Tissue Engineering of Heart Valves
Decellularized Valve Scaffolds

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In 1956, Gordon Murray first reported the human clinical surgical use of fresh aortic valve homografts (cadaveric) transplanted into the descending thoracic aorta for clinical amelioration of the consequences of native aortic valve insufficiency.1 His initial operation preceded by 5 years the availability of mechanical aortic valve prostheses. Although the operation was only partly successful hemodynamically, these “homograft valves” had remarkable durability and performance. Some patients had no calcification or transvalvular gradients for up to 20 years, whereas others ultimately failed as a consequence of progressive fibrosis and calcification.2,3 In 1962, the initial use of aortic valve homografts in the orthotopic position were reported independently by Sir Donald Ross of England and Sir Brian Barrett-Boytes of New Zealand.4–6 As manufactured prosthetic valves gradually evolved in design and range of choices, homograft use began to decline because of issues of acquisition logistics, banking, transport, sizing, infectious disease transmission, and others. With the development of organized organ and tissue donation for transplantation in the 1980s and 1990s, cardiovascular allograft tissues became increasingly available primarily as cryopreserved valves with variably retained native cell viability. Importantly, the terrific surgical and specific performance advantages of allograft semilunar cardiac valves have been recognized by reconstructive surgeons worldwide.7 These valve “transplants” have by and large crossed histocompatibility and ABO constraints, however. Although they perform well in the short- to mid-term, they have been associated with ultimate fibrosis and failure in a significant proportion of cases, especially in patients for whom the desirability of a living transplant would be the greatest: neonates, infants, and young children (ie, for whom retained growth and repair functions would be ideal). 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; even with these durability constraints, for many indications the allograft valve remains the best option for reconstructing certain cardiac lesions in patients such as destroyed ventricular outflow tracts resulting from bacterial endocarditis and complex reconstructions for congenital structural heart disease.8

The obvious advantages of the allograft valve combined with the inevitable durability issues have led to more than a decade of research at many centers around the world to develop a “tissue-engineered” heart valve. This “holy grail” of cardiac valve surgery has yet to be achieved, but advances suggest we are close to such a construct, and certainly the barriers are being better defined.9 The critical issues facing tissue engineering of heart valves are the definition of such a valve, where to start in terms of scaffold material, and how to accomplish the requisite recellularization. Although perhaps a complex field, actually the decision tree is relatively linear and dichotomous. One must begin with an acellular scaffold design, which replicates functionally, if not structurally, the performance of a native semilunar heart valve. Choosing the design of the evolved mammalian heart semilunar valves avoids many mechanical engineering issues and is burdened only by having to relate performance of the tissue-engineered construct to the performance characteristics of fresh human heart valves. Unfortunately, this is a problem. Highly sophisticated descriptions of the viscous-elastic properties, relevant strength testing, flexural performance, mechanisms for growth, matrix degradation and turnover, parameters that control relative protein synthesis (eg, matrix metalloproteinase activity, collagen-to-elastin ratios responding to varying pressure, flow, or sheer stresses) have only recently begun to be defined.10–13 Choosing a scaffold is a dichotomous decision between a derived human extracellular matrix (ECM) functional semilunar valve (ie, decellularized homografts) and a fabricated valve from polymers or polymer ECM hybrids. Polymer-based fabrication is appealing from a manufacturing standpoint, with its ability to create an infinite variety of sizes, lengths, and so forth, but it suffers from 2 difficult barriers. The polymer fabrication must replicate the performance characteristics of the fresh native valve, and no such polymer or fabrication process has yet been developed.14 Even if such a process could be discovered, the degradation of a polymer requires inflammatory destruction by macrophages and replacement of the polymer structure with host materials. Although this has been accomplished in relatively inert structures such as bone and cartilage, current knowledge is insufficient to control the foreign body scar response to do
anything more than create a fibroblast reaction rather than establish a normal trilaminar structure consisting of appropriate proportions of ECM, structural proteins, and multiple cell-type populations distributed in density and location typical for a functioning heart valve.\textsuperscript{15} This makes the biological semilunar valve perhaps the easiest pathway to pursue (at least initially) to set up the next series of bifurcated decisions.

