

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.ⁱ

During cardiac development, the valve cusps and leaflets originate as outgrowths (*endocardial cushions*) from mesodermal connective tissue (*mesenchyme*).ⁱⁱ 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- β , these newly formed mesenchymal cells remodel the cushions into leaflets and cusps.ⁱⁱⁱ

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.^{iv} 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^v 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.^{vi} 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.^{vii,viii} For example, when valves are subjected to changes in mechanical loading in disease (e.g., myxomatous mitral valves)^{ix} 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.^{x,xi} 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.^{xii}

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.^{xiii} 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.^{xiv,xv} BAV was related to specific mutations in the signaling and transcriptional regulator NOTCH1 in several kindred families with aortic valve and congenital heart disease.^{xvi} 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.^{xvii}

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.^{xviii} With the rising average age of the population the prevalence of aortic stenosis, estimated at 2%, is increasing.^{xix} 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.^{xx} 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.^{xxi,xxii,xxiii} 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.^{xxiv,xxv}

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.^{xxvi} 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)*.^{xxvii} 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.^{xxviii} The most recently identified locus on 13 has several important candidate genes related to extracellular matrix remodeling^{xxix}, which is clearly abnormal in this condition. Moreover, a recently developed mouse model of MVP has suggested that TGF- β

dysregulation in connective tissue likely plays an important role in Marfan syndrome-related and possibly other forms of MVP.^{xxx}

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.^{xxxii} Although studies have not yet indicated that survival of this patient group is improved by reduction of MR^{xxxiii}, 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.^{xxxiii} With contemporary valve replacements, patient prognosis is good at 15-20 years.^{xxxiv} 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.^{xxxv} *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,^{xxxvi} and directed translational investigation based on pathological studies has enabled clinical progress.^{xxxvii} 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^{xxxviii}); 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.^{xxxix}

Percutaneous Mitral Valve Repair

There is intense interest in endovascular repair of the mitral valve.^{xl,xli} 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.^{xlii} The era of percutaneous valve replacement in humans started in 2000 with percutaneous pulmonary valve replacement^{xliii}; the first clinical percutaneous aortic valve implantation was done in 2002.^{xliiv} 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.^{xliiv}

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.^{xlii,xliii} 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.^{xliiii,xliii}

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*.¹ 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.^{ii,iii} 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^{liii}, and the use of bone-marrow derived mesenchymal stem cells as a potentially efficacious cell source for TEHV.^{liv}

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,^{lv} and in human studies by covering implanted ventricular assist devices^{lvi} and homing to denuded arteries following balloon injury.^{lvii} Appropriate cell-signaling molecules on the surface of the scaffold may encourage EPC adhesion, differentiation and function.^{lviii} Moreover, recent experimental evidence suggests that human bone marrow may contribute smooth muscle like cells to adult human heart valves.^{lix, lx}

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.^{lxi} Although early animal studies suggest efficacy, an initial trial of decellularized porcine valves implanted in humans was frustrated by structural failure.^{lxii} Nevertheless, this concept continues to be enthusiastically investigated.

References

- i. Yacoub MH, Cohn LH. Novel approaches to cardiac valve repair. From structure to function: Parts I and II. *Circulation* 2004;109:942 and 1064.
- ii. Eisenberg LM, Markwald RR. Molecular regulation of atrioventricular valvuloseptal morphogenesis. *Circ Res* 1995;77:1.
- iii. Schroeder JA, et al. Form and function of developing heart valves: Coordination by extracellular matrix and growth factor signaling. *J Mol Med* 2003;81:392.
- iv. Schoen FJ: Aortic valve structure-function correlations: Role of elastic fibers no longer a stretch of the imagination (Editorial). *J Heart Valve Dis* 1997; 6:1.
- v. Durbin AD, Gotlieb AI. Advances towards understanding heart valve response to injury. *Cardiovasc Pathol* 2002;11:69.
- vi. Aikawa E, et al. Human cardiac valve remodeling from fetal to adult: Implications for postnatal adaptation and tissue engineering. *Circulation* 2005;112:II-714.
- vii. Walker GA, et al. Valvular myofibroblast activation by transforming growth factor beta: Implications for pathological extracellular matrix remodeling in heart valve disease. *Circ Res* 2004;95:253.
- viii. Rabkin-Aikawa E, et al: Dynamic and reversible changes of interstitial cell phenotype during development and remodeling of cardiac valves. *J Heart Valve Dis*. 2004;13:841.
- ix. Rabkin E, et al. Activated interstitial myofibroblasts express catabolic enzymes and mediate matrix remodeling in myxomatous heart valves *Circulation* 2001; 104:2525.
- x. Butcher JT, et al. Unique morphology and focal adhesion development of valvular endothelial cells in static and fluid flow environments. *Arterioscler Thromb Vasc Biol* 2004;24:1429.

