ELSEVIER

Progress in Pediatric Cardiology 21 (2006) 137 – 152

Progress in Pediatric Cardiology

www.elsevier.com/locate/ppedcard

From cadaver harvested homograft valves to tissue-engineered valve conduits

Richard Hopkins *

Collis Cardiac Surgical Research Laboratory, Rhode Island Hospital, and Brown Medical School, Division of Cardiothoracic Surgery, Department of Surgery, Providence, RI 02906, United States

Available online 9 March 2006

Abstract

As cardiac surgery enters its 4th Era, the era of bioengineering, tissue engineering and biotechnology, surgical solutions will embrace living tissue transplants, hybrid structures (inert+living) and biological implants that are really endogenous protein factories. The evolution of conduit surgery from Dacron tubes with pig valves through the wet stored homograft and thence cryopreserved homograft era, is also now entering the tissue-engineered heart valve era. Such constructs will likely resolve many of the lingering issues, which limit the durability and usefulness of heart valved conduits. The saga of this evolution captures in microcosm the revolution that will ultimately result in better treatments for children and adults with structural heart disease. The biological, developmental, and regulatory challenges are described so that an appreciation can be developed for the complexity of this new science and its integration into clinical use. The promise, however, far outweighs the barriers and represents no less than a dramatic new Era in cardiac surgery.

Keywords: Aortic valve; Congenital heart disease; Tissue engineering; Homografts

1. Introduction

Cardiac surgery is now on the dawn of the fourth major Era for the field. Since the initiation of true open-heart surgery utilizing cardiopulmonary bypass by Gibbon in 1953, there have been 3 distinct Eras [162]. The first Era, I term the *Anatomical Era*, as this was the age of recognition of the normal and pathologic anatomy of congenital and acquired cardiac disease. Surgeons developed numerous procedures and endeavored to restore normal cardiac anatomy assuming that normal function would follow. Ultimately, this included the repair and replacement of heart valves, the closure of septal defects, the opening of stenosis (e.g., coarctation repairs) and the closure of abnormal shunts (ligation of PDA, repair aorta pulmonary window). The *Pathophysiologic Era II* was marked by an increasing understanding of cardiac physiology/pharmacology and the exploitation of this knowledge to reduce perioperative mortality and morbidity, to increase overall safety of surgery and to establish surgical goals that were more physiologic rather than purely anatomic (e.g., cardioplegia, deep hypothermia total circulatory arrest, single ventricle Fontan physiology, Rastelli type conduit repairs and of course, coronary artery bypass surgery to improve myocardial perfusion). During *Era II* mortality rates ranged from 10% to 25% for various lesions. *Era III* (*Biochemical and Space Age Era*) built on the physiologic progress of *Era II* with increased understanding of the biochemistry of myocardial intermediate metabolism, pulmonary hypertension, ventricular biomechanics, etc. and also incorporated the rapidly expanding space age technologies of electronics, computer and chip controls circuitry, feedback algorithms, miniaturization, and the overall improvement of engineering designs. This impacted cardiac surgery with improved prosthetic heart valves; smaller and smaller pacemakers that did more and more, miniaturized and improved heart–lung bypass circuitry, better materials interfaces, and markedly improved anesthetic and postoperative care support systems and multi-organ pharmacology. In addition, imaging modalities progressed from cardiac catherization

* Tel.: +1 401 444 7700; fax: +1 401 444 3911.
E-mail address: RAHopkins@lifespan.org.

1058-9813/$ - see front matter © 2005 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.ppedcard.2005.11.002

and chest X-ray to high fidelity 3-D echocardiography, high resolution-fast CAT scanning and magnetic resonance imaging. It is now rare for the surgeon to go to the operating room without a very clear picture of not only the patient's anatomy but also physiology and an assessment of cardiopulmonary (and other organ systems) reserve. Increasingly adventuresome structural heart repairs have been developed utilizing multiple prosthetic, xenograft, and autologous materials (hypoplastic left heart, MAPCA unifocalizations, etc.). Concomitantly, statistically valid risk analysis and outcomes assessment have provided appropriate measurement methods for individual surgeons and programs to improve results and by which to assess the therapeutic value of newer techniques, drugs, modalities and materials. Raw mortality rates for many congenital lesions now approach zero, and overall rank between 1.5 and 4.7%. We are now entering *Era IV*, which I believe will be the era of *bioengineering*, *tissue engineering*, and *biotechnology*. Having surpassed the space-age electronic and computer engineering revolution, we are now entering a time when the engineering of living tissues, the construction of hybrid component parts (living cells+scaffolds-renewable or inert platforms) and the evolution of genetic understanding and the promise of genetic modification including cell-based gene therapy set the stage for an entirely new approach to surgery. Cardiac surgery will combine excellent technical skills with the always required superb decision-making capability of the heart surgeon leveraged with a thorough understanding of the science and methodology of molecular genetics, bioengineering, biotechnology, and overall adherence to principles which reduce surgical trauma, shorten and simplify surgical interventions, and yet reconstruct the heart in ways that are not only anatomically and physiologically salubrious but also recruit the benefits of living tissue perhaps genetically modified to reestablish cell and tissue populations to replace or support pathologic components of the cardiopulmonary and vascular systems.

This special monograph issue of *Progress in Pediatric Cardiology* is designed to provide both clinicians and academicians with a series of articles from world experts who are on the cutting edge of *Era IV*. Inherent to this new era is the development of cell, tissue and gene-based therapies that will be utilized with a surgical mindset to rebuild hearts in ways and dimensions that were previously unimaginable. The cardiac surgeon of the future will be truly the epitome of the clinician scientist. This author has pursued research for over 18 years aimed at developing a living, growing personal heart valve replacement specifically to resolve the issues surrounding conduit and valve surgery in infants and children but which might also be applicable to adults for certain diagnostic entities. However, tissue engineering goes far beyond valve replacements and includes small caliber arterial grafts, living heart muscle tissue replacements, bridging to transplant with a replacement hybrid ventricular power pumps, cell-based gene therapy, etc. A review of valve related tissue engineering follows and functions as an introduction to the articles in this issue by world experts in the field.

2. Tissue-engineered heart valve

The quest for the ideal cardiac valve replacement has been ongoing for approximately five decades; however, an FDA approved viable biological heart valve containing functional cuspal cells which are retained for the life of the patient, is not available. Cryopreserved allograft cardiac valves have viable cuspal cells present at the time of implantation; however, they are markedly reduced in number after implantation and eventually are lost resulting in an acellular valve. Despite acellularity and antigenicity, cryopreserved cardiac valves demonstrate surgical advantages compared to bioprosthetic xenografts [1,2]. Unfortunately, the appeal of these valves due to excellent hemodynamic performance is diminished by calcification, immune and inflammatory reactions, and ultimately structural failure [116]. A valve that retains the surgical and design advantages without the biological disadvantages of allografts and which has or promotes restoration of recellularization with appropriate host cells should represent the ideal valve replacement. The primary motivation is to develop a recell cardiac valve constructed from decellularization of allograft tissues suitable for ventricular outflow tract valve replacement, which will be seeded with cells that are genetically identical to those of the patient and will remain free from materials-related structural deterioration for a lifetime. Engineering design and material properties advantages are accrued by using native valves as decellularization scaffolds but will require replacing donor cells with specific recipient cells while avoiding degradation of either the material properties of the scaffold or the phenotypic and mitotic potential of the seeded or attracted (autologous recell) cells which would introduce new and novel failure mechanisms. The systematic identification of potential failure modes will lead to further bioengineering, materials science, cell and molecular biology, experimental pathology and surgical research to reduce or eliminate identified failures modes and risks impacting the safety and performance of a tissue-engineered aortic valve. Morbidity and mortality reductions will be significant. Reduced medical costs will be realized. The ultimate significance of this will be the acquired basic knowledge necessary to create a safe, biologically rational and hemodynamically functional tissue-engineered aortic valve for replacement of defective valves in children and young adults.

Heart disease remains the most frequent cause of death in Americans. Valvular heart disease is the primary etiology or a secondary component in at least 30% of patients with surgical heart disease. Safety and effectiveness of current generation of mechanical and bioprosthetic replacement cardiac valves have met thresholds of acceptability, but choice is often limited by specific anatomic and patient-related factors [4].

Despite antigenicity, acquisition/banking issues, cryopreserved allografts remain the best clinical option for many pediatric complex cardiac reconstructions [5]. In infants and children requiring ventricular outflow tract reconstructions, the availability of suitable valve conduits is restricted, while size and lack of growth are real clinical problems [6]. Manufactured valves are poor choices in children due to accelerated calcification of porcine valves [115] and need for anticoagulation with mechanical options. Cryopreserved allograft heart valves remain the most flexible option for complex ventricular outflow tract reconstruction in infants, children and young adults [7]. None of the currently available clinical options is perfect and all exhibit aspects of "prosthetic valve disease", sometimes with accelerated and catastrophic fibrocalcific failure.

The accomplishment of a viable personal heart valve will represent advancement, not only in the options for clinical cardiac surgical repairs, but also for the entire field of bioengineering. It clearly requires a combination of expertise in multiple disciplines including cell and molecular biology, tissue engineering, clinical surgery, tissue mechanics, cell signaling, chronic animal modeling, and thus represents a great challenge for scientists, clinicians and regulators. Discussions of evolving guidelines with the FDA (Hilton Head Conferences 2002–2005) recognized that this new era is fast approaching and proposed replacing traditional safety and efficacy engineering criteria (e.g., accelerated wear testing) with risk management and failure mode analysis approaches focusing on unique valve designs. The creation of a tissue-engineered aortic valve will establish, as a paradigm, an approach for tissue-engineered solutions to other clinical problems. The health significance is great as a living TE AoV could provide a permanent replacement for both children and young adults resulting in a reduction of re-operative surgeries, reduced mortality/morbidity, and improved hemodynamic performance combining resistance to infection with biological integrity, while limiting the need for anticoagulation. Conservative estimate of health care costs saved by development of a tissue-engineered aortic valve to substitute for current generation prosthetic aortic valves (n=30,000/year) and eliminating most re-operations to replace degenerated cryopreserved allografts totals 36 billion dollars/10 years for just US healthcare [8]. Eliminating multiple re-operations for infants and children will reduce their mortality and suffering.

2.1. Prosthetics and tissue engineering

An ideal replacement heart valve should have the following characteristics: 1) elicit no inflammatory or foreign body response; 2) non-immunogenic; 3) viable, having long-term durability and the capability to repair degenerated components; 4) available; 5) non-thrombogenic; 6) capable of somatic growth; and 7) design features adaptable to individual patient requirements [9]; 8) capable of remodeling to changing stresses both normal (e.g., aging related) and abnormal (e.g., pathological hypertension). Design and fabrication of previous generations of replacement cardiac valves (i.e., mechanical, bioprosthetic and allografts) have been limited by design features constrained by manufacturing capabilities, the availability of suitable biomaterials and regulatory demands [10,11]. A viable heart valve will be capable of somatic growth, renewal of the cell population, regeneration of extracellular matrix components and adequate hemodynamic performance for the lifetime of the patient have yet to be met, significant advances have been made [12–25]. Distinct heart valve design approaches are being explored in various laboratories, around the world using either biodegradable polymeric materials [12–28], ECM constructs, ECM hybrids or decellularization semilunar valve conduit scaffolds [19–25]. Current heart valve designs make use of a three dimensional structure (referred to as a scaffold) consisting of either a biodegradable *polymer* (e.g., polyglycolic acid, polylactic acid, polyhydroxyoctanoate) or a tissue-derived biomaterial. In addition to the use of polymeric and decellularization tissues, fibrin gel, gelatin, folded cell sheets (myofibroblasts; collagen) and elastin-collagen composite scaffolds are also being designed [26–28]. These heart valve designs are based on the expectation that scaffold material will recell with patient's autologous cells either before implantation (in vitro cell seeding) or after implantation (in vivo recellularization). Such procedures will make previously unexplained biological demands on the requisite cells; see article by Drs. Rhim and Niklason [164]. Such recellularized scaffolds would theoretically be capable of proliferating valvular cells and synthesizing extracellular matrix (ECM) components resulting in a viable tissue-engineered aortic valve satisfying the ideal requirements, on an ongoing basis.