In this issue of *Circulation*, Rieder and colleagues address the next bifurcation point: Assuming that the tissue-engineered valve will be based on a biological ECM scaffold derived from a functional valve, should it be an allograft only (ie, human), or can xenografts be modified by the decellularization process to work satisfactorily? Obviously, the latter would simplify acquisition issues, although they may be complicated by disinfection criteria. This important article emphasizes with elegant laboratory methodology the previously suspected antigenicity of xenograft (ECM) proteins.\textsuperscript{16} This has been previously suggested by in vitro and in vivo animal studies and human assays.\textsuperscript{17–19} The unfortunate clinical experiment with the implantation of decellularized xenograft heart valves (Synergraft) before the completion of sufficient preregulatory studies only emphasized the risk of proceeding down pathways absent full understanding of the potential for ECM provocation of the immune as well as the innate nonspecific inflammatory responses.\textsuperscript{20} In fact, a “decellularized” xenograft scaffold may actually be more inflammatory than present versions of cryopreserved homografts (ie, with retained donor cells). Human or “processed” xenograft scaffolds are almost certainly more proinflammatory when cells are disrupted and cellular debris, cytokines, and other inflammatory moiety are not thoroughly removed from the matrix.\textsuperscript{17} Such concerns are supported by the studies of Rieder et al.\textsuperscript{16} They demonstrated that the lowest level of stimulation was with thoroughly “decellularized” human tissues. In fact, the decellularized porcine leaflets were far more attractive (stimulated macrophage response) than the extracts of human native pulmonary cusps that had not been decellularized. Thus, this article along with other studies suggest that this bifurcation decision path leads to the selection of the human allograft, not xenografts, as a basis for decellularization technologies designed to obtain functioning heart valve ECM scaffolds for tissue engineering of heart valves.

A series of laboratory small- and large-animal implant/ explant studies are needed to determine the best means for recellularization. Again, this is essentially a bifurcated decision tree: Should the recellularization be performed before implantation or not? The former would assume some form of bioreactor-based cell seeding. Such experiments have been performed, but the parameters necessary to establish a phenotypically appropriate cell population and distribution density (including, at minimum, the trilaminar microanatomy of conduit wall and valve leaflets) are not yet fully defined.\textsuperscript{21} Numerous studies have indicated that bioreactor environments must 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).\textsuperscript{22} All of this will have to be accomplished within the context of the clinical realities 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, and banking. Each of these steps is again a bifurcation with typically yes or no decisions that require simply performing the appropriate sequence of experiments. The authors of the present study have been active in this field for many years, and along with others of us who are interested in achieving the tissue-engineered heart valve have sequentially worked through this decision tree; for this they should be congratulated. Clearly, the preponderance of evidence suggests that an earlier realization of a tissue-engineered heart valve construct will be accomplished with some form of a decellularized human heart valve as a scaffold.

It would be remiss not to mention some of the potential difficulties with the ECM decellularized allograft. The removal of the cells and, ostensibly at least, some of the soluble proteins by definition must result in some alterations of physical properties of the valve itself. These degradations need to be carefully defined, measured, and related to appropriate standards derived from similar measurements of fresh human functioning valves. Although it seems attractive, and early evidence suggests that the material properties of these ECM scaffolds approach those of normal valves, there is no a priori reason why this should be so. Certainly, if such a decellularization process weakens relevant physical properties of the putative transplant valve below which safety can be ensured, then additional steps will need to be taken to “[strengthen]” the ECM platform. Once again, this is a bifurcated decision tree. Is it strong enough? Is it not strong enough? The methodology to measure it will need to be relatively sophisticated and will rely on far more complex engineering approaches than typical stress–strain curves and yield stress measurements.\textsuperscript{11} Subsequent to mechanical properties characterization, a putative scaffold will need to be assessed for its ability to recellularize in vitro or in vivo and methods developed (either physiological or biological) to drive the recellularization process to reestablish a native multicellular host population that organizes living, functioning heart valve tissue that can grow, remodel, heal, and defend itself against destruction. Each of these steps likely can be accomplished, and such a “tissue/cell engineering” project 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, and cell differentiation. The “no-lose” aspect of this line of investigation is that even if a tissue-engineered heart valve by strict definition is not achieved, studies such as these will, at minimum, rationalize the process for improving allograft valves for use in complex cardiac reconstructions.

Both the Food and Drug Administration’s prosthetic valve guidance document of 1994 and ISO 5840:1996 suggest that before such a biological heart valve can be considered for a human trial it should be replicated in a large animal model. Studies such as that by Rieder et al demonstrate that the...
animal model will have to use a tissue-engineered valve based on an allograft scaffold within species. The methods must then be completely replicated, with technology transferred to human valve constructs, which only then can be tested in humans. Without following such a dichotomous and yet sequential “road map” to a tissue-engineered aortic valve, mistakes will be made such as that made with Synergraft. Thus, the regulatory structures relevant to tissue, cell, and gene engineering also will need to be developed by FDA and other regulatory agencies to promote design-specific testing with construct-specific failure modes and effects analysis to appropriately define safety and efficacy pass/fail criteria without creating unnecessary barriers by equating bioengineered constructs to the classical categories of either devices or biologics. The tissue-engineered heart valve is the prototype multidisciplinary collaborative project that is virtually ideal as the archetype for the new era of bioengineered solutions to complex cardiac diseases.

References


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