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- xi. Butcher JT, et al. Transcriptional profiles of valvular and vascular endothelial cells reveal phenotypic differences. Influence of shear stress. *Arterioscler Thromb Vasc Biol* 2005; Epub ahead of print.
 - xii. Simmons CA, et al. Spatial heterogeneity of endothelial phenotypes correlates with side-specific vulnerability to calcification in normal porcine aortic valves. *Circ Res* 2005;96:792.
 - xiii. Fedak PW, et al. Clinical and pathophysiological implications of a bicuspid aortic valve. *Circulation* 2002;106:900.
 - xiv. Cripe L, et al. Bicuspid aortic valve is heritable. *J Am Coll Cardiol* 2004;44:138.
 - xv. Horne BD, et al. Evidence for a heritable component in death resulting from aortic and mitral valve diseases. *Circulation* 2004;110:3143.
 - xvi. Garg V, et al. Mutations in *NOTCH1* cause aortic valve disease. *Nature* 2005;437:270.
 - xvii. Fedak PW, et al. Vascular matrix remodeling in patients with bicuspid aortic valve malformations: implications for aortic dilatation. *J Thorac Cardiovasc Surg* 2003;126:797.
 - xviii. Schoen FJ: The heart. In: Robbins/Cotran Pathologic Basis of Disease, 7th Ed., Kumar V, Fausto N, Abbas A (eds.), Philadelphia: Saunders, 2004, p. 555.
 - xix. Freeman RV, Otto CM. Spectrum of calcific aortic valve disease: pathogenesis, disease progression, and treatment strategies. *Circulation* 2005;111:3316.
 - xx. Kim KM. Apoptosis and calcification. *Scanning Microscopy* 1995;9:1137.
 - xxi. Otto CM, et al. Characterization of the early lesion of 'degenerative' valvular aortic stenosis: histological and immunohistochemical studies. *Circulation* 1994;90:844.
 - xxii. Demer LL. Mineral explorations: search for the mechanism of vascular calcification and beyond. *Arterioscler Thromb Vasc Biol* 2003;23:1739-1743.
 - xxiii. Mohler ER III. Mechanisms of aortic valve calcification. *Am J Cardiol* 2004;94:1396-1402.
 - xxiv. Cowell SJ, et al. A randomized trial of intensive lipid-lowering therapy in calcific aortic stenosis. *N Engl J Med* 2005;352:2389.
 - xxv. Verma S, et al. Can statin therapy alter the natural history of bicuspid aortic valves? *Am J Physiol Heart Circ Physiol* 2005;288:H2547.
 - xxvi. Freed LS et al. Prevalence and clinical features of mitral valve prolapse. *New Engl J Med* 1999;341:1.
 - xxvii. Weyman AE, Scherrer-Crosbie M. Marfan syndrome and mitral valve prolapse. *J Clin Invest* 2004;114:1543.
 - xxviii. Roberts R. Another chromosomal locus for mitral valve prolapse. Close but no cigar. *Circulation* 2005;112:1924.
 - xxix. Nesta F, et al. New locus for autosomal dominant mitral valve prolapse on chromosome 13. Clinical insights from genetic studies. *Circulation* 2005;112:2022.
 - xxx. Ng CM, et al. TGF- β -dependent pathogenesis of mitral valve prolapse in a mouse model of Marfan syndrome. *J Clin Invest* 2004;114:1586.
 - xxxi. Trichon BH, et al. Relation of frequency and severity of mitral regurgitation to survival among patients with left ventricular systolic dysfunction and heart failure. *Am J Cardiol* 2003;91:538.
 - xxxii. Wu AH, et al. Impact of mitral valve annuloplasty on mortality risk in patients with mitral regurgitation and left ventricular systolic dysfunction. *J Am Coll Cardiol* 2005;45:381.
 - xxxiii. Schoen FJ. Pathology of heart valve substitution with mechanical and tissue prostheses. In *Cardiovascular Pathology*, 3rd Ed, Silver MD, Gotlieb AI, Schoen FJ (eds.), Churchill Livingstone, 2001, p. 629.
 - xxxiv. Rahimtoola SH. The next generation of prosthetic heart valves needs a proven track record of patient outcomes at \geq 15 to 20 years. *J Am Coll Cardiol* 2003;42:1717.
 - xxxv. Hammermeister K, et al. Outcomes 15 years after valve replacement with a mechanical versus a bioprosthetic valve: Final report of the Veterans Affairs randomized trial. *J Am Coll Cardiol* 2000;36:1152.
 - xxxvi. Schoen FJ. Approach to the analysis of cardiac valve prostheses as surgical pathology or autopsy specimens. *Cardiovasc Pathol* 1995; 4:241
 - xxxvii. Schoen FJ, Levy RJ. Calcification of tissue heart valve substitutes: progress toward understanding and prevention. *Ann Thorac Surg* 2005; 79:1072.
 - xxxviii. Marshall AC, et al. Aortic valvuloplasty in the fetus: technical characteristics of successful balloon dilation. *J Pediatr* 2005;147:424.
 - xxxix. Vahanian A, et al. Percutaneous approaches to valvular disease. *Circulation* 2004;109:1572.
 - xl. Sousa JE, et al. New frontiers in interventional cardiology. *Circulation* 2005;111:671.