2.2. Polymeric scaffolds

Initial efforts to develop a biodegradable polymeric scaffold consisted of the evaluation of polyglycolic acid (PGA) alone or in combination with polylactic acid (PLA) fabricated into the shape of a single pulmonary valve (PV) cusp, seeded with autologous vascular cells (mixed cell population — endothelial cells, fibroblasts, smooth muscle cells) and implanted in lambs [12–14]. The in vivo preclinical evaluation of PGA-PLA single pulmonary leaflet replacements was encouraging; however, attempts to replace the PV with a trileaflet PGA-PLA valve were not successful. Although rapid PGA-PLA degradation was observed (e.g., within 6 weeks), this leaflet design was limited by the rigid materials properties of PGA, which stimulated a search for a more flexible biodegradable polymer. Polyhydroxyoctanoate (PHO) was next identified as a possible leaflet material. PHO is a member of a family of polymers polyhydroxalkanoates (PHA). PHAs are linear polyester thermoplastics, biosynthesized by various microorganisms as well as by fermentation commercially. PHAs have the advantage of being quite flexible (e.g., % elongation, 1000)

as compared to PGA and PLA (% elongation < 5%). PHO can be made porous (potentially increasing cell adhesion and tissue ingrowth) by using a salt leaching technique [15]. Valve conduits have been fabricated from PHO and evaluated following implantation as a PV interposition grafts in sheep. After 24 weeks of implantation hemodynamic performance was noted to be adequate with mild regurgitation observed. Histologically fibrous encapsulation and ingrowth were observed in the conduit wall; however, PHO was still present in the leaflets after 6 months of implantation [16]. Although the materials properties of PHO were better suited for the fabrication of heart valve leaflets, the long biodegradation time exceeding 6 months is problematic. Residual PHO may impede recell and stimulate a chronic inflammatory fibrotic response. The ideal biodegradable polymeric scaffold material has not been identified. Efforts are continuing to discover novel materials possessing the requisite materials properties suitable for construction of a flexible leaflet tissue-engineered heart valve, resorbable within 6 weeks of implantation, yet provide a suitable scaffold for in vivo formation of a leaflet [29] without stimulating a chronic inflammatory response. Such barriers suggest that a polymer based scaffold will require more time to achieve than using a decellularization allograft "scaffold", but will provide a more flexible platform. See article by Dr. Fong and colleagues [165].

2.3. Tissue-derived scaffolds

Research on ECM (extracellular matrix) scaffolds has been occurring in parallel with biodegradable polymers. See article by Drs. Taylor, Cass and Yacoub [166]. Limitations imposed by synthetic biodegradable materials may be circumvented by the selection of a tissue-derived biomaterial capable of accommodating the mechanical stress of a semilunar valve. Decellularization (also referred to as acellular or devitalized) valves have emerged as useful tissues for the creation of a tissue-engineered aortic valve scaffold, especially since valve functional design issues are not an issue. Various approaches have been reported to remove the cellular components from semilunar valve tissue while retaining the majority of the ECM components (primarily collagens, elastin and the less water soluble proteoglycans). The following decellularization methods effectively remove endothelial and cuspal interstitial cells; however, cardiac myocytes and arterial wall smooth muscle cells are variably present after processing: 1) anionic non-denaturing detergents; 2) non-ionic detergents; 3) trypsin/EDTA and 4) deionized water. In addition to removing the cellular components, residual nucleic acids are also cleared from the tissue by DNase/RNase digestion. Tissues are washed to facilitate removal of cellular remnants and tissue processing reagents. Decellularization may also reduce the immunogenicity of tissues. Choosing the design of the evolved mammalian heart semilunar valves avoids many mechanical engineering issues and burdened only by having to relate performance of the tissue-engineered construct to performance characteristics of fresh human heart valves. Highly sophisticated descriptions of the viscous-elastic properties, relevant strength testing, flexural performance, mechanisms for growth, matrix degradation and turnover, parameters which control relative protein synthesis (e.g., MMP activity, collagen to elastin ratios responding to varying pressure, flow or sheer stresses) have only recently begun to be defined [30–39,116–157]. See article by Dr. Merryman and colleagues [167].

2.4. In vitro cell seeding versus autologous in vivo recellularization

Decellularization allograft valves with trypsin/EDTA have been reported followed by static in vitro seeding of the upper surface of the valve with autologous myofibroblasts (6 days) followed by endothelial cells (2 days) before implantation in sheep (pulmonary artery interposition graft). This method resulted in complete cuspal recell, fibrous sheath formation and a confluent endothelial cell layer on the surface of the cusp. Partial degeneration was observed in the unseeded decellularization control valves without evidence of cuspal interstitial cell repopulation [23]. Cell seeding in vitro has been observed to facilitate in vivo recell of tissue-engineered scaffolds independent of the type of biomaterial used [16–23]. A finding, which has stimulated our interest in the identification of autologous clinical cell sources for in vitro cell seeding of tissue, engineered scaffolds. Vascular tissues (carotid artery, jugular vein) have been used as cell sources in the majority of the animal studies reported. Dermal fibroblasts have recently been considered as an alternative cell source for seeding biodegradable scaffolds. Unfortunately, after 8–10 weeks of implantation (replacement of a single pulmonary valve cusp) the dermal fibroblast seeded leaflets are thickened and contracted [18], suggesting that these are not interchangeable with vascular myofibroblasts. Other investigations have explored bioreactor-based approaches to cell seeded CV structures and have developed early data on gene expression and ultimately protein synthesis by using either extracellular matrix proteins or mechanical factors. Phenotypic expression of cells presumably may be "directed" by the in vitro preconditioning environment. Alterations in extracellular adhesion proteins have been reported to change the phenotypic expression of smooth muscle cells [39]. Mechanical factors have been reported to alter intracellular cytoskeletal features, protein synthesis and proliferation: hydrostatic pressure, shear stress and stretch [28,40–46]. Such experiments have been performed but the parameters necessary to establish a phenotypically appropriate cell population and distribution density (including the trilaminar microanatomy of conduit wall and valve leaflets) are not yet fully defined [47]. Numerous studies have indicated that bioreactor environments will have to be carefully tuned to regulate phenotypic expression, migration, and distribution of such cells. The information in this field is

in its early phases of acquisition. Assuming that in vitro cell seeding could be avoided, then, the alternative pathway is to direct in vivo (after implant) autologous recellularization to reestablish the normal distribution of phenotypically appropriate cells within the valve complex (leaflet and conduit) [48]. This will be accomplished within the context of the clinical realties of valve replacement surgery and the limitations of tissue transplantation including the logistics of supply, preparation at the time of surgery, availability of appropriate sizes, banking, etc. Clearly the preponderance of evidence suggests that an earlier realization of a tissue-engineered heart valve construct will be accomplished by utilizing some form of a decellularized human heart valve as scaffold [49], which may ultimately be replaced by hybrid "manufactured" scaffolds.

2.5. Immune response in cryopreserved homografts (containing viable and dead cells, cell debris, proteins)

A major myth of the cryopreserved cardiac valve transplant era was that these allografts were essentially "immunologically privileged", thought in part due to myofibroblasts being "buried" within the matrix. This mythology led to some commercial processing performed with modifications intended to enhance viability, not only of the myofibroblasts, but even of endothelium. This approach contrasted with other techniques (also used clinically and promulgated by us) in which human allograft valves were intentionally exposed to solutions to remove endothelium, while the remainder of the processing steps preserved smaller populations of viable myofibroblasts. It has now been shown in both human and animal studies that allografts with excessively retained cellular material typically elicit immune responses [76]. Cryopreserved allografts have decreased durability in younger children (especially neonates), which has been attributed to an accelerated immune response-mediated valve failure [50]. Both T cell and B cell infiltration has been demonstrated [50,51] as well as a persistence of human leukocyte antigen (HLA) antibodies in children receiving cryopreserved allografts [52–55]. Proliferative and inflammatory responses provoke myo-intimal proliferation, which either may be a passive fibrous sheathing phenomenon attributed to inflammation or to a low grade immune response [50,53,56]. Shaddy and coworkers did not find ABO compatibility to be associated with shortened durability in their study of allografts in the pulmonary position [57] but other more compelling studies suggest that ABO is important. See article by Drs. Christenson and Kalangos [168]. Other workers have demonstrated persistently positive panel reactive antibodies suggesting a significant HLA antibody response to implantation of allograft valves in both children and adults which supports an immune component to the inflammatory response [58–66]. Hawkins has presented a follow-up of their earlier study on the response of HLA antibodies to cryopreserved valved allografts in pediatric heart surgery by performing panel reactive antibodies before and after surgical implantation of partially decellularized allografts [67]. In this study in which human allograft valves were supposedly decellularized to an effective cellular debris removal of between 80 and 87%, there still was a moderate HLA antibody response, suggesting that even minimal retention of nuclear or cell wall material elicits antibodies; this has been confirmed by our own studies. Thus, the concept of immune privilege has been shown to be untrue and any cells, which might be spared metabolic, morphologic or necrotic doom, are susceptible to immune attack [68]. Mitchell [69] assessed the post-transplantation status of cryopreserved allograft valves compared to those transplanted as a part of whole heart replacement, and demonstrated that the cryopreserved valves showed early cell ultrastructural degeneration with complete acellularity at later time-points and morphological alterations of matrix; while, in contrast, the aortic valves of orthotopic heart transplants retained near normal cell and matrix morphology with no evidence of the injuries exhibited by cryopreserved valves. The valves within the heart transplant displayed apparently normal cellularity, protected by antirejection medication as well as having short harvest to transplant time and no preimplantation processing. In addition to the obvious allograft immune issues related to cells and retained cellular fragments, one group [70] from France has demonstrated immunogenicity of extracellular matrix in xenograft cardiovascular tissues. Importantly, despite immunogenicity, the terrific surgical and specific performance advantages of allograft semilunar cardiac valves have been recognized by reconstructive surgeons worldwide [2,84–86]. However, these valve "transplants" have by and large crossed histocompatibility and ABO constraints and while performing well in the short–medium term have been associated with ultimate fibrosis and failure in a significant proportion of cases, especially in those patients for whom the desirability of a living transplant would be the greatest: neonates, infants, and young children, i.e. for whom retained growth and repair functions would be ideal [101,112,114,158]. Thus, there have been many attempts to modify homograft valve transplants at either the donor or recipient level to achieve relative or actual immune tolerance with the thought that this would retain the outstanding engineering design of the native semilunar valve while avoiding the inevitable foreign body reaction seen when antigenic or proinflammatory materials are transplanted. No truly satisfactory solution has been achieved for current clinically used cryopreserved valves, which clearly remain proinflammatory and antigenic [71–75,161].

