Pediatric Cardiac Surgery Annual

Cardiac Surgeon's Primer: Tissue-Engineered Cardiac Valves

Richard Hopkins

KEYWORDS Tissue engineering, cardiac valves, aortic valve

What is a Tissue-Engineered Heart Valve?

In 1993, Langer and Vacante,[1] described cardiovascular tissue engineering as "an interdisciplinary field that applies the principles of engineering and life sciences toward the development of biological substantives that restore, maintain, or improve tissue function." In the textbook the "*Principles of Tissue Engineering,*" Dr. Robert Nerem[2] described tissue engineering as "the use of a combination of cells, engineering materials, and suitable biochemical factors to improve or replace biological functions in an effort to affect the advancement of medicine." In 2005, MacArthur and Oreffo[3] described the field as "understanding the principles of tissue growth, and applying these to produce functional replacement tissues for clinical work" in the journal *Nature*. Tissue engineering involves applications to repair or replace structural tissues (eg, bone, cartilage, blood vessels, and so on) that function by and on the basis of their mechanical properties. It differs from cell transplantation, in which only the cells are transplanted (as distinct from their milieu), with the expectation that they will perform a specific cell-based function. Regenerative medicine typically emphasizes the use of stem cells to produce tissues. Functional tissue, engineered devices, or functional organs can be constructed using principles of cell biology and material science with or without the use of synthetic biomaterials as scaffolds. The underlying supposition of tissue engineering is to allow the natural biology of a system to thrive yet effecting replacement, repair, or maintenance of cell and tissue function. Therapeutic strategies can be developed to enhance the cell and tissue function or to enhance certain characteristics of the cell and tissue function while still maintaining the anatomic structural replacement component.

Tissue engineering of heart valves implies a matrix that is seeded or repopulated with cells. These can be autologous cells. This requires harvesting from the donor recipient, expansion or amplification in vitro, maintenance of differentiation or dedifferentiation in vitro with redifferentiation in vivo. It also requires the development of seeding protocols. Allogenic cell sources include foreskin, Wharton's jelly, or certain multi-potential or stem cells. All of which have immunologic issues, while the multi-potential cells have oncologic issues. By definition, xenogeneic cell sources are antigenic cells; even more importantly, such sources often contain cell debris such as proteins, lipoproteins, and membrane fragments that are often even more antigenic than intact xenogeneic cells. Syngeneic cell sources are useful for inbred research animal models to identify nonimmune issues, but have limited application clinically except for twins.

The scaffold for a tissue-engineered heart valve or other construct is built on matrix material.[4-6] The scaffold is defined as a 3-dimensional acellular structure onto which cells are implanted (or seeded). The purposes of a scaffold includes: (1) defining functional structural design; (2) enhancing structural properties; (3) delivering biochemical factors; (4) ensuring access to vital cell nutrients; and (5) exerting appropriate mechanical and biological influences to drive cell behavior. Synthetic matrices require degradation to disappear. This degradation can be either inflammatory or hydrophilic dissolution. Examples of synthetic matrices include polylactic acid which degrades in vivo to lactic acid, polyglycolic acid which degrades relatively fast, and polycaprolactone which degrades relatively slowly. Matrices can also be derived from extracellular structural matrix natural materials either within or outside of species including collagen, elastin, or bone. Proteins and other moieties can be added such as fibrin, polysaccharides, insulin, and glylosaminoglycons. Current issues with collagen structure include undesirable

Brown Medical School and the Collis Cardiac Surgical Research Laboratory, Division of Cardiothoracic Surgery, Providence, RI.

Dr Hopkins' academic activities are supported in part by the Mary and Karl Karlson Endowed Professorial Chair and by the Ellen and Charles Collis Endowment of Dr Hopkins Cardiac Surgical Research Laboratory. Lifenet Tissue Services (Virginia Beach, VA), St. Jude Medical (MN), Carbomedics, Inc. (Austin, TX), and Medtronics Corp. (MN) have supported valve tissue engineering research with unrestricted research grants Dr. Hopkins' Collis Research Laboratories

Address correspondence to Richard A. Hopkins, MD, Cardiothoracic Surgery, Brown Medical School, Division of Cardiac Surgery, 2 Dudley St #500, Providence, RI 02905; e-mail: RAHopkins@CMH.edu

1092-9126/07/$-see front matter
doi:10.1053/j.pcsu.2007.01.004

Table 1 Engineered Scaffolds- Polymers

Textiles
Synthetic woven and non-woven meshes of polymers
Solvent casting and particular leading
Gas foaming
Emulsification
Liquid phase separation
CAD/CAM – 3-dimensional structures computer designed

cross-linking or, conversely, the inability to calibrate cross-linking (leading to alterations in strength in the latter case), but, in the former, a fixed tissue that cannot contribute to protein, turnover, or remodeling. One exciting area of great promise includes hybrid matrices, which include polymers with added extracellular matrix (ECM) components including structural and soluble proteins.[7]

