage. *Infective endocarditis* is an infrequent but serious potential complication with all types of valves. *Structural deterioration* uncommonly causes failure of contemporary mechanical valves. However, structural deterioration manifest as primary tissue failure is a major failure mode of bioprostheses, usually with calcification and/or non-calcific structural damage and/or tearing, which causes secondary regurgitation. Examination of retrieved valves has contributed to understanding and managing these problems,<sup>xxxvi</sup> and directed translational investigation based on pathological studies has enabled clinical progress.<sup>xxxvii</sup> Experimental and clinical studies have shown that calcification is a dystrophic process caused by reaction of calcium in the serum with the residual phospholipids of the cells of the tissue valve matrix made non-viable by glutaraldehyde treatment during valve fabrication. New prostheses pretreated with anticalcification therapies targeted toward disrupting calcification mechanisms are being used in several commercial valves with apparently favorable results.

### **Balloon Valvuloplasty**

Percutaneous treatment with balloon dilatation is the treatment of choice for many patients with mitral stenosis, pulmonary stenosis, and congenital aortic stenosis (including in-utero<sup>xxxviii</sup>); however, these valvular lesions constitute only a small portion of the valvular heart disease spectrum. The major complications result from leaflet tearing. In contrast, aortic valve balloon valvuloplasty has had only a limited efficacy and high mortality and morbidity in adult calcific aortic stenosis.<sup>xxxix</sup>

### **Percutaneous Mitral Valve Repair**

There is intense interest in endovascular repair of the mitral valve.<sup>xl,xli</sup> Experimental studies have used different devices, either “rings” to stabilize and tighten the mitral annulus or a “clip” mimicking the Alfieri operation (edge-to-edge suturing of the mitral valve leaflets, producing a double-orifice mitral valve without stenosis). Percutaneous approaches in posterior annuloplasty might be accomplished by device placement in the coronary sinus, left atrium (or both), or by device placement behind the posterolateral wall of the LV. Several groups have exploited the proximity of the coronary sinus (CS) to the posterior mitral annulus to perform percutaneous annuloplasty but there are considerable challenges, including anatomic variability in the relationship of the coronary sinus to the mitral annulus, potential damage to the circumflex coronary artery, muscle stretch and relaxation of the constricting force over time, device design and the potentially deleterious effects of a device within the coronary sinus for an extended period.

### **Percutaneous Valve Replacement**

To date, the open surgical approach has been the only option for valve replacement. Percutaneous valve implantation uses catheter-based techniques to deliver a foldable heart valve, mounted within an expandable stent, to a diseased valve annulus.<sup>xlii</sup> The era of percutaneous valve replacement in humans started in 2000 with percutaneous pulmonary valve replacement<sup>xliii</sup>; the first clinical percutaneous aortic valve implantation was done in 2002.<sup>xliv</sup> The applicability of percutaneous valve replacement will depend on the development of collapsible and compressible valve prostheses, large diameter and durable stents, and innovative valve fixation technology. In the case of treatment for aortic stenosis, access will of necessity be either retrograde arterial or trans-septal, the obstruction will have to be alleviated prior to valve implantation, and coronary flow cannot be compromised. Thus, percutaneous pulmonary valve implantation in humans has proceeded more rapidly than aortic; indeed, a recent report described encouraging results using this procedure in 59 young patients with pulmonary regurgitation following right ventricular outflow tract obstruction for congenital heart disease.<sup>xlv</sup>

### **Heart Valve Regeneration and Tissue Engineering**

As discussed above, tissue remodeling during valve development, maturation and adaptation are similar to those occurring in pathological conditions. Normal and pathological cardiac valvular tissue responds to altered environmental conditions, particularly mechanical loading, by cell activation and matrix remodeling, processes which are probably regulated by stresses in the tissue (see above). The relationships of gross valve stress to cellular stimuli are under investigation.<sup>xlvi,xlvii</sup> The goal is to harness these mechanisms to induce regeneration of diseased or deficient valve structure. Innovative work to generate a living valve replacement is active in many laboratories. A *tissue engineered* (living) valve replacement could have the ability to assume normal function and the capacity to repair structural injury and potentially grow.<sup>xlviii,xlix</sup>

In the general paradigm of tissue engineering, cells are seeded on a synthetic polymer or natural material that serves as a scaffold and then a tissue is matured *in vitro* (in a *bioreactor* that provides a suitable metabolic and mechanical environment), until proliferating cells produce a sufficient amount and quality of ECM to form the *construct*.<sup>1</sup> In the second step, the construct is implanted in the appropriate anatomic location, where further remodeling *in-vivo* may occur to

recapitulate the normal functional architecture of an organ or tissue. Key processes occurring during the *in vitro* and *in vivo* phases of tissue formation and maturation are 1) cell proliferation, sorting and differentiation, 2) extracellular matrix production and organization, 3) degradation of the scaffold, and 4) remodeling and potentially growth of the tissue.

We have collaborated with a group at Children's Hospital, Boston to fabricate tissue engineered heart valves (TEHV) using autologous cells seeded onto biodegradable synthetic polymers. Constructs formed *in vitro* have functioned as pulmonary valve replacements in sheep for up to five months, and evolved into a specialized layered structure resembling native semilunar valve.<sup>ii,iii</sup> These studies demonstrate that a tissue grown *in vitro* can function as a valve replacement *in vivo* and serve as a template for remodeling of valve tissue. Recent studies extending these concepts have demonstrated the ability of a pulmonary artery conduit prepared using similar technology to achieve appropriate functional growth over a two-year period in growing lambs<sup>liii</sup>, and the use of bone-marrow derived mesenchymal stem cells as a potentially efficacious cell source for TEHV.<sup>liv</sup>

Accumulating evidence suggests that circulating endogenous progenitor cells can be recruited in-vivo to sites where needed. Endothelial progenitor cells (EPCs) promote endothelial regeneration in dog models by covering implanted Dacron grafts,<sup>lv</sup> and in human studies by covering implanted ventricular assist devices<sup>lvi</sup> and homing to denuded arteries following balloon injury.<sup>lvii</sup> Appropriate cell-signaling molecules on the surface of the scaffold may encourage EPC adhesion, differentiation and function.<sup>lviii</sup> Moreover, recent experimental evidence suggests that human bone marrow may contribute smooth muscle like cells to adult human heart valves.<sup>lix, lx</sup>

Thus, a most exciting possibility is that a scaffold derived from decellularized tissue (e.g., valve or cell-free porcine small intestinal submucosa) or degradable polymer could be implanted without prior cell seeding and be a suitable substrate to attract the appropriate cells to regenerate a living valve composed of autologous cells.<sup>lxi</sup> Although early animal studies suggest efficacy, an initial trial of decellularized porcine valves implanted in humans was frustrated by structural failure.<sup>lxii</sup> Nevertheless, this concept continues to be enthusiastically investigated.

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