3. Scaffold design driven by immunology and materials properties

Choosing a scaffold can be a dichotomous decision between a derived human extracellular matrix (ECM)

functional semilunar valve (i.e. decellularization homografts) versus a fabricated valve from polymers or polymer ECM hybrids. Polymer based fabrication is quite appealing from a manufacturing standpoint with the ability to create an infinite variety of sizes, lengths, and etc. but suffers from two very difficult barriers. The polymer fabrication will have to replicate the performance characteristics of the fresh native valve and no such polymer or fabrication process has yet been developed [73]. Even if such a process could be discovered, the degradation of a polymer requires inflammatory destruction by macrophages, WBCs, etc., and replacement of the polymer structure with host proteins. While such has been accomplished in relatively inert structures such as bone and cartilage, there is insufficient knowledge currently available to control the foreign body scar response to do anything more than create a fibroblast reaction rather than establishing a normal trilaminar structure consisting of appropriate proportions of extracellular matrix, structural proteins and multiple cell-type populations distributed in density and location typical for a functioning heart valve [74]. This leaves the biological semilunar valve as perhaps the easiest initial pathway to pursue. Assuming that the tissue-engineered valve will be based upon a biological extracellular matrix scaffold derived from a functional valve, should it be an allograft only (i.e. human) or can xenografts be modified by the decellularization process to work satisfactorily? Obviously the latter would simplify acquisition issues although perhaps complicated by disinfection criteria. The group from Vienna emphasized with elegant laboratory methodology the previously suspected antigenicity of xenograft (ECM) proteins [75]. See article by Dr. Simon and colleagues [169]. This has been previously suggested by in vitro, in vivo animal studies, and human assays [37,76–78]. The clinical experiment with the implantation of decellularized *xenograft* heart valves (Synergraft®) has only emphasized the risk of proceeding down pathways absent full understanding of the potential for extracellular matrix provocation of the immune as well as innate non-specific inflammatory responses [79]. In fact, a partially "decellularized" xenograft scaffold is likely to be far more inflammatory than current versions of cryopreserved homografts (i.e. with partially retained but intact and viable matrix donor cells). Human or "processed" xenograft scaffolds are almost certainly more proinflammatory when cells are disrupted and necrotic cellular debris, cytokines, and other inflammatory moieties are not thoroughly removed from the matrix [76]. Such concerns are supported by our own studies using a sensitized monocyte cytokine assay [75]. These demonstrated that the lowest level of stimulation was with thoroughly "decellularized" human tissues, while partially decellularized leaflets were far more attractive (stimulated macrophage response) than human native pulmonary cusps that had not been decellularized. Thus, the Vienna work along with our own studies, suggests this decision path leads to the selection of human allograft (and not xenografts) as basis for decellularization technologies designed to obtain functioning valve ECM scaffolds for tissue engineering of heart valves. See article by Dr. Simon and colleagues [169], and Drs. Taylor, Cass, and Yacoub [166]. Such "tissue/cell engineering" projects will result in a large amount of knowledge that will be acquired at all levels including surgical technique, physiology, bioengineering, molecular biology, cell–cell signaling, immunology, cell differentiation, etc. The "no lose" aspect of this line of investigations is such that even if a tissue-engineered heart valve (by strict definition of such) is not achieved, studies such as these will, at the minimum, rationalize the process for improving allograft valves for use in complex cardiac reconstructions, as well as providing the knowledge base for other constructs [17,82,83]. See article by Johnson, Barabino, and Nerem [170].

The Synergraft® experience illustrates the first two putative major failure modes. This proprietary "decellularization" method was marketed as an antigen reduction method, thinking it resulted in an otherwise equivalent valve to the clinically familiar cryopreserved homograft. Clinical implants performed with xenografts treated by Synergraft® technology were often followed by rapid fibrocalcification, rejection and accelerated valve failure as might have been predicted by surrogate marker assays — including MHC I and II staining of preimplant materials, provocable PRA titers in vivo and sensitized macrophage cytokine release assay [79]. Interestingly, the human allograft version of "Synergraft" seems to also fail in some patients with (especially pediatric) accelerated fibrous scarring (in some cases resulting in atretic arches within months of Norwood I repairs) or acute ruptures with hemorrhage leading to death and disability suggesting weakened tissue material properties (personal communications Congenital Heart Surgeon's Society 2003, 2004). Somewhat more encouraging short-term results have been reported in adult recipients of the human allograft Synergrafts® [163].

Both the FDA Prosthetic Valve Guidance document: 1994, and ISO 5840: 2005 suggest that before a biological heart valve be considered for a human trial, it should be tested in a large animal model and strongly recommend juvenile sheep for tissue-engineered valves. This seems especially prudent for valves in which collagen is not cross-linked [158,159]. Macrophage stimulation studies demonstrate that the animal model will have to utilize a tissue-engineered valve based on an allograft scaffold *within species*. The methods must then be replicated with technology transferred to human valve constructs, which only then can be tested in humans. Without following such a sequential "road map" to a tissue-engineered aortic valve, mistakes can occur [49,79]. The juvenile sheep implant is a harsh test for biological valves as any propensity for inflammation is rewarded with calcifications and thus it has been the most reliable surrogate marker animal implant model for ultimate performance in humans [3]. While it might be argued that a primate model could be required

before approval of a tissue-engineered valve for human trials, emphasizing the use of ovine cells, tissues, and recipients will pave the way for future studies [49,80,81].

4. Cryopreserved homografts

In 1956 Gordon Murray reported the use of a fresh aortic valve homograft transplanted into the descending thoracic aorta for amelioration of the consequences of native aortic valve insufficiency [92]. His initial operation preceded by 5 years the availability of the Starr Edwards mechanical aortic valve prosthesis. In 1962, the initial clinical use for aortic valve homografts was reported independently by Sir Donald Ross of England and Sir Brian Barratt-Boyes of New Zealand. Limitation of donor availability led to preservation attempts to increase cadaveric harvest and to increase storage times by establishing homograft valve banks. Multiple storage techniques were evaluated and ultimately the most successful proved to be cryopreservation using cryoprotectants (particularly DMSO) and storage in liquid nitrogen vapor at −180 °C. Just as the development of conduit surgery revolutionized the field of congenital heart surgery, the development of human tissue-based valves and conduits simplified conduit surgery and complex pediatric valve replacements such that very complex operations could be developed for which previous available clinical materials were unsatisfactory (e.g., the homograft patch use in the "Norwood" operation, Ross procedures, homograft conduits with unifocalization, etc.). Multiple clinical series have demonstrated outstanding architectural outcomes but unfortunately for the subgroups most needing such homograft materials, the durability was the shortest (neonates, infants and young children) [5].

Initial studies noted that clinical harvesting of allograft cardiac valves necessitates a time period of warm ischemia, corresponding to the time from cessation of donor heartbeat to the time of transport, first characterized in porcine aortic valve leaflets by magnetic resonance spectroscopy [84–87]. Cryopreserved processing related metabolic markers of cell injury were assessed biochemically via HPLC analysis of adenine nucleotide pool [88–90]. These experiments suggested that while many cells within cryopreserved homografts might be morphologically intact at the time of implantation, they were likely "doomed" to early death simply from metabolic depletion. Similar findings were confirmed in human leaflet cells [91–93]. These experiments supported the concept of a *"stunned" leaflet cell population* [94,95]. Incubation of thawed cryopreserved homograft valves in culture (37°C, media with 15% serum) for 8 days could restore normal cell population, energy reserves and matrix composition to the leaflets [96].

We have developed multiple large animal models to establish the implant biology of *cryopreserved "homografts"* [97]. Using the ovine model, fresh and cryopreserved allograft cardiac valves were explanted after various time intervals ranging from 2 days to 20 weeks. After 20 weeks of implantation, the cryopreserved cusps were essentially acellular and the valvular surfaces covered by fibrous sheath. This is similar to the repeatedly documented fate of cryopreserved homografts in humans (Fig. 1). Following 30 days of implantation, few viable leaflet cells were present; however, apoptotic bodies were observed in the cuspal tissue [97]. Leaflet interstitial cells exhibited losses in proliferating cell nuclear antigen (a marker of mitotic function), positive nick-end labeling, nuclear condensation, pyknosis, and formation of apoptotic bodies containing remnants of nuclear material. This evidence of mitotic cessation and apoptosis was detectable by 2 days following implantation. It reached a peak at 10–14 days, and by 20 weeks, grafts were essentially acellular. Explanted leaflets were routinely encapsulated with fibrous sheath — a finding also seen in human explants. Our work indicates that long-term viability is limited by two mechanisms. First, the early phase cell survival is likely low following transplantation due to limited cell energy reserves. Second, either the stress of preimplantation processing or the abnormal environment in which the injured leaflet cells exist following transplantation triggers apoptosis [84–86,98–100]. Of course, non-necrotic apoptotic cell death would by definition limit host inflammatory response and thus minimize fibrocalcification.

So why do homografts work [109]? The current evidence suggests that the better cryopreservation processing protocols can be designed to strip endothelium and reduce acute matrix cell viability to approximately 40% presumably by leaching and removing the most superficial cells. When this occurs with minimal stimulation post-transplantation, although panel reactive antibodies may rise, the slow foreign body response of the body is to sheath the implanted valve with pliable layers of fibroblasts and ultimately a pseudointima or even true endothelial cells. This further segregates the antigenic cells of which the vast majority ultimately die an apoptotic cell death, which in of itself is non-inflammatory. Thus, the homograft valve actually functions as a mandrel on which the body constructs its own fibrous functioning heart valve. Ultimate durability is limited by calcification. The more donor endothelium or HLA-ABO rich endothelial and matrix cells that remain after the processing, the greater the rejection phenomenon and the faster fibrocalcific deteriora-

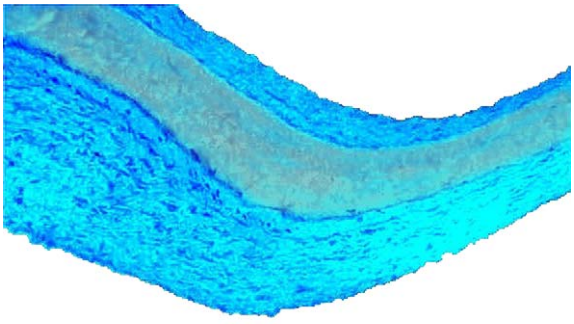

Fig. 1. Cryopreserved leaflet explanted after 20 weeks. Note acellular donor leaflet "sheathed" with recipient fibrous tissue.

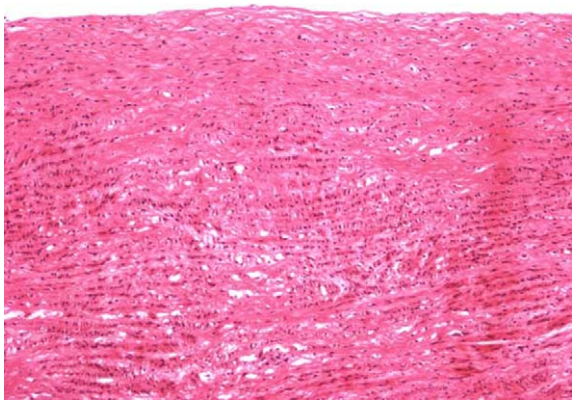

Fig. 2. Aortic valve (fresh ovine) sinus wall pretreatment H&E. 200×.

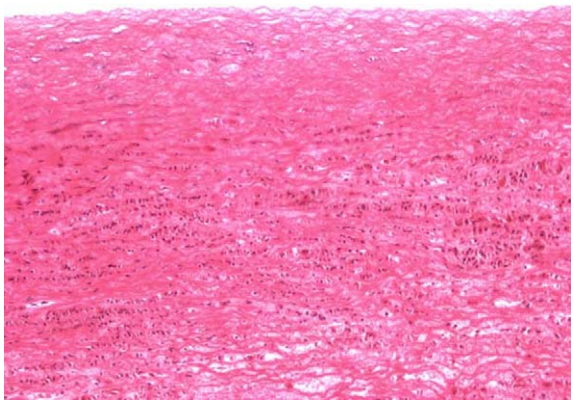

Fig. 4. Aortic valve (fresh ovine) sinus wall s/p water wash H&E. 200×.

tion will occur. In addition, the age of the patient has clearly demonstrated a relationship to the aggressivity of the fibrocalcific degeneration suggesting a heightened immune response the younger the patient. This theory will account for the extreme variability seen in valve function and durability in both the right and left ventricular outflow tract positions which varies from lifelong durability to very rapid deterioration (especially in neonates with effective performance limited to 1–2 years). The evidence for this theory is overwhelming but also provides a scientific basis for pursuing a decellularized homograft as a useful scaffold material that in of itself would not meet the criteria of a tissue-engineered heart valve (i.e. scaffold + seeded cells) but would certainly qualify as an improved homograft as long as degradation of the structural matrix protein during processing has not resulted in material safety issues due to structural weakening or enhanced propensity for deterioration [155].

