Tissue Engineering

Heart Valves

The aortic valve is a light, durable, and fast-reacting tissue. Its unique design enables it to separate the left ventricle from the aorta, allowing unimpeded forward flow of blood when open and permitting no retrograde flow when closed. Its three semilunar leaflets open and shut passively, responding directly to pressure gradients in the cardiac cycle.

The aortic valve responds to the demands of a normal, active lifestyle by handling large pressure variances (10 to 80 mm Hg before the valve opens) and large volumes of blood (70 mL is ejected past the open valve) throughout 30 to 40 million cycles per year. Efficient, reliable valve function is crucial for healthy heart function throughout life.

When a congenital defect or a disease such as rheumatic fever or endocarditis compromises valve function, the leaflets become scarred and stiffened. As a result, malfunctioning valves typically exhibit some combination of stenosis, which is restriction of forward flow, and regurgitation, which is backward flow caused by incomplete closure. Cardiologists can hear these structural defects as heart murmurs and visualize them by echocardiography.

Valve malfunction can lead eventually to hypertrophy of the upstream chamber, compensating for increased blood volume but ultimately giving rise to heart failure. To restore proper heart function, aortic valve defects are typically managed by valve replacement, involving open-heart surgery with cardiopulmonary bypass. With about 200,000 valve replacement procedures being performed each year worldwide, a long-lasting, high-functioning valve is essential and in great demand.

A valve replacement must be easy to implant, nonobstructive when it is open, competent when closed, with low inertia of moving parts, low bulk to minimize interactions with retained heart structures, and minimal associated pressure gradients. In addition, a valve replacement needs to be nonthrombogenic, non-injurious to blood cells, non-immunogenic, free from calcification, strong, flexible, and durable.

Current choices for replacement include mechanical valves of ball and cage or bileaflet types, bioprosthetic xenografts made from processed porcine or bovine tissue, and homografts, which are cryopreserved after being harvested from human donors.

Autografts are also used in the Ross double-valve replacement procedure, in which the patient's own pulmonary valve replaces the aortic valve and one of the other valve choices replaces the pulmonary valve, positioning the native pulmonary valve to best advantage in the more demanding, higher-pressure aortic circuit.

Although current valve substitutes afford adequate function, associated disadvantages include a need for lifetime anticoagulation therapy with mechanical valves, gradual structural damage over time with bioprosthetic xenografts, and technically challenging surgery and limited availability with homografts.

An ideal valve replacement would have the characteristics already described and also would resist infection, continuously remodel its extracellular matrix components, repair itself after injury, and grow. The ability to increase in size over the lifetime of the recipient is especially important for pediatric patients, who presently require multiple operations as they outgrow their valves.

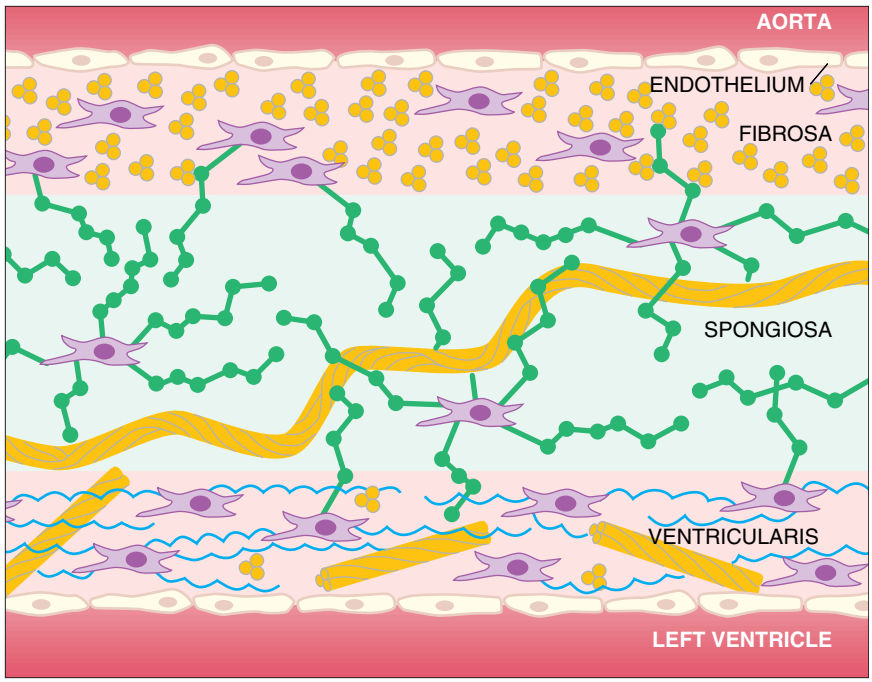
The native aortic valve functions reliably and efficiently by virtue of its distinctive structure. Its main components include myofibroblasts, endothelial cells, and extracellular matrix, including collagen, elastin, and glycosaminoglycans. Endothelial cells form an external blood-contacting layer, and there are three distinct internal layers, each containing myofibroblasts and extracellular matrix.

