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TRANSLATIONAL PERSPECTIVES

JOURNAL WATCH

Engineering Vessels as Good as New?

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SUMMARY

Blood vessels convey essential nutrients to end organs, and when diseased, they must be replaced or bypassed. Traditionally plastic and synthetic materials have been used but are susceptible to thrombosis, stenosis, and poor patency rates. A recent report in *Science Translational Medicine* describes a decellularized matrix grown in vitro from commercially sourced fibroblasts that can be used as a vascular graft. Fibroblasts are grown for several weeks on a fibrin scaffold, laying down a collagen layer. After decellularization and transplantation as an arteriovenous fistula, this group showed that grafts remained patent for several weeks. The lack of cellular material in this graft at the time of transplantation reduced the risk of immune rejection. The matrix laid down by the fibroblasts can serve as a scaffold for recipient cells to colonize after implantation, but also provides structural support for arterial blood flow. Other tissue-engineered grafts of decellularized matrices have recently been tested in clinical trial. For these strategies, the cell type, scaffold material, and culture conditions are key components that dictate not only the type and quality of the end product, but also allow standardization and quality control necessary for widespread translation into clinical use. These off-the-shelf decellularized products may be the first in a new generation of therapies for patients with cardiovascular disease. (J Am Coll Cardiol Basic Trans Science 2018;3:119–21) © 2018 The Author. Published by Elsevier on behalf of the American College of Cardiology Foundation. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Dysfunctional vasculature is a common underlying cause of cardiovascular diseases because the inflammatory, metabolic, and proliferative changes leading to atherosclerosis are extremely common in Western society. In advanced cases, native vessels are not adequate to supply the needed blood to tissues of the heart, legs, and other organs, and surgical bypass is needed. In the case of hemodialysis, conduits are used to create high-flow arterial-venous fistulas needed for thrice-weekly canalization. Artificial conduits of polytetrafluoroethylene or polyester (Dacron) are in widespread use for these applications and over time can be endothelialized by migrating and circulating endothelial (progenitor) cells. However, these materials are subject to thrombosis and stenosis, especially at anastomotic sites. Generally, the patency of grafts made of artificial materials is inferior to those made of native patient tissues such as veins (1). Therefore, many groups are investigating novel materials to manufacture vessels with greater patency, longevity, and biocompatibility. The cost, time delay, and scalability of these strategies have been significant barriers.

In the November 1, 2017, issue of *Science Translational Medicine*, Syedain et al. (2) describe an advance in the development of tissue-engineered vessel conduits. They describe the functionality, patency, and histologic features of this conduit in baboons as a preclinical validation study. The manufacturing process starts with human dermal fibroblasts obtained from a commercial vendor that were encased in a fibrin gel (**Central Illustration**). The gel is pre-formed into a cylindrical, vessel-like shape that guides the cellular growth of the fibroblast in a pulsed flow bioreactor. As the fibroblasts grow, they digest the fibrin and deposit their own extracellular

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CENTRAL ILLUSTRATION Decellularized Vessel Manufacturing Workflow

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matrix (ECM) composed mainly of collagen. After growing for 5 weeks, the new matrix is decellularized by detergent digestion, and the resulting ECM construct is stored in phosphate-buffered saline until use (3). The underlying premise of this strategy is that the collagen ECM manufactured by the fibroblasts creates a biological scaffold for host cell engraftment and that this scaffold is superior to synthetic materials. The lack of residual cellular components as well as digestion of fibrin is designed to prevent immune activation. The collagen is strong enough to withstand the pressures and deformations of surgical implantation as well as the intravascular arterial pressures. The components of this strategy—cell type, culture conditions, bioreactor, and fibrin scaffold—are the result of 15 years of research and development by this group.

The decellularized scaffolds were implanted in the forearms of 7 baboons as an arteriovenous graft and evaluated at 3 and 6 months. After some technical optimization, the 3-month patency rate was 83% at 3 months and 60% at 6 months. Grafts were accessed 4 times throughout the course of the experiment to mimic dialysis cannulization and, importantly, achieved hemostasis without thrombosis. Encouraging results were also seen on mechanical testing of the vessels. Before implantation and after 3 or 6 months, burst pressure and suture retention strength were similar or better compared with native arteries. At the time of explantation at either 3 or 6 months, re-endothelialization was seen as a monolayer of $CD31^+$ endothelial cells on the luminal surface. There was no ectopic calcification seen by von Kossa staining. There was neither anastomotic stenosis nor hyperplasia as is often seen in grafts of synthetic materials. Most grafts lacked significant inflammatory cell infiltration, and markers of systemic inflammatory response were not elevated. While not all grafts functioned perfectly, these promising results show the biocompatibility, recellularization, and functionality in a primate model.

