The Future of Cell-Based Vascular Grafts for Hemodialysis Access

A new technology that seeks to reduce complications and costs.

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Synthetic vascular grafts made of Dacron (Invista, Wichita, KS) and Gore-Tex (Gore & Associates, Flagstaff, AZ) have performed well in large- and medium-diameter vascular reconstructions. Although recent advances in surface coatings have improved their performance in smaller-diameter applications,[1] synthetic grafts remain a foreign material that, despite remarkable inertness, still triggers a chronic inflammatory and scarring response.

In the specific context of hemodialysis access grafts, synthetic materials have the disadvantage of being irremediably damaged by repeated puncture, leading to thrombosis and false aneurysm. They also offer a microenvironment that is particularly favorable for microorganism development and are four- to 12-fold more likely to become infected than a fistula made from native tissue.[2]

Nonautologous sources of blood vessels, such as homografts (allografts) and animal-derived xenografts, have been used as alternative hemodialysis access conduits and have shown to be resistant to infection.[3] However, these materials trigger immune and inflammatory responses that can cause graft aneurysm or lead to immunosensitization and disqualification of patients from transplant.[4]

DEVELOPMENT OF NEW GRAFTS

With the advent of mammalian cell culture, the idea of producing a cell-based blood vessel was rapidly proposed, but the apparent impossibility of creating a mechanically sound construct without the inclusion of a synthetic scaffold significantly detracted from the potential benefits of this approach.[5] More recently, biodegradable scaffolds have been used in combination with various cell types and bioreactors to produce successful animal models.[6-8] However, transitioning to the use of human cells proved difficult and was just recently realized with the support from good xenogeneic preclinical data.[9] Biodegradable scaffolds present challenges, such as the host's ability to rapidly and heavily remodel the implant before the scaffold loses its mechanical strength. In addition, degradation byproducts can cause local changes in the environments that can adversely affect cell proliferation or phenotype.[10]

We developed an approach that allows the production of mechanically strong tissues that are composed solely of human cells and the extracellular matrix (largely collagen) produced by these cells.[11] Because this approach excludes any foreign materials and avoids any chemical processing of the extracellular matrix, as is required for animal-sourced tissues, the

Figure 1. More effective cell amplification can be achieved by using modern cultureware options, such as this HyperFlask (Corning, Tewksbury MA), which uses approximately one-tenth of the space of traditional flask cultures.

resulting constructs offer an unparalleled potential for biocompatibility and tissue integration.

Cytograft Tissue Engineering, Inc. (Novato, CA) was founded around this technology, termed "tissue engineering by self-assembly," with the goal of rapidly bringing tissue-engineered blood vessels (TEBVs) to the clinic. Although other groups aimed toward coronary artery bypass grafting or distal reconstruction, we initially identified hemodialysis access as the most challenging application, but the one with the most urgent clinical need.

INITIAL CLINICAL SUCCESS

Completely biological TEBVs have intrinsic advantages over synthetic access grafts because they: (1) will naturally resist infection; (2) can rapidly seal after puncture without the need for maturation; (3) can easily be remodeled by the host; and (4) can have an antithrombogenic endothelium. In 2005, we were the first to implant a TEBV in the high-pressure circulation of a patient.[12] This patient with end-stage renal disease (ESRD) had a history of failed hemodialysis access, but with the Lifeline graft (Cytograft Tissue Engineering, Inc.), she was dialyzed for 13 months, with only one event at 11 months, until she received a transplant. An expanded study with these endothelialized, living, autologous grafts showed a nearly fourfold reduction in graft-related events in a patient population with advanced ESRD.[13]

TRANSITIONING TO COMMERCIAL RELEVANCE

Now that the proof-of-concept has been demonstrated, the next challenge for cell-based vascular grafts will be the development of products that can be produced at an acceptable cost and address multiple markets. Developing a cost-effectiveness strategy requires a multifaceted approach. One important aspect that is necessary for this transition is product simplification that can lead to streamlined manufacturing. We are investigating three possible simplifications of our TEBV.

Figure 2. A compact bioreactor design will be key in producing tissue-engineered products at an acceptable cost. This system can typically support 60 vessels (up to 120) in 77 L of heated controlled atmosphere. The modular closed system provides both increased safety and ease of sampling for quality control.

Although the endothelium may be critical for the patency of small-diameter vessels in lower-flow applications like coronary artery bypass grafting and lower limb bypass, we anticipate that endothelial cells may not be needed for hemodialysis access grafts, considering the very high shear forces at the luminal surface of these vessels. In addition, a TEBV made of a natural, unprocessed, human extracellular matrix may spontaneously re-endothelialize, either from the anastomosis or from circulating cells, like native arteries after endovascular procedures. This simplification would eliminate the need for a separate biopsy, endothelial-specific culture media, many of the cell-amplification steps, and the graft-seeding steps, as well as the associated quality control and release-testing steps. Maybe more importantly, eliminating the endothelial cells introduces the possibility of an important second simplification: using an allogeneic approach.

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Although endothelial cells strongly express class II major histocompatibility complex and are a key player in organ rejection, fibroblasts (the cells used to produce the tubular part of the TEBV) have been shown to be well tolerated in a widely used, US Food and Drug Administration–approved, allogeneic tissue–engineered skin (Apligraf, Organogenesis, Inc., Canton, MA). The use of an allogeneic platform has key manufacturing advantages. This approach eliminates the needs of biopsy for each patient, performing multiple strictly segregated cell expansions, validating every patient-specific batch, and managing patient-to-patient variability. It should be noted that from a single skin biopsy, enough normal adult fibroblasts can be cultured to produce hundreds of thousands of TEBVs without the need for genetic modification or pooling of multiple donors. But for the patient, the most significant advantage is that allogeneic TEBVs can be produced in advance and be available "off the shelf" to serve the emergent need often encountered in ESRD management.

Another simplification that can improve graft availability and also decrease cost is the use of a nonliving graft. We hypothesize that the implantation site can supply host fibroblasts to repopulate the graft. Because we have observed that the collagen of our grafts is not significantly degraded after implantation for at least 9 months,[14] we expect that the graft will easily withstand the mechanical loads without aneurysm formation during graft repopulation. We have recently announced that first human use of an allogeneic TEBV. This non-endothelialized, nonliving, frozen, allogeneic TEBV was successfully used in patients with ESRD without any signs of immune rejection.[15] In addition, this TEBV can be stored for more than 1 year without losing mechanical strength.

IMPROVING THE MANUFACTURING PROCESS

An important part of transitioning cell-based approaches from academic research projects to commercially viable products is to take advantage of the various tools that have become available for mass production of cells, such as amplification in cell "factories" and the implementation of automation in cell and bioreactor feeding (Figure 1). Bioreactor design is also an important component of the production process that will affect the manufacturing costs. In our case, we have consciously avoided designs that include external pumps to deliver fluid flow, pulsating pressures, or dynamic mechanical stimulations in favor of a very compact design (Figure 2). Although complex bioreactors are interesting scientific endeavors, external devices and connected tubing are prone to failure, contamination, and above all, take up valuable footprint.

CONCLUSION

After 2 decades of hype, and some spectacular failures, a second generation of tissue-engineering companies is at the threshold of commercialization. With a focus on large markets and life-saving applications, TEBVs designed for hemodialysis access are among the most promising products poised to bring tissue engineering into widespread clinical use. ■

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