5. Decellularized scaffolds for tissue-engineered valves

Since our original publications investigating pulsatile material properties of the pulmonary artery [104–108], we and others have increasingly focused on viable living heart valve replacements. Initially the focus was on the RVOT reconstructions and methods for cryopreservation [87,110–114]. As part of this process it became apparent that the dominant aortic valve interstitial cell had dual structural and functional phenotypes and were properly typed myofibroblast [47]. Our laboratory in collaboration with Dr. Hilbert went on to establish that the cell fate of cryopreserved viable leaflet cells was apoptosis rather than necrosis [97]. This led us to develop cell restoration in vitro [87–91,96,114,132]. Our laboratory embarked on developing appropriate large animal models for testing both recellularized as well as acellular tissue-engineered valves in the ventricular outflow tract positions beginning with echo and cardiac catherization protocols for establishing appropriate functionality [121,131,157]. We further defined the high fidelity pressure flow measurement computations for relating hydraulic impedance elasticity and geometry in the pulmonary arteries [120]. Subsequently, we developed an approach for sourcing autologous cells for cell seeding [48,121] and further evaluated and modified the decellularization technologies currently extant [35–38] as well as investigating other materials such as photo-oxidized pericardium and sheep intestinal submucosa [131]. And finally our laboratory has established the inflam-

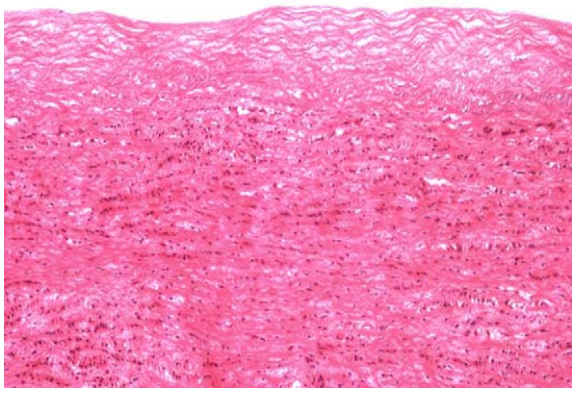

Fig. 3. Aortic valve (fresh ovine) sinus wall s/p detergent Rx 1 H&E. 200×.

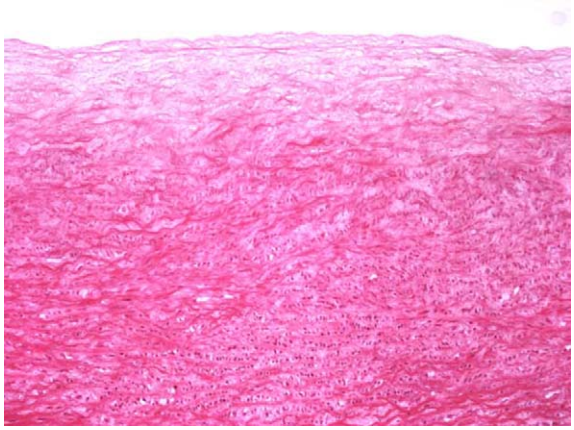

Fig. 5. Aortic valve (fresh ovine) sinus wall s/p endonuclease Rx H&E. 200×.

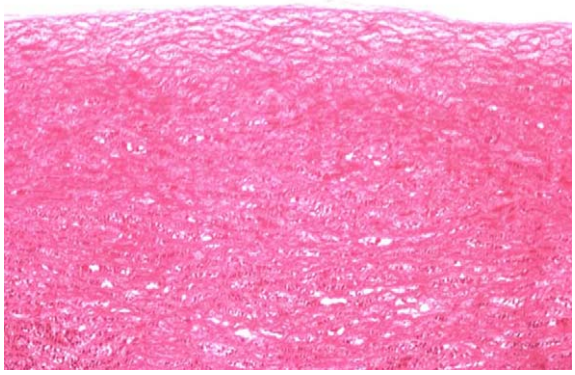

Fig. 6. Aortic valve (fresh ovine) sinus wall/p detergent Rx 2 H&E. 200×.

Fig. 8. Macroscopic appearance of decellularized valve preimplantation.

matory potential of scaffolds which are not fully decellularized and free of all antigenic debris by relating the retention of MHC I and II stained material to the development of ovine panel reactive antibodies in chronically implanted sheep valves [37]. Decellularized scaffolds retain inflammatory potential when not adequately prepared. We have demonstrated this both in vivo and with in vitro surrogate markers establishing separate assays for the immune as well as the innate inflammatory stimulation. Conversely, we have established that appropriately decellularized semilunar valve material will recellularize following chronic implantation into sheep [38] (Figs. 2–14). And further we have defined that recellularization appears to occur in waves with the majority of the myofibroblasts migrating in from the adventitial side of the implanted valve as a wavefront (Figs. 12–17). In-migration appears to be initiated by a wave of macrophages followed by a wave of myofibroblasts with pronounced αsma at the front end of the wave and increasing collagen production at the back end of the wave as marked by the collagen chaperone protein colligin HSP-47. With the current versions of decellularized scaffolds, no calcification occurs except in the areas of suture injury. We have investigated subtle hydraulic energy consequences of differences in compliance and changing dimensions during the cardiac cycle and established that unstented valves (i.e. homografts, tissue-engineered valves) have significant advantages in terms of ventricular work requirements. While teleologically it makes sense that the valves in the higher-pressure environments are expressing increased stiffness and which correlate with increased expression of αsma and HSP-47, the exact mechanisms by which the cell "knows" to respond require further elucidation [160].

These assays have demonstrated a correlation between developing ovine panel reactive antibodies titers following "decellularization" valves implanted chronically in juvenile sheep and the amount of residual HLA stainable antigenic debris (MHC I and II IHC) [37]. In addition, a quantitative surrogate marker assay for non-specific inflammatory potential has been developed based on cytokine release (ELISA, Western blots) by sensitized monocytes [71]. These indicate that refined decellularization processing methodologies result in non-antigenic decellularization scaffolds, which provoke a macrophage infiltration but *not* lymphocytes. As a surrogate marker for collagen synthesis we have developed IHC, Western blots, and ELISA methods for determining HSP-47 (colligin) protein expression as a

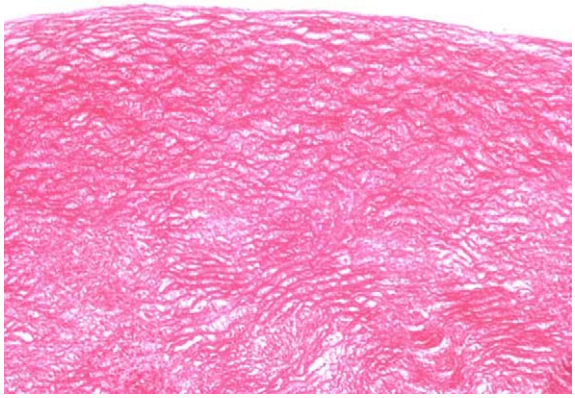

Fig. 7. Final decellularized H and E ovine sinus wall (aortic valve) H&E. 200×.

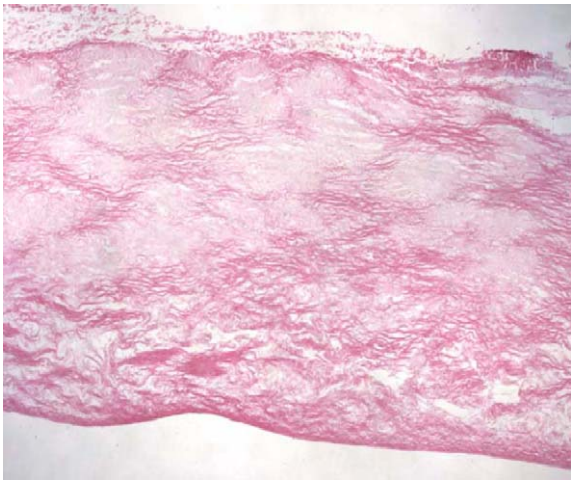

Fig. 9. Preimplant decellularized PA conduit wall H&E. 400×. Decellularization method different from that depicted in Figs. 2–8.

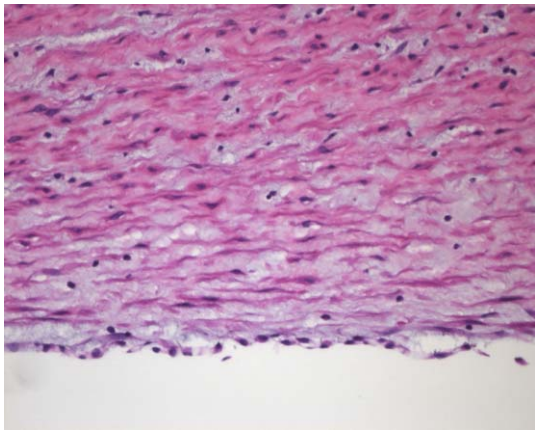

Fig. 10. Decellularized conduit wall (PA) explanted s/p 10 weeks from ovine aortic position.

measure of synthetic activity for collagen types I and III (which together account for >98% of valve collagen). HSP-47 is an endoplasmic reticulum chaperone protein linked to procollagen expression with augmentation of collagen production by increased HSP-47 levels such that any perturbation that selectively alters the amount of HSP-47 in the ER "resets" the amount of collagen produced [102,103,31]. This makes it an excellent quantitative and qualitative surrogate marker for identifying myofibroblasts responding to increased mechanical stresses by increasing structural protein (collagen) synthesis [160] (Figs. 12–17).

We have also focused on developing a series of tests (i.e. *surrogate markers*) to qualify (actually to disqualify) putative scaffolds. Qualifying criteria for scaffolds are based on failure modes and end effects analysis (FMEA) and our experience with biological heart valves (processing effects and related explant pathology), to identify appropriate surrogate markers for successful tissue self-regeneration that include:

Appropriate cell phenotypes
Cell densities

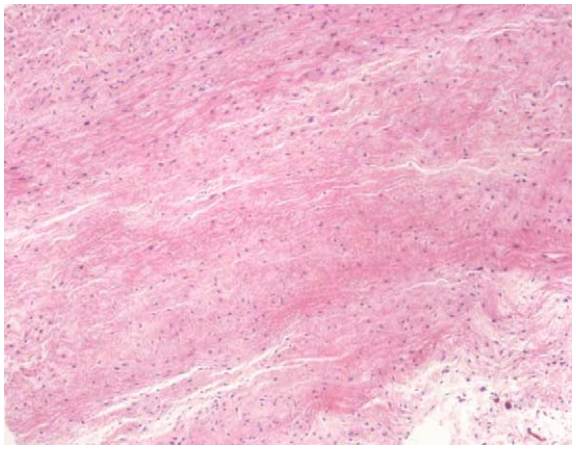

Fig. 11. Note the myofibroblasts migrating into sinus wall, neointima formation and abluminal healing H&E. 400×.