Matrix requirements include porosity-pore size, cell seeding, migration, and nutrition. Typically, it should be biodegradable so that the molecules can be absorbed and disposed of by the body or dissolve with minimal to no residual, especially avoiding any inflammatory scar formation. Obviously, degradation rates of a biodegradable matrix must not exceed the tissue formation rate or material failure would occur. If matrix material is permanent, it must enhance material properties and not interfere with the structural or biological functionality of the valve. If it is extracellular matrix protein, it must be available for turnover (degradation-protein synthesis cycle) and must not induce inflammatory responses. For polymers, there are numerous categories (see Table 1).[5,6]

The use of cells for seeding requires cell sourcing.[8,9] The source must be convenient, functional, plastic, viable, and able to be cultured in vivo and must not dedifferentiate in vitro. Seeding of such cells includes engineering not just the cell but the cell cycle to enhance migration, proliferation, and protein expression. There are a number of techniques for specific cell types. The unmodified cells are presumed to know what to do but often they do not; this emphasizes the need for large animal modeling before human trials. In addition, the tissue engineer must very specifically define the phenotype of the desired cell type, and also must create a classification and identification scheme that can clearly establish whether the desired cell is being seeded and is replicating. Such methodology includes isolation using selective media or cell sorting and then assaying protein expression by various techniques. Comparing the expressed genes or proteins synthesized allows definition of the cell's capabilities and phenotype. However, cell behaviors of such cells can change in vitro as well as in vivo, and these characteristics must be defined for the seeding cells. Thus, bioreactor cell seeding (which is an ex-vivo technique) must have the various conditions defined and indicate whether matrix maturation or degradation occurs during this phase. Such bioreactors can be static or dynamic and can have various time-dependent recipes, all of which must be validated. In vivo, the body tends to respond to any implant with wound healing and thus, in the case of tissue engineering, this needs to be directed rather than undirected. Local cell migration is always going to happen. Thus attracting a larger percentage of the required cells or replacing them to selectively prevent undesired cells from entering the matrix is critical. Such manipulation of matrices is termed "scaffold pretreatment."[10]

The issue of matrix maturation (ie, structural and soluble ECM components changing composition, disappearing, or recycling) must be assessed for any such assembled cell and matrix tissue-engineered valve. There are mass transport limitations to all tissue-engineered structures because initially there is no blood supply. In the case of heart valves, this is not an issue for the leaflets, because leaflet interstitial cells live off passive diffusion. However, the base of the leaflets and the wall of the aortic or pulmonary sinuses do have a vaso vasorum, and thus implantation of such a construct without adequate nutrient supply will often result in apoptosis of the cells resulting in hypocellularity and/or hypoxic necrosis with resultant scar formation.[11-13] Cell migration and cell proliferation both play a role in the recellularization process.

After assembly, the tissue engineering heart valve requires surgical implantation.[14] As in all surgical procedures, this provokes inflammation of three types: (1) nonspecific foreign body effect in which macrophages predominate; (2) immune driven effects in which T cells and B cells respond to antigenic simulation; and (3) classical wound healing with fibrous scarring leading to fibrocalcific degeneration. Recellularization requires not only the appropriate cell phenotypes (as discussed above), but also a re-establishment of the location, density of repopulation, cell-based tissue reconstruction, creation of a dynamic and appropriate milieu in which cell signaling and extracellular matrix formation occurs in response to appropriate triggers, and demonstration that the cells re-establish synthesis capability for critical structural and soluble proteins as well as lipoproteins.[15] It is likely that such reformation will be optimized by replicating the trilaminar native leaflet structure (Fig. 1).

The *sine quo non* of a viable extracellular matrix tissue-engineered valve is the establishment of a cellularized living tissue capable of growth, remodeling, healing, and adaptation.[15] In tissue engineering language, this means three different things: (1) constructive remodeling occurs as the tis-

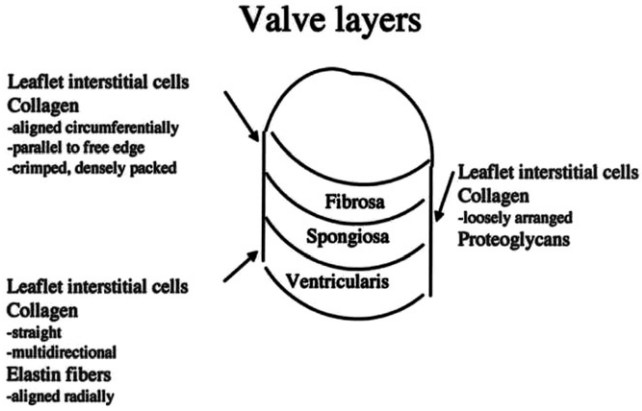

Figure 1 Diagrammatic representation of semilunar valve leaflet trilaminar structure. There is a thin, endothelial lining on both the outflow and inflow surfaces.