In the middle of those three layers, the arrangement of cells and associated glycosaminoglycans absorbs bending stresses. In the adjacent layers, more concentrated myofibroblasts and differentially oriented collagen and elastin provide strength under changing pressures. This structure is critical to the valve's combined flexibility, strength, and endurance. Living cells, moreover, modulate their synthesis of extracellular matrix components in response to changing hemodynamics.

The challenging goal of replicating the structure and function of the native valve can best be achieved by creating a living valve. For this endeavor, researchers have turned to tissue engineering.

At its most fundamental level, a tissue-engineered valve can be considered to be composed of two well-integrated components: a scaffold and cells. The scaffold may be permanent, or it may degrade over time as incorporated cells synthesize extracellular matrix to replace

"Tissue Engineering" is edited by Jeffrey R. Morgan and Martin L. Yarmush of the Center for Engineering in Medicine, Massachusetts General Hospital and Harvard Medical School, Boston.



Heart valve leaflets are composed of distinct layers with different densities of myofibroblasts (*purple*) and extracellular matrix components. Collagen (*yellow*) is dense and circumferential in the fibrosa, loose in the spongiosa, and multidirectional in the ventricularis. Proteoglycans (*green*) predominate in the spongiosa, and radial elastin fibers (*blue*) are prominent in the ventricularis.

it. The cells may be derived from a variety of sources, as will be described, and may be incorporated into the scaffold in vitro, prior to implantation, or recruited in vivo following implantation.

Current scaffold options under development include polymer matrices and decellularized tissues. Synthetic polymers include degradable polymers such as poly-lactic acid and poly-glycolic acid, used in resorbable sutures, and polyhydroxyalkanoate, a newer polymer produced by fermentation. Researchers are also investigating natural polymers such as collagen, chitosan, and fibrin because of their natural strength and biocompatibility.

Polymeric valves have the difficult task of replicating a complex valve geometry. In contrast, scaffolds based on decellularized human or animal valves provide optimal geometric and biocompatibility properties, if only their immunogenic cells can be removed. Decellularization procedures vary and are often proprietary; most include treatment of tissue with hypotonic buffers, detergents, and enzymes, with the goal of removing not only cells but immunogenic cellular fragments as well.

A valve that grows and repairs itself requires the presence of living cells within it. The dual function of the native valve's myofibroblasts, namely contraction and synthesis of extracellular matrix, is important for the valve's optimal function. So are the multiple blood-responsive functions of endothelial cells.

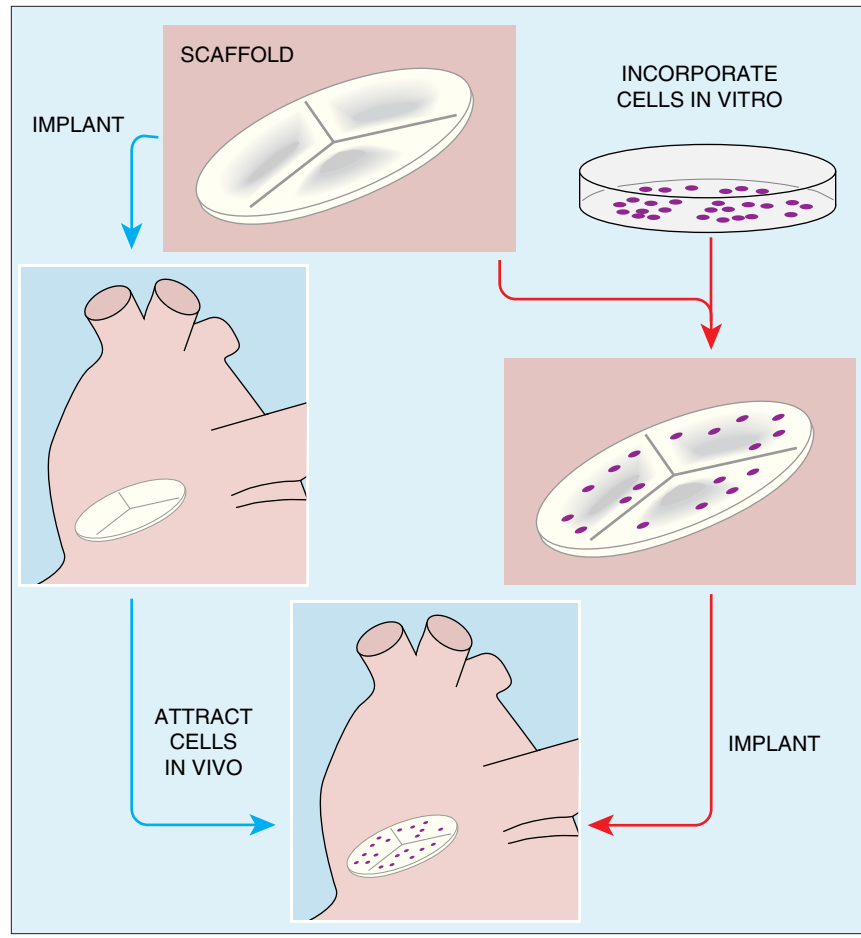
One current strategy for a tissue-engineered valve is to implant a scaffold that can attract the patient's own cells to migrate into it and populate it in vivo. Such a scaffold could contain biological signals to promote cell adhesion or proliferation, such as ascorbate, fibroblast growth factor, and extracellular matrx. Another approach is to harvest the patient's cells, expand their population in vitro, combine the cells with a scaffold, and implant a cell-containing valve.