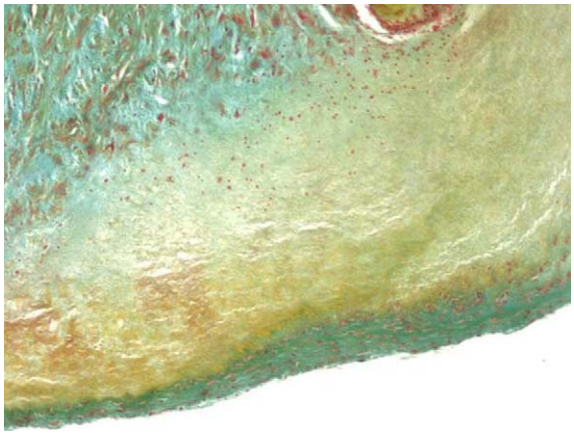

Fig. 12. Separate sections of 20-week explanted decellularized pulmonary conduit wall ECM scaffold: the Movat's stain demonstrates myofibroblasts and monocytic cells infiltrating the acellular scaffold. Newly synthesized ground substance (i.e. soluble proteins, etc.) stains blue-green and collagen stains yellow (Fig. 13); vimentin monoclonal marks fibroblasts that are also colligin (HSP-47) positive (Fig. 14), while the acellular unpenetrated portion of ECM scaffold is devoid of markers for viable myofibroblasts. 100×.

Cell location
Cell migration and orientation–structural protein synthesis
Protein turnover and renewal moieties
Soluble proteins synthesis
Cell proliferative capacity

For the reasons established above, models must be developed using the same species for the donor scaffold and host–recipient in vivo testing. Thus, the allograft semilunar valve as the classic study allograft valve for scaffolds must first be defined in the ovine model to

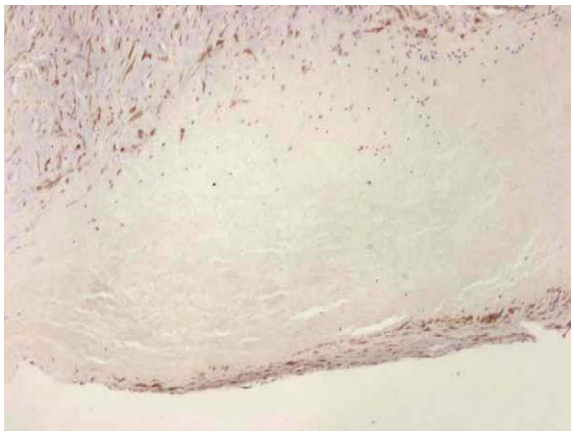

Fig. 13. Separate sections of 20-week explanted decellularized pulmonary conduit wall ECM scaffold: (Fig. 12) the Movat's stain demonstrates myofibroblasts and monocytic cells infiltrating the acellular scaffold. Newly synthesized ground substance (i.e. soluble proteins, etc.) stains blue-green and collagen stains yellow; (Fig. 13) vimentin monoclonal marks fibroblasts that are also colligin (HSP 47) positive (Fig. 14) , while the acellular unpenetrated portion of ECM scaffold is devoid of markers for viable myofibroblasts. 100×.

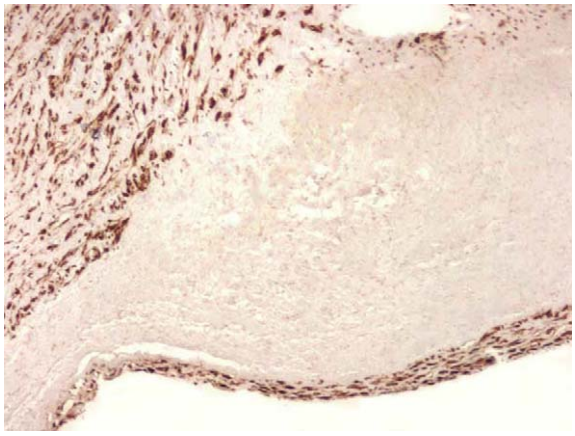

Fig. 14. Separate sections of 20-week explanted decellularized pulmonary conduit wall ECM scaffold: (Fig. 12) the Movat's stain demonstrates myofibroblasts and monocytic cells infiltrating the acellular scaffold. Newly synthesized ground substance (i.e. soluble proteins, etc.) stains blue-green and collagen stains yellow; (Fig. 13) vimentin monoclonal marks fibroblasts that are also colligin (HSP 47) positive (Fig. 14), while the acellular unpenetrated portion of ECM scaffold is devoid of markers for viable myofibroblasts. 100×.

validate the surrogate markers for extension to human cells and tissues. Such qualifying tests are grouped into three groups, which are serially tested moving from in vitro surrogate markers for failure to in vivo functional testing, this sequence is efficient and since the scaffold must qualify on all tests to be considered, each is necessary but not sufficient alone to pass a putative ECM scaffold [37,38].

There are basically three primary failure modes for living valve transplants and tissue-engineered valves include 1) calcification and fibrosis leading to stenosis and scarring (i.e. clinically analogous to developing degenerative or inflammatory aortic stenosis and prosthetic valve stenosis), 2) early material failure and fatigue (clinically analogous to aneurysm formation and rupture),

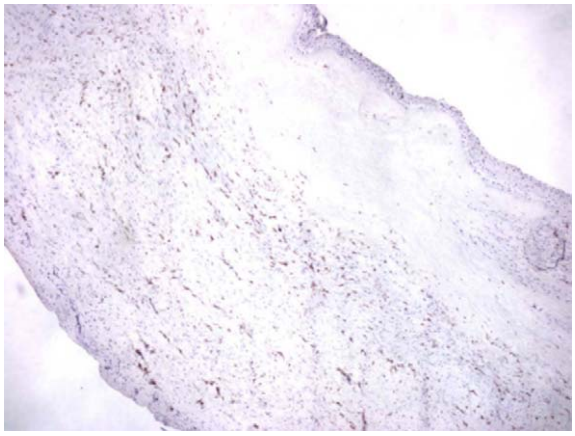

Fig. 15. Immunohistochemical staining for macrophages in explanted (20 weeks) decellularized tissue demonstrates cells throughout the fibrous sheath with higher cell densities along a "wavefront" at the migration front into the decellularized tissue interface. 200×.

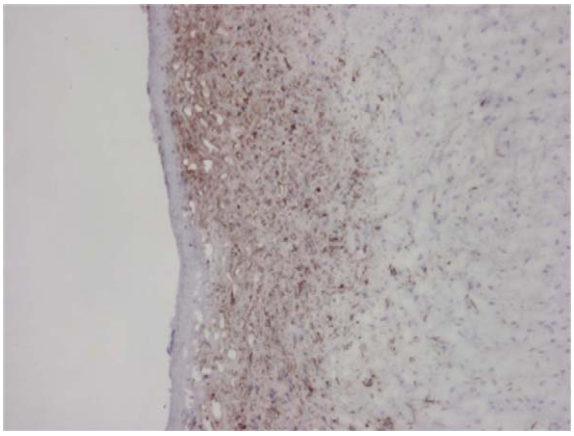

Fig. 16. Alpha smooth muscle actin immunohistochemical staining of 20-week explanted decellularized conduit wall demonstrates an advancing wavefront of biologically active cells from the adventitia to the luminal aspect. Cells "behind" wavefront demonstrate less smooth muscle actin staining especially at the origin of the cells for migration within the adventitia. 200×.

3) failure to recellularize or maintain recellularization and thus failure to establish a true living tissue-engineered aortic valve with regenerative capacity thereby leading to chronic deterioration and failure.

6. Decellularization methodologies

After testing numerous decellularization strategies in both ovine and human tissues with the critical endpoints of retaining strength characteristics and also being devoid of cells and cellular debris by MHC I and II staining, we have determined that such a strategy is achievable starting with fresh valves and results in scaffolds that are minimally stimulatory by our sensitized macrophage cytokine release assay, do not provoke measurable PRA responses in sheep,

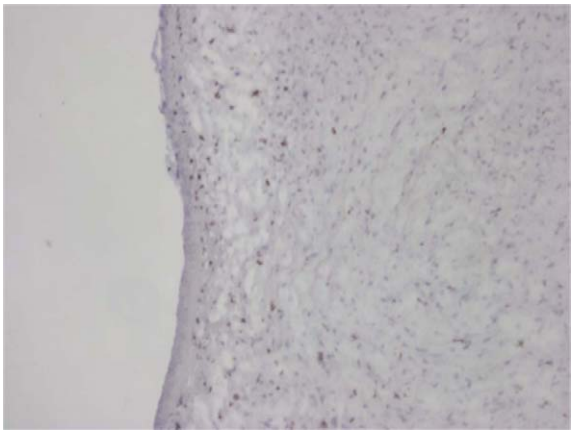

Fig. 17. Immunohistochemical macrophage staining of 20-week decellularized tissue explants demonstrates macrophages. While distributed throughout tissue matrix, the higher densities are at the "advancing edge" of recellularization wavefront suggesting that the macrophages "lead" or at least accompany the migrating myofibroblasts. 200×.

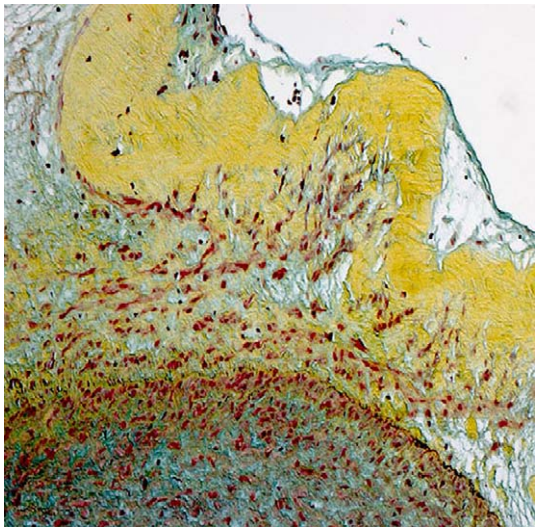

Fig. 18. Decellularized ovine pulmonary valve sinus wall and leaflet in RVOT. Note cells streaming into base of leaflet from sinus wall. Myofibroblasts repopulating spongiosa. 200×.

have no cell toxicity in vitro, are attractive static cell culture substrates for valve interstitial cells (vics) and endure the rigors of implants as functioning valves in the ovine large animal model [71]. Methods such as those developed by LifeNet Tissue Services (Norfolk, VA), which are anionic detergent-based result in excellent decellularization and progressive in-vivo recellularization with a scaffold expressing requisite salubrious characteristics (Patent #US 6,743,574) (Fig. 18).

7. Hybrid and bioengineered solutions

If there is a primary focus to this volume of *Progress in Pediatric Cardiology*, it is the tissue-engineered heart valve. I have chosen this specifically because of my own research and clinical experience in the area and also that it functions well as a paradigm for such technologies of the future. The application of knowledge gained in the development of the tissue-engineered heart valve is, like so many things, almost unimaginable in their depth and breadth. Solving the problems and challenges of this type of tissue engineering opens the field to tissue-based gene therapy, hybrid components, replacing tissues with constructs capable of growth and healing. Such additional wonders of *Era IV* are suggested in the review of *State-of-the-Art for Ventricular Assist Devices for Children* by Tim Baldwin and Brian Duncan of the National Heart, Lung and Blood Institute [171]. Bioengineered muscle constructs as a basis for both tissue reconstruction as well as cell-based gene and protein therapy are reviewed by Dr. Vandenburgh [172]. Such constructs in and of themselves and as platforms for even more awesome therapies will find development and clinical applications more rapidly than can be imagined. This will not only benefit patients but will also stress the regulatory and product development cycles as they raise theoretical risk and failure modes not applicable to traditional devices or biologics.