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- xli. Block PC. Percutaneous mitral valve repair. Are they changing the guard? *Circulation* 2005;111:2154.
 - xliv. Lutter G, et al. Percutaneous valve replacement: Current state and future prospects. *Ann Thorac Surg* 2004;78:2199.
 - xlvi. Bonhoeffer P, et al. Percutaneous insertion of the pulmonary valve. *J Am Coll Cardiol* 2002;39:1664.
 - xlvii. Cribier A, et al. Early experience with percutaneous transcatheter implantation of heart valve prosthesis for the treatment of end-stage inoperable patients with calcific aortic stenosis. *J Am Coll Cardiol* 2004;43:698.
 - xlviii. Khambadkone S, et al. Percutaneous pulmonary valve implantation in humans. Results in 59 consecutive patients. *Circulation* 2005;112:1189.
 - xlix. Merryman WE, et al. The effects of cellular contraction on aortic valve leaflet flexural stiffness. *J Biomech* 2006;39:88. Epub 2005 Jan 7.
 - l. Merryman WD, et al. Correlation between heart valve interstitial cell stiffness and transvalvular pressure: Implications for collagen biosynthesis. *Am J Physiol Heart Circ Physiol* 2005. Epub ahead of print.
 - li. Rabkin-Aikawa E, et al. Heart valve regeneration. *Adv Biochem Eng Biotechnol* 2005;94:141.
 - lii. Vesely I. Heart valve tissue engineering. *Circ Res* 2005;97:743.
 - liii. Rabkin E, Schoen FJ: Cardiovascular tissue engineering. *Cardiovasc Pathol* 2002;11:305.
 - liiii. Hoerstrup SP, et al. Functional living trileaflet heart valves grown in vitro. *Circulation* 2000;102:III44.
 - liiii. Breuer CK, et al. Application of tissue-engineering principles toward the development of a semilunar heart valve substitute. *Tissue Eng* 2004;10:1725
 - liiii. Hoerstrup SP, et al. Functional growth in tissue engineered living, vascular grafts: Follow up at 100 weeks in a large animal model. *Circulation* 2006 (in press).
 - liiii. Sutherland FWH, et al. From stem cells to viable autologous semilunar heart valve. *Circulation* 2005; 111:2783
 - liiii. Shi Q, et al. Evidence for circulating bone marrow-derived endothelial cells. *Blood* 1998;92:362.
 - liiii. Frazier OH, et al. Immunochemical identification of human endothelial cells on the lining of a ventricular assist device. *Tex Heart Inst J* 1993;20:78.
 - liiii. Rafii S, et al. Characterization of hematopoietic cells arising on the textured surface of left ventricular assist devices. *Ann Thorac Surg* 1995;60:1627.
 - liiii. Lutolf MP, Hubbell JA. Synthetic biomaterials as instructive extracellular microenvironments for morphogenesis in tissue engineering. *Nat Biotechnol* 2005;23:47.
 - liiii. Deb A, et al. Bone marrow-derived myofibroblasts are present in adult human heart valves. *J Heart Valve Dis* 2005;14:674.
 - liiii. Simper D, et al. Smooth muscle progenitor cells in human blood. *Circulation* 2002;106:1199.
 - liiii. Matheny RG, et al. Porcine small intestine submucosa as a pulmonary valve leaflet substitute. *J Heart Valve Dis* 2000;9:769.
 - liiii. Simon P, et al. Early failure of the tissue engineered porcine heart valve SYNERGRAFT in pediatric patients. *Eur J Cardiothorac Surg* 2003;23:1002.