For in vitro cell incorporation, many options exist with respect to cell source, phenotype, and number. Joseph Vacanti and John Mayer at Harvard Medical School, and others, have seeded polymer valves with 10 million cells cultured from skin, venous, and arterial sources. The group of Richard Hopkins at Brown University School of Medicine has expanded a single 1-mm-diameter biopsy of the tricuspid valve into more than a million cells in 1 month, demonstrating the potential utility of that source.

Studies have suggested that cardiac and venous cells are superior to arterial and dermal cells with regard to extracellular matrix production. Genetically modified cells and stem cells are also under investigation because of their potential versatility and their ability to replicate desired endothelial and myofibroblast function.

Another strategy for a tissue-engineered valve that contains cells at their peak of function is physical conditioning of valves in vitro following cell incorporation. It is well known that endothelial cells, smooth muscle cells, and fibroblasts respond to flow conditions by modulating their physical and chemical properties, including orientation and extracellular matrix synthesis. Various bioreactors have been designed to expose the cell-containing valve to physiologic pressures and flows, with the goal of modulating the cell phenotype to most closely approximate that of native valve cells.

The myriad options of building blocks for a tissue-engineered valve make for an exciting field of



Two pathways toward a tissue-engineered heart valve are being explored. Scaffolds may be natural or synthetic, and cells can be derived in various ways.

research. Recalling the basic elements of the tissue-engineered valve, the scaffold and its incorporated cells, one might well wonder which combinations of choices have come the farthest on the journey toward clinical usefulness, and which will give rise to the valve with the best function.

Answering the first question is more straightforward. Using the strategy of a decellularized homograft scaffold with cells incorporated in vivo, researchers at CryoLife, Inc. have developed the CryoValve SG, a human pulmonary valve decellularized by the proprietary SynerGraft process and then cryopreserved. Human recipients of CryoValves in the pulmonary position have exhibited normal valve function at 3 months postoperatively, and analogous sheep recipients of sheep SynerGraft valves have shown some amount of host cell invasion at 6 months following implantation.

Using the contrasting strategy of a biodegradable polymer scaffold with cells incorporated and conditioned in vitro, workers in Joseph Vacanti and John Mayer's group have implanted a poly-hydroxyalkanoate scaffold containing arterial cells into the sheep pulmonary position. They observed the presence of cells with associated increased collagen content over 6 months after implantation.

Determining which design choices give rise to the best functioning valve is a more difficult question that remains to be answered. Balances need to be established between a number of factors, including valve reproducibility, time to complete cell incorporation, and time from diagnosis to implant availability.

Many academic and industrial groups around the world are engaged in pushing the limits of tissue engineering to meet the challenge of creating a valve to last the lifetime of the patient. Given the size of the stakes and the depth of the science, it is appropriate to hold the hope that tissue engineers will, in the not too distant future, provide patients and surgeons with a successful living, growing heart valve.

DIANE HOFFMAN-KIM

Department of Molecular Pharmacology, Physiology, and Biotechnology
Brown University
Providence , Rhode Island