That such is the future is also suggested by numerous and readily apparent changes within the field of cardiac surgery. First, engineering undergraduates are avidly sought by medical schools. Secondly, virtually all of the major journals in cardiac surgery have added sections for manuscripts dealing with “evolving technologies”. And finally additional journals are being born that transcend traditional fields and are read by engineers, cardiac surgeons, cardiologists, fundamental biologists and converge on this evolving knowledge of base (e.g., *Tissue Engineering* and *Journal of Heart Valve Disease*). It has been a privilege to guest edit this monograph issue of *Progress and Pediatric Cardiology* and it is my wish that this will stimulate interest in cross-disciplinary discovery, research and development. The ultimate goal is to improve our options for treatment of congenital and structural heart disease.

References

[1] Grunkemeier GL, Bodnar E. Comparison of structural valve failure among different “models” of homograft valves. J Heart Valve Dis 1994;3:556–60.
[2] Grunkemeier GL, Bodnar E. Comparative assessment of bioprosthesis durability in the aortic position. J Heart Valve Dis 1991;6:534.
[3] Gallegos RP, Nockel PJ, Rivard AL, Bianco RW. The current state of in vivo pre-clinical animal models for heart valve evaluation. J Heart Valve Dis 2005;14:423–32.
[4] Schoen FJ, Levy RJ. Tissue heart valves: current challenges and future research perspectives. J Biomed Mater Res 1999;47:439–65.
[5] Hopkins RA. Cardiac reconstructions with allograft tissues. New York: Springer-Verlag; 2005. 655 pages.
[6] Mayer Jr JE. Uses of homograft conduits for right ventricle to pulmonary artery connections in the neonatal period. Semin Thorac Cardiovasc Surg 1995;7:130–2.
[7] Yacoub M, Rasmi NRH, Sundt TM, Lund Oea. Fourteen-year experience with homovital homografts for aortic valve replacement. J Thorac Cardiovasc Surg 1995;110:186–94.
[8] Clarke DR. Presidential address: value, viability and valves. J Thorac Cardiovasc Surg 2002;124(1):1–6.
[9] Harken DW, Curtis LE. Heart surgery: legend and a long look. Am J Cardiol 1967;9:393–400.
[10] Gross L, Kugel MA. Topographic anatomy and histology of the valves in the human heart. Am J Pathol 1931;7:445–73.
[11] Walther T, Walther C, Falk V. Early clinical results after stentless mitral valve implantation and comparison with conventional valve repair or replacement. Circulation 1999;100(19 Supplement):II78–83.
[12] Shinoka T, Ma PX, Shum-Tim D, et al. Tissue-engineered heart valves: autologous valve leaflet replacement study in a lamb model. Circulation 1996;94:II-164.
[13] Shinoka T, Shum-Tim D, Ma PX, et al. Creation of viable pulmonary artery autografts through tissue engineering. J Thorac Cardiovasc Surg 1998;115:536–46.
[14] Stock U, Nagashima M, Khalil PN, et al. Tissue-engineered valve conduits in the pulmonary circulation. J Thorac Cardiovasc Surg 2000;119:732–40.
[15] Shum-Tim D, Stock U, Harkach J, et al. Tissue engineering of autologous aorta using a new biodegradable polymer. Ann Thorac Surg 1999;68:2298–304.

[16] Hoerstrup SP, Sodian R, Daebritz S, et al. Functional living trileaflet heart valves grown in vitro. Circulation 2000;102(suppl):III-44–9.
[17] Hoerstrup SP, Sodian R, Sperling JS, Vacanti JP, Mayer JE. New pulsatile bioreactor for in vitro formation of tissue engineered heart valves. Tissue Eng 2000;6:75–9.
[18] Shinoka T, Shum-Tim D, Ma PX, et al. Tissue-engineered heart valve leaflets: does cell origin affect outcome? Circulation 1997;96:II 102–7.
[19] Wilson GJ, Courtman DW, Klement P, Lee JM, Yeger H. Acellular matrix: a biomaterials approach for coronary artery bypass and heart valve replacement. Ann Thorac Surg 1995;60:S353–8.
[20] Elkins RC, Dawson PE, Goldstein S, Walsh SP, Black KS. Decellularized human valve allografts. Ann Thorac Surg 2001;71: S428–32.
[21] O'Brien M, Goldstein S, Walsh S. The Synergraft recellularization: first studies before clinical implantation. Semin Thorac Cardiovasc Surg 1999;13:87–92.
[22] Elkins RC, Goldstein S, Hewitt CW, et al. Recellularization of heart valve grafts by a process of adaptive remodeling. Semin Thorac Cardiovasc Surg 2001;13:87–92.
[23] Steinhoff G, Stock U, Karim N, et al. Tissue engineering of pulmonary heart valves on allogenic acellular matrix conduits: in vivo restoration of valve tissue. Circulation 2000;102:III-50–5.
[24] Booth C, Korossis SA, Wilcox HE. Tissue engineering of cardiac valve prostheses: I. Development and histologic characterization of an acellular scaffold. J Heart Valve Dis 2002;11:457–62.
[25] Korossis SA, Booth C, Wilcox HE. Tissue engineering of cardiac valve prostheses: II. Biomechanical characterization of decellularized porcine valves. J Heart Valve Dis 2002;11:463–71.
[26] Jockenhoevel S, Zund G, Hoerstrup SP. Fibrin gel—advantages of a new scaffold in cardiovascular tissue engineering. Eur J Cardiothorac Surg 2001;19:424–30.
[27] Sakai T, Li RK, Weisel RD, et al. The fate of tissue-engineered cardiac graft in the right ventricular outflow tract of the rat. J Thorac Cardiovasc Surg 2001;121:932–42.
[28] Shi Y, Ramanurthi A, Vesley I. Towards tissue engineering of a composite aortic valve. Biomed Sci Instrum 2002;38:35–40.
[29] Hoerstrup SP, Kadner A, Breymann C, Morris C. Living, autologous pulmonary artery conduits tissue engineered from human umbilical cord cells. Ann Thorac Surg 2002;74:46–52.
[30] Sacks MA, He A, Baijens L, et al. Surface strains in the anterior leaflet of the functioning mitral valve. Ann Biomed Eng 2002;30(10):1281–90.
[31] Merryman WD, YounI HD, Lukoff H, et al. Correlation between heart valve interstitial cell stiffness and transvalvular pressure: implications for collagen biosynthesis", AJP. Heart Circ Physiol 2006 (January);290.
[32] Butcher JT, Penrod AM, Garcia AJ, Nerem RM. Unique morphology and focal adhesion development of valvular endothelial cells in static and fluid flow environments. Arterioscler Thromb Vasc Biol 2004;24:1–6.
[33] Xing Y, He Z, Warnock JN, Hilbert SL, Yoganathan AP. Effects of constant static pressure on the biological properties of porcine aortic valve leaflets. Ann Biomed Eng 2004;32(4):555–62.
[34] Rabkin E, Padera RF, Schoen FJ. Phenotypic modulation of interstitial cells of human cardiac semilunar valves from fetal through adult life. Third Biennial Meeting of the Society for Heart Valve Disease, Vancouver.
[35] Hilbert S, Yanagida R, Linthurst-Jones A, et al. Decellularized allograft valve conduits used for RVOT reconstructions in juvenile sheep: valve function and explant pathology findings. Proceedings of the Eighth Annual Georgia Institute of Technology Workshop: Cardiovascular Tissue Engineering from Basic Biology to Cell-Based Therapies, Hilton Head, S.C., 2004 (March).
[36] Hilbert SL, Yanagida R, Souza J, et al. Prototype anionic detergent technique used to decellularized allograft valve conduits evaluated in the right ventricular outflow tract in sheep. J Heart Valve Dis 2004;13:831–40.
[37] Ketchedjian A, Krueger P, Lukoff H, et al. Ovine panel reactive antibody assay of HLA response to allograft bioengineered vascular scaffolds. J Thorac Cardiovasc Surg 2005;129:159–66.
[38] Ketchedjian A, Linthurst-Jones A, Krueger P, et al. Recellularization of decellularized allograft scaffolds in ovine great vessel reconstructions. Ann Thorac Surg 2005;79:888–96.
[39] Hayward IP, Bridle KR, Campbell GR. Effect of extracellular matrix proteins on vascular smooth muscle cell phenotype. Cell Biol Int 1995;19:727–34.
[40] Weston MW, Yoganathan AP. Biosynthetic activity in heart valve leaflets in response to in vitro flow environments. Biomed Eng 2001;29:752–63.
[41] Vouyouka AG, Powell RJ, Ricotta J. Ambient pulsatile pressure modulates endothelial cell proliferation. J Mol Cell Cardiol 1998;30:609–15.
[42] Predel HG, Yang Z, von Segesser Z. Implications of pulsatile stretch on growth of saphenous vein and mammary artery smooth muscle. Lancet 1992;340:878–9.
[43] Juliano RL, Haskill S. Signal transduction from the extracellular matrix. J Cell Biol 1993;120:577–85.
[44] Salwen SA, Szarowski DH, Turner JN. Three-dimensional changes of the cytoskeleton of vascular endothelial cells exposed to sustained hydrostatic pressure. Med Biol Eng Comput 1998;36:520–7.
[45] Vandenburgh HH. Mechanical forces and their second messengers in stimulating cell growth in vitro. Am J Physiol 1992;262(3 pt. 2): R350–5.
[46] Hoerstrup SP, Sodian R, Sperling JS, Vacanti JP, Mayer JE. New pulsatile bioreactor for in vitro formation of tissue engineered heart valves. Tissue Eng 2000;6:75–9.
[47] Messier Jr R, Bass B, Aly H, et al. Dual structural and functional phenotypes of the porcine aortic valve interstitial population: characteristics of the leaflet myofibroblast. J Surg Res 1994;57: 1–21.
[48] Hoffman-Kim D, Maish M, Krueger P, et al. Comparison of three myofibroblast cell sources for the tissue engineering of cardiac valves. Tissue Eng 2005 (Jan–Feb);11(1–2):288–301.
[49] Hopkins RA. Tissue engineering of heart valves: decellularized porcine and human valve scaffolds. Invited Editorial. Circulation 2005;111:2712–4.
[50] Rajani B, Uree RB, Ratliff NB. Evidence for rejection of homograft valves in infants. J Thorac Cardiovasc Surg 1998;115:111–7.
[51] Vogt P, Stallmach T, Niederhauser U, et al. Explanted cryopreserved allografts: a morphological and immunohistochemical comparison between arterial allografts and allograft heart valves from infants and adults. EJCTS 1999;15:639–45.
[52] Shaddy RE, Thompson DD, Osborne KA, Hawkins JAFT. Persistence of human leukocyte antigen (HLA) immunogenicity of cryopreserved valved allografts used in pediatric heart surgery. Am J Cardiol 1997;80:358–9.
[53] Motomura N, Imakita M, Yutani C, Kitoh Y, Kawashima Y, Oka T. Histological change in cryopreserved rat aortic allograft. J Cardiovasc Pathol 1995;36:53–60.
[54] Neves JP, Gulbenkian S, Ramos T, Martins AP, Caldas MC, Mascarenhas R. Mechanisms underlying degeneration of cryopreserved vascular homografts. J Thorac Cardiovasc Surg 1997;113: 1014–21.
[55] Neves J, Monteiro C, Santos R. Histologic and genetic assessment of explanted allograft valves. Ann Thorac Surg 1995;60:141–5.
[56] Hoekstra FM, Witvliet M, Knoeep CY, et al. Immunogenic human leukocyte antigen Class II antigens on human cardiac valve induce specific antibodies. Ann Thorac Surg 1998;66:2022–6.
[57] Wagner E, Roy R, Marois Y, Douville Y, Guidoin R. Fresh venous allografts in peripheral arterial reconstruction in dogs: effects of histocompatibility and of short-term immunosuppression with cyclosporine A and mycophenolate mofetil. J Thorac Cardiovasc Surg 1995;110:1723–44.