TRANSLATIONAL RELEVANCE

The field of tissue-engineered vascular conduits has made considerable progress since early attempts dating back to the 1960s when materials such as porous metals, fiberglass, pericardium, and tendon were implanted with variable success (4). In 2009, the results of first human studies of tissue-engineered vascular conduit in the arterial system were reported. These constructs were grown from patient fibroblasts isolated from skin and endothelial cells from superficial vein (5). Cells were grown in culture in a production process taking 7 to 9 months. Three of 10 patients had dysfunction of the graft within the first 3 months, but other grafts functioned well for a year despite being punctured hundreds of times for hemodialysis access. The cost, quality control, and time delay in manufacturing this type of construct is not feasible for widespread clinical translation.

In the 1980s and 1990s, tissue engineering of vessels became more refined. Researchers and engineers understood the critical importance of the endothelium as an interface with the coagulation and inflammatory systems and designed constructs seeded with endothelial cells and supported by collagen, fibrin, and synthetic matrices (6). Later, decellularization gained traction for its ability to use complex ECM manufactured by cells, but avoiding immune recognition of foreign cellular antigens (2,7). The most advanced example of this is a construct in clinical trials developed by Niklason et al. (8). Similar to Syedain et al. (2), this construct is a decellularized matrix created by donor cells. The construct is grown from deceased donor vascular smooth muscle cells seeded on a synthetic polyglycolic acid polymer scaffold (9). The cells

were grown for 8 weeks and subjected to pulsatile cyclic distension in a bioreactor. The smooth muscle cells produce a matrix containing collagen types I and III, fibronectin, and vitronectin. Biopsy specimens of grafts implanted in patients showed progressive recellularization in vivo with endothelial and smooth muscle cells (8,9). A phase III clinical trial is underway to compare patency and safety of these constructs with synthetic grafts in dialysis patients (Comparison of the Human Acellular Vesses [HAV] With ePTFE Grafts as Conduits for Hemodialysis; NCT02644941).

Tissue-engineering approaches to vessel manufacturing are gaining traction, reaching clinical trial and large animal and primate feasibility studies (2,9,10). These materials are showing long-term functionality and safety in vivo. Cell type, culture conditions, scaffolds, and graft ECM vary and it is not known which strategy has better in vivo functionality. Common to all strategies has been the dependence of growth in carefully controlled "bioreactor" systems (**Central Illustration**). These systems can impart physical stressors on cells such as pulsatile flow, stretch, and strain that direct and support the growth of constructs during manufacturing. These bioreactors are also key quality control systems needed for U.S. Food and Drug Administration approval. They can provide standardization across batches of grafts as well as be tested at critical stages for quality control parameters such as sterility. Bioreactors can also be a source of intellectual property or company proprietary information that can provide some financial incentives for commercialization and translation. Reproducibility, testability, and standardization are essential for cost-effective manufacturing of cell-derived ECM products.

There are several reasons why hemodialysis access has been an early application of tissue-engineered vessels. Forearm graft location is straightforward from a surgical perspective, and there are clinical strategies to manage catastrophic malfunction such as bleeding or occlusion. Coronary artery bypass grafts are an area of clinical need but more technically challenging to implant. Acute graft dysfunction would cause cardiac ischemia or be fatal. However, the poor patency of saphenous vein grafts compared with arterial bypasses clearly shows the need for new strategies. As technical innovations and clinical experience advance, decellularized matrices will find applications for cardiovascular disease. Although they are more complex to manufacture than synthetic materials, these products may have significant advantages that justify the cost. Manufacturing and quality control are areas of rapid technical innovation and development and will continue to rely on the skill and persistence of biomedical engineers, translational researchers, and clinicians. However, if successful, these emerging technologies may open new therapeutic avenues for a variety of patients with cardiovascular diseases.

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