[58] Hoekstra F, Knoop C, Vaessen L. Donor-specific immune response against human cardiac valve allografts. J Thorac Cardiovasc Surg 1996 (Aug);112(2):281–6.
[59] Hoekstra F, Knoop C, Jutte C. Effect of cryopreservation and HLA-DR matching on the cellular immunogenicity of human cardiac valve allografts. J Heart Lung Transplant 1994;13:1095–8.
[60] Hoekstra FM, Witvliet M, Knoop C. Donor-specific anti-human leukocyte antigen class I antibodies after implantation of cardiac valve allografts. J Heart Lung Transplant 1997;16:570–2.
[61] Wychulis AR, Kincaid OW, Wallace RB. Primary dissecting aneurysms of peripheral arteries. Mayo Clin Proc 1969;44:804–10.
[62] McLaughlin JS. The renegade fact. Ann Thorac Surg 2002;73:1031.
[63] Gott VL, Cameron DE, Alejo DE, et al. Aortic root replacement in 271 Marfan patients: a 24-year experience. Ann Thorac Surg 2000;73:438–43.
[64] Shaddy RE, Hunter DD, Osborn KA. Prospective analysis of HLA immunogenicity of cryopreserved valved allografts used in pediatric heart surgery. Circulation 1994;94:1063–7.
[65] Holmes JH, Bloch RD, Hall RA, Carter YM, Karmy-Jones RC. Natural history of traumatic rupture of the thoracic aorta managed nonoperatively: a longitudinal analysis. Ann Thorac Surg 2002;73:1149–54.
[66] Morishita K, Murakami G, Koshino T, et al. Aortic root remodeling operation: how do we tailor a tube graft. Ann Thorac Surg 2000;73:1117–21.
[67] Kirali K, Mansuroglu D, Omeroglu SN, et al. Five-year experience in aortic root replacement with the flanged composite graft. Ann Thorac Surg 2002;73:1130–7.
[68] Hogan PG, Brien O'M. Improving the allograft valve: does the immune response matter? J Thorac Cardiovasc Surg 2003;126:1251–3.
[69] Mitchell RN, Jonas RA, Schoen FJ. Pathology of explanted cryopreserved allograft heart valves: comparison with aortic valves from orthotopic heart transplants. J Thorac Cardiovasc Surg 1998;115:118–27.
[70] Allaire E, Bruneval P, Mandet C, Becquemin JP, Michel JB. The immunogenicity of the extracellular matrix in arterial xenografts. Surgery 1997;122:73–81.
[71] Ketchedjian A, Lukoff H, Krueger P, Hopkins RA. Human in vitro macrophage assay to identify the relative innate pro-inflammatory potential of bioengineered vascular constructs. Proceedings of the Ninth Annual Hilton Head Workshop: Engineering Tissues 2005;19. p. 50.
[72] Hopkins RA. Cardiac reconstructions with allograft valves. New York: Springer-Verlag; 1989. 194 pages.
[73] Breuer CK, Mettler BA, Anthony T, Sales VL, Schoen FJ, Mayer JE. Application of tissue-engineering principles toward the development of a semilunar heart valve substitute. Tissue Eng 2004;10(11/12):1725–36.
[74] Flanagan TC, Pandit A. Living artificial heart valve alternatives: a review. Eur Cell Mater 2003;6:28–76.
[75] Rieder E, Seebacher G, Kasimir MT, et al. Tissue engineering of heart valves: decellularized porcine and human valve scaffolds differ importantly in residual potential to attract monocytic cells. Circulation 2005 (May 31);111(21):2792–7.
[76] Oei FB, Stegmann AP, van der Ham F, Zondervan PE, Vaessen LM, Baan CC. The presence of immune stimulatory cells in fresh and cryopreserved donor aortic and pulmonary valve allografts. J Heart Valve Dis 2002;11:315–25.
[77] Hogan P, Duplock L, Green M, et al. Human aortic valve allografts elicit a donor-specific immune response. J Thorac Cardiovasc Surg 1996;112:126–7.
[78] Hawkins JA, Hillman ND, Lambert LM, et al. Immunogenicity of decellularized cryopreserved allografts in pediatric cardiac surgery; comparison with standard cryopreserved allografts. J Thorac Cardiovasc Surg 2003;126:247–52.
[79] Simon P, Kasimir MT, Seebacher G, et al. Early failure of the tissue engineered porcine heart valve Synergraft in pediatric patients. Eur J Cardiothorac Surg 2003;23:1002–6.
[80] Stock UA, Schenke-Layland K. "Performance of decellularized xenogenic tissue in heart valve replacement". Biomaterials 2006 (Jan);27(1):1–2.
[81] Hilbert SL, Yanagida R, Souza J, et al. Prototype anionic detergent technique used to decellularized allograft valve conduits evaluated in the right ventricular outflow tract in sheep. J Heart Valve Dis 2004;13:831–40.
[82] Hopkins RA, Reyes A, Imperato DA. Ventricular outflow tract reconstructions with cryopreserved cardiac valve homografts: a single surgeon's 10-year experience. Ann Surg 1996;223:544–53.
[83] Clarke DR, Campbell DN, Hayward AR, Bishop DA. Degeneration of aortic valve allografts in young recipients. J Thorac Cardiovasc Surg 1993;105:934–42.
[84] Aklog L, Carr-White GS, Birks EJ, Yacoub MH. Pulmonary autograft versus aortic homograft for aortic valve replacement: interim results from a prospective randomized trial. J Heart Valve Dis 2000;9:76–89.
[85] Wang A, Jaggers J, Ungerleider RM, Lim CS, Ryan T. Exercise echocardiographic comparison of pulmonary autograft and aortic homograft of replacements for aortic valve disease in adults. J Heart Valve Dis 2003;12:202–8.
[86] Laforest I, Dumesnil JG, Briand M, Cartier PC, Pibarot P. Hemodynamic performance at rest and during exercise after aortic valve replacement: comparison of pulmonary autografts versus aortic homografts. Circulation 2002;106:157–62.
[87] St Louis J, Corcoran P, Rajan S, et al. Effects of warm ischemia following harvesting of allograft cardiac valves. EJCTS 1991;5:458–64 [discus].
[88] Messier Jr RH, Domkowski PW, Aly HM, et al. High-energy phosphate depletion in leaflet matrix cells during processing of cryopreserved cardiac valves. J Surg Res 1992;52(5):483–8.
[89] Domkowski PW, Messier RH, Crescenzo DG, et al. Preimplantation alteration of adenine nucleotides in cryopreserved heart valves. Ann Thorac Surg 1993;55:1–7.
[90] Abd-Elfattah AS, Messier RH, Domkowski PW, Crescenzo DG, Wallace RB, Hopkins RA. Inhibition of adenosine deaminase and nucleoside transport: utility in a model of homograft cardiac valve preimplantation processing. J Thorac Cardiovasc Surg 1993;105:1095–105.
[91] Crescenzo DG, Hilbert SL, Messier Jr RH, et al. Human cryopreserved allografts: electron microscopic analysis of cellular injury. Ann Thorac Surg 1993;55:25–31.
[92] Murray G. Homologous aortic valve segment transplants as surgical treatment for aortic and mitral insufficiency. Angiology 1956;7:466–71.
[93] Brockbank KGM, Dawson PE, Carpenter JF. Heart valve leaflet revitalization. Tissue Cell Rep 1993;1:18–9.
[94] VanDerKamp AWM, Visser WJ, van Dongan JM, Nauta J, Galjaard H. Preservation of aortic heart valves with maintenance of cell viability. J Surg Res 1981;30:47.
[95] O'Brien MF, Stafford G, Gardner M, et al. The viable cryopreserved allograft aortic valve. J Card Surg 1987;2:153–67.
[96] Messier R, Bass BL, Domkowski PW, Hopkins RA. Interstitial cellular matrix restoration of cardiac valves following cryopreserved. J Thorac Cardiovasc Surg 1999;118:36–49.
[97] Hilbert SL, Luna RE, Zhang J, et al. Allograft heart valves: the role of apoptosis-mediated cell loss. J Thorac Cardiovasc Surg 1999;117:454–62.
[98] Findley WN, Lai JS, Onaran K. Creep and relaxation of nonlinear viscoelastic materials. New York, NY: Dover; 1976.
[99] Hilbert SL, Barrick MK, Ferrans VJ. Porcine aortic valve bioprosthesis: a morphologic comparison of the effects of fixation pressure. J Biomed Mater Res 1990;24:773–87.
[100] Hilbert SL, Sword LC, Batachelder KC, Barrick MKFV. The simultaneous assessment of bioprosthetic heart valve biomechanical

properties and collagen crimp length. J Biomed Mater Res 1996;31:503–9.
[101] Bechtel JF, Bartels C, Schmidtke C, Skibba W, Muller-Steinhardt M, Kluter H. Does histocompatibility affect homograft valve function after the Ross procedure? Ann Thorac Surg 2001 (Jun);71(6): 2003–7.
[102] Rocnik EF, van der Veer E, Cao H, Hegele RA, Pickering JG. Functional linkage between the endoplasmic reticulum protein HSP-47 and procollagen expression in human vascular smooth muscle cells. J Biol Chem 2002;277(41):38571–8.
[103] Tasab M, Batten MR, Bulleid NJ. Hsp47: a molecular chaperone that interacts with and stabilizes correctly-folded procollagen. EMBO J 2000;19:2204–11.
[104] Hopkins RA, Hammon JW, Smith PK, McHale PA, Anderson RW. Adaptation of the pulmonary circulation to acute and chronic high flow stress. Circulation 1978;58(Suppl. II):81.
[105] Hopkins RA, Hammon JW, Smith PK, McHale PA, Anderson RW. Response of the pulmonary circulation to chronic pulmonary venous hypertension. Circulation 1978;58(Suppl. II):81.
[106] Hopkins RA, Hammon JW, McHale PA, Smith PK, Anderson RW. Pulmonary vascular impedance analysis of adaptation to chronically elevated blood flow in the awake dog. Circ Res 1979;45:267–74.
[107] Hopkins RA, Hammon JW, McHale PA, Smith PK, Anderson RW. An analysis of the pulsatile hemodynamic responses of the pulmonary circulation to acute and chronic pulmonary venous hypertension in the awake dog. Circ Res 1980;47:902–19.
[108] Domkowski P, Cockerham J, Crescenzo D, et al. Pulmonary hydraulic impedance responses to hypoxia and hypercapnia in newborn pigs. J Appl Physiol 1994;77:386–96.
[109] Hopkins RA. Right ventricular outflow tract reconstructions—the role of valves in the viable allograft era. Ann Thorac Surg 1988;45:593–4.
[110] Hu J, Gilmer L, Hopkins RA, Wolfinbarger L. Effects of antibiotics on cellular viability in porcine heart valve tissue. Cardiovasc Res 1989;23:960–4.
[111] Hu J, Gilmer L, Hopkins RA, Wolfinbarger L. Assessment of cellular viability in cardiovascular tissue as studied with 3H-Proline and 3H-Inulin. Cardiovasc Res 1990;24:528–31.
[112] Smith JD, Hornick PI, Rasmi N, Rose ML, Yacoub MH. Effect of HLA mismatching and antibody status on "homovital" aortic valve homograft performance. Thorac Surg 1998;66:S212–5.
[113] Crescenzo D, Hilbert S, Barrick M, Messier R, Wallace R, Hopkins R. Donor heart valves: electron microscopic and morphometric assessment of cellular injury induced by warm ischemia and cryopreserved. J Thorac Cardiovasc Surg 1992;103:253–8.
[114] Dignan R, O'Brien M, Hogan P, Passage J, Stephens F, Thornton A. Influence of HLA matching and associated factors on aortic valve homograft function. J Heart Valve Dis 2000;9:504–11.
[115] Jones M, Eidbo EE, Hilbert SL, Ferrans VJ, Clark RE. Anticalcification treatments of bioprosthetic heart valves: in vivo studies in sheep. J Card Surg 1989;4:69–73.
[116] Gao ZB, Pandya S, Hosein N, Sacks MS, Hwang NHC. Bioprosthetic heart valve leaflet motion monitored by dual camera stereo photogrammetry. J Biomech 2000;33:199–207.
[117] Nielsen PMF, Hunter PJ, Smaill BH. Biaxial testing of membrane biomaterials: testing equipment and procedures. J Biomech Eng 1991;113:300.
[118] Gloeckner D, Billiar KL, Sacks MS. The bending behavior of fixed porcine aortic cusp. Proceedings of the Third World Congress of Biomechanics. Japan: Hokaiddo; 1998.
[119] Sacks MS, Billiar KL. Biaxial mechanical behavior of bioprosthetic heart cusps subjected to accelerated testing. In: Gabbay S, Frater RW, editors. Advances in anticalcific and antidegenerative treatment of heart valve prostheses. Austin, TX: Silent Partners; 1997.
[120] Domkowski P, Cockerham J, Kot P, et al. Relationship of hydraulic impedance, elasticity, and geometry in the pulmonary artery of maturing newborn pigs. J Surg Res 2001;100:116–26.
[121] Maish M, Hoffman-Kim D, Krueger PM, Souza JM, Harper JJ, Hopkins RA. Tricuspid valve biopsy—a potential source of cardiac myofibroblast cells for tissue engineered cardiac valves. J Heart Valve Dis 2003;12:264–9.
[122] Billiar KL, Sacks MS. Biaxial mechanical properties of the natural and glutaraldehyde treated aortic valve cusp: Part I. Experimental results. J Biomech Eng 2000;122:23–30.
[123] Sacks MS. Biaxial mechanical evaluation of planar biological materials. J Elast 2000;61:199–246.
[124] Takemura H, Yamamoto H, Kunishima H, et al. Evaluation of a human monocytic cell line THP-1 model for assay of the intracellular activities of antimicrobial agents against *Legionella pneumophila*. J Antimicrob Chemother 2000;46:589–94.
[125] Xing Y, He Z, Warnock JN, Hilbert SL, Yoganathan AP. Effects of constant static pressure on the biological properties of porcine aortic valve leaflets. Ann Biomed Eng 2004;32:555–62.
[126] Xing Y, Warnock JN, He Z, Hilbert SL, Yoganathan AJ. Cyclic pressure affects the biological properties of porcine aortic valve leaflets in a magnitude and frequency dependent manner. Ann Biomed Eng 2004;32:1461–70.
[127] Lipke DW, Couchman JR. Increased proteoglycan synthesis by cardiovascular system of coarctation hypertensive rats. J Cell Physiol 1991;147:479–86.
[128] Willems IE, Havenith J, Mits JF, Daemen MJ. Structural alterations in heart valves during left ventricular pressure overload in the rat. Lab Invest 1994;71:127–33.
[129] Quick DW, Kunzelman S, Kneebone JM, Cochran RP. Collagen synthesis is upregulated in mitral valves subjected to altered stress. ASAIO J 1997;43:181–6.
[130] Butcher JT, Penrod AM, Garcia AJ, Nerem RM. Unique morphology and focal adhesion development of valvular endothelial in static and fluid flow environments. Arterioscler Thromb Vasc Biol 2004 (Aug);24(8):1429–34.
[131] Ara Ketchedjian, George Cooper, Steve Hilbert, et al. "Chronic sheep implant study of small intestinal submucosa: fabricated pulmonary and mitral valve replacements" presented at American College of Surgeons October, 2004.
[132] Messier Jr R, Domkowski P, Aly H, et al. Adenine nucleotide depletion in cryopreserved human cardiac valves: the 'stunned' leaflet interstitial cell population. Cryopreserved Biol 1995;32:199–208.
[133] Schenke-Layladm k, Riemann I, Optiz F, Konig K, Halbhuber KJ, Stock UA. Comparative study of cellular and extracellular matrix composition of native and tissue engineered heart valves. Matrix Biol 2004;23:113–25.
[134] Roy A, Brand NJ, Yacoub MH. Expression of 5-hydroxytryptamine receptor subtype messenger RNA in interstitial cells from human heart valves. J Heart Valve Dis 2000;9:256–60.
[135] Roy A, Brand NJ, Yacoub MH. Molecular characterization of interstitial cells isolated from human heart valves. J Heart Valve Dis 2000;9:459–64.
[136] Salwen S, Szarowski DH, Turner JN, Bizios R. Three-dimensional changes of cytoskeleton of vascular endothelial cells exposed to sustained hydrostatic pressure. Med Biol Eng Comput 1998;36:520–7.
[137] Schneider PJ, Deck JD. Tissue and cell renewal in the natural aortic valve of rats: an autoradiographic study. Cardiovasc Res 1981;15: 181–9.
[138] Katwa LC, Tyagi SE, Campbell DJ, Lee G, Cicila GT, Weber KT. Valvular interstitial cells express angiotensinogen and cathepsin D, and generate angiotensin peptides. Int J Biochem Cell Biol 1996;28: 807–21.
[139] Dreger SA, Taylor PM, Allen SP, Yacoub MH. Profile and localization of matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs) in human heart valves. J Heart Valve Dis 2002;11:875–80.
[140] Sacks MS, Schoen FJ. Collagen fiber distribution occurs independent of calcification in clinically explanted bioprosthetic heart valves. Biomed Mater Res 2002 (Dec 5);62(3):359–71.

[141] Billiar KL, Sacks MS. Biaxial mechanical properties of the natural and glutaraldehyde treated aortic valve cusp: Part I. Experimental results. J Biomech Eng 2000;122:23–30.
[142] Billiar KL, Sacks MS. Biaxial mechanical properties of the native and glutaraldehyde-treated aortic valve cusp: part II. A structural constitutive model. J Biomech Eng 2000;122:327–35.
[143] Wells SM, Sacks MS. Effects of fixation pressure on the biaxial mechanical behavior of porcine bioprosthetic heart valves with long-term cyclic loading. Biomaterials 2002;23:2389–99.
[144] Sacks MS, Gloeckner DC, Vyanvahare N, Levy RJ. Alterations in the flexural behavior of porcine bioprosthetic heart valves with fatigue. J Heart Valve Dis (submitted for publication).
[145] Sacks MS, Chuong CJ. Orthotropic mechanical properties of chemically treated bovine pericardium. Ann Biomed Eng 1998;26: 892–902.
[146] Engelmayer GC, Hildebrand DK, Sutherland WH, Mayer JE, Sacks MS. Novel bioreactor for the dynamic flexural stimulation of tissue engineered heart valve biomaterials. Biomaterials 2003 (Jun);24(14):2523–32.
[147] Hildebrand DK. A novel testing system for fatigue studies of heart valve biomaterials. Annals of Biomedical Engineering (submitted for publication).
[148] Eliktar D, Nerem RM, Galis ZS. The role of matrix metalloproteinase-2 in the remodeling of cell-seeded vascular constructs subjected to cyclic strain. J Biomech Eng 2001;29:923–34.
[149] Levesque MJ, Nerem RM. The elongation and orientation of cultured endothelial cells in response to shear stress. J Biomech Eng 1985;107:341–7.
[150] Weston MW, Yoganathan A. Biosynthetic activity in heart valve leaflets in response to in vitro flow environments. Ann Biomed Eng 2001;29:752–63.
[151] Simon P, Kasimir MT, Seebacker G, et al. Early failure of the tissue engineered porcine heart valve Synergraft in pediatric patients. Eur J Cardiothorac Surg 2003 (Jun);23(6):1002–6.
[152] Lu Y, Shansky J, Del Tatto M, et al. Therapeutic potential of implanted tissue-engineered bioartificial muscles delivering recombinant proteins to the sheep heart. Ann N Y Acad Sci 2002;961: 78–82.
[153] Filip DA, Radu A, Simionescu M. Interstitial cells of the heart valves possess characteristics similar to smooth muscle cells. Circ Res 1986;59:310–9.
[154] Rabkin E, Aikawa M, Stone JR, Fukumoto Y, Libby P, Schoen FJ. Activated interstitial myofibroblasts express catabolic enzymes and mediate matrix remodeling in myxomatous heart valves. Circulation 2002;104:2525–32.
[155] Bernacca GM, Gibson SA, Wilkenson R, Wheatley DJ. Confocal laser scanning microscopy of calcified bioprosthetic heart valves. J Heart Valve Dis 1994;3:205–11.
[156] Hoerstrup SP, Zünd G, Ye Q, Schoeberlein A, Schmid AC, Turina M. Tissue engineering of a bioprosthetic heart valve: stimulation of extracellular matrix assessed by hydroxyproline assay. ASAIO J 1999;45:397–402.
[157] Hong T, Maish MS, Cohen J, et al. Reproducible echocardiography in juvenile sheep and its application in the evaluation of a pulmonary valve allograft implant. Contem Top Lab Anim Sci 2000;39:15–21.
[158] Piatosa B, Birbach M, Fedorowicz M, Maruszewski B. Presence of IgG anti-HLA antibodies in fourteen patients after cryopreserved allogenic heart valve implantation. Transplant Proc 2002;34:643–4.
[159] Magid R, Murphy TJ, Galis ZS. Expression of matrix metalloproteinase-9 in endothelial cells is differentially regulated by shear stress. Role of C-Myc. J Biol Chem 2003 (Aug);29;278(35): 32994–9.
[160] Merryman WD, Youn HDI, Lukoff H, et al. "Correlation between heart valve interstitial cell stiffness and transvalvular pressure: implications for collagen biosynthesis", AJP: Heart and Circulatory Physiology, 290: (January); 2006.
[161] Koolbergen Dr, Hazekamp MG, de Heer E, Bruggemans EF, Huysmans HA, Dion RA. The pathology of fresh and cryopreserved heart valves: an analysis of forty explanted homograft valves. J Thorac Cardiovasc Surg 2002;124:689–97.
[162] Gibbon JH. Application of a mechanical heart and lung apparatus to cardiac surgery. Minn Med 1954;37:171–4.
[163] Zehr KJ, Yagubyan M, Connolly HM, Nelson SM, Schaff HV. Aortic root replacement with a novel decellularized aortic homograft: postoperative immunoreactivity and early results. J Thorac Cardiovasc Surg 2005;130:1010–5.
[164] Rhim C, Niklason LE. Tissue engineered vessels: cells to telomeres. Prog Pediatr Cardiol 2005.
[165] Fong P, Shin'oka T, Lopez-Soler RI, Breuer C. The use of polymer based scaffolds in tissue-engineered heart valves. Prog Pediatr Cardiol 2005.
[166] Taylor PM, Cass AEG., Yacoub MH. Extracellular matrix scaffolds for tissue engineering heart valves. Prog Pediatr Cardiol 2005.
[167] Merryman WD, Engelmayr Jr. GC, Liao J, Sacks MS. Defining biomechanical endpoints for tissue engineered heart valve leaflets from native leaflet properties. Prog Pediatr Cardiol 2005.
[168] Christenson JT, Kalangos A. Blood group antigenicity and clinical durability of cryopreserved homografts. Prog Pediatr Cardiol 2005.
[169] Simon P, Kasimir M-T, Rieder E, Weigel G. Tissue engineering of heart valves—immunologic and inflammatory challenges of the allograft scaffold. Prog Pediatr Cardiol 2005.
[170] Johnson TL, Barabino GA, Nerem RM. Engineering more physiologic in vitro models for the study of vascular biology. Prog Pediatr Cardiol 2005.
[171] Baldwin JT, Duncan BW. Ventricular assist devices for children. Prog Pediatr Cardiol 2005.
[172] Vandenburgh HH. Bioengineered muscle constructs and tissue-based therapy for cardiac disease. Prog Pediatr Cardiol 2005.