First human use of an allogeneic tissue-engineered vascular graft for hemodialysis access

Wojciech Wysytrychowski, MD, PhD, Todd N. McAllister, PhD, Krzysztof Zagalski, MD, Nathalie Dusserre, PhD, Lech Cierpka, MD, PhD, and Nicolas L’Heureux, PhD

An arteriovenous fistula is the current gold standard for chronic hemodialysis access. Tunneled catheters or synthetic grafts have poorer outcomes and much higher risks of infection. This report presents the first clinical use of a completely biological, allogeneic, nonliving, and human tissue-engineered vascular graft. Tissue-engineered vascular grafts built from allogeneic fibroblasts were implanted as shunts in three hemodialysis patients. The tissue-engineered vascular graft was stored for 9 months, without loss of mechanical strength. Implanted grafts showed no signs of degradation or dilation, with time points up to 11 months. Results of panel-reactive antibody and cross-reactivity tests showed no evidence of immune responses. (J Vasc Surg 2014;60:1353-7.)

With the advent of cell culture, Weinberg and Bell and Zilla et al pioneered the idea of using cells to confer physiologic qualities to prosthetic vascular grafts. L’Heureux et al. realized the original vision of Weinberg and Bell by implanting the first completely biological, living, laboratory-grown graft in the high-pressure circulation of a human, using an approach termed tissue engineering by self-assembly. Clinical use of this autologous graft showed remarkable results, with high primary patency rates and no access-related infections. However, this approach was encumbered by long production times and 3) were dependent on a groin catheter for hemodialysis access at the time of enrollment. The use of the Lifeline allogeneic graft was recognized as a lifesaving procedure by the Medical University of Silesia Bioethics Committee (Katowice, Poland). All patients signed informed consents after the details and risks of the study were described to them by the principal investigator.

Vessels were produced, as previously described, but were not endothelialized, from cells of two informed male donors (aged 72 and 81 years) with end-stage renal disease. Vessels were dehydrated and stored at −80°C for 6 (Allo 1) and 9 months (Allo 2 and 3) before use in this allogeneic safety trial. All graft lots met release criteria previously established for the Lifeline graft. Preimplantation burst pressure was 6407 ± 633 and 5877 ± 896 mm Hg for donor 1 (Allo 1) and donor 2 vessels (Allo 2 and 3), respectively (n = 4 and 8). Suture retention was 261 ± 20 gram-force (gf) and 319 ± 48 gf, respectively (n = 4 and 8). Predehydration values for burst and retention strength were available for donor 2 vessels and were not significantly different from preimplant values (Student t-test, P < .08). Vessels from a third donor (not implanted) were stored for 13 months, without loss of strength (burst, 5066 ± 777 mm Hg [n = 8]; suture, 402 ± 28 gf [n = 8]). Preimplantation compliance ranged from 2.0% to 3.1%/100 mm Hg (standard deviation, 0.4-0.7 [n = 4 to 8]). The grafts were implanted using standard surgical techniques and required no special handling or instrumentation.

Ultrasound examinations at days 2 and 4, at weeks 1, 2, and 4, and monthly thereafter, showed no signs of graft degradation or aneurysm formation (Figs 1-3). To demonstrate functionality, the graft of patient Allo 1 was punctured for hemodialysis at 12 weeks, patient Allo 2 at 7 weeks, and patient Allo 3 at 8 weeks. Blood flows ranging from 1.3 to 1.9 L/min exceeded values for good vascular access, and low dynamic venous pressures were observed.

Immunologic and inflammatory blood markers were within expected values. Total T-lymphocyte values, as well as CD4+ and CD8+ subsets, were not influenced by the allogeneic implantation. Panel reactive antibody (PRA) values at 4, 7, and 24 weeks were not elevated (Allo 2 was not tested after 7 weeks, and Allo 1 was not elevated).
Fig 1. Graft of patient Allo 1, who was an 80-year-old woman with glomerulonephritis and coronary artery disease. She had been receiving hemodialysis for 4.5 years, with previous failures of an ambilateral brachial-cephalic access and of tunneled hemodialysis catheters in multiple locations, recurrent catheter infections and thromboses, hospitalizations for pneumonia, and lower limb deep venous thrombosis. A, Preimplantation hematoxylin and eosin staining of the devitalized graft showed the same organization as a living graft, but with dense nuclear remnants in the outer layers. B, Macroscopic view shows the implanted graft. Doppler ultrasound results at (C) 1 month, (D) 6 months, and (E) 11 months (composites from scan of entire length of graft) demonstrated no evidence of aneurysm or wall degradation. At 11 months, restenosis was noted near the medial anastomosis (MA), 6 months after percutaneous transluminal angioplasty. Flow was 1.3, 0.7, and 0.4 L/min at 1, 6, and 11 months, respectively. Blood test results before implantation and after 2 and 7 weeks: white blood cells (normal reference range, 4.0-10.0 × 10^9/µL): 5.1, 5.1, and 5.7 × 10^9/µL; lymphocytes (normal reference range, 20.5%-45.5%): 22.3%, 35.4%, and 23.7%; T-lymphocytes (normal reference range, 59.0%-85.0%): 77.8%, 73.3%, and 78.3%; T-helper (CD3^+4^+) cells (normal reference range, 29.0%-57.0%): 42.1%, 42.9%, and 40.7%; and T-suppressor/cytotoxic (CD3^+8^+) lymphocyte (normal reference range, 11.0%-38.0%): 32.8%, 33.3%, and 32.9%. Donor-specific cross-match and panel reactive antibody (PRA) test results at 7 and 24 weeks after implantation were negative. AA, Arterial anastomosis; VA, venous anastomosis.
tested at 4 weeks), showing that the allogeneic tissue-engineered graft did not sensitize patients to human leukocyte antigen antibodies. In addition, lymphocytotoxic cross-reactivity tests between donor 1 and Allo 1 were negative at 7 weeks and at 6 months.

To ensure secondary graft patency, interventions were required at 3 months in Allo 3 and at 5 months in Allo 1. Stenoses of axillary and central veins were observed in both patients, partly as a result of previous placements of catheters and shunts. Stenoses at the medial anastomosis of the Lifeline graft were also noted.

Mechanical thrombolysis and thrombectomy, followed with intraluminal balloon dilatation (Allo 1) or hybrid intervention with surgical thrombectomy and axillary vein stenting (Allo 3), were performed successfully. In all three patients, no symptoms of graft-related infection with access site inflammation were observed, which is consistent with previous observations from autologous Lifeline graft implants.

Patient Allo 1 ultimately lost graft patency 11 months after implantation as the result of an automobile accident. The resulting
injuries led to shunt constriction and occlusion. Patient Allo 2, who had previous access-related infections and C-reactive protein levels >100 mg/L before implantation, showed systemic signs of infection 6 weeks after implantation. A semipermanent catheter was removed, leaving the Lifeline graft as the sole vascular access for the last 7 days before the patient died of sepsis. Autopsy revealed multiple renal abscesses with lung abscess. The graft of patient Allo 3 functioned for 7 months and failed due to thrombosis.

DISCUSSION

Infections of synthetic access devices are particularly important complications for end-stage renal disease patients aged >65 years. Infection in this population is associated with a mortality rate hovering near 10% per event. In contrast, fistulas made from native vessels, when available, show infection rates that are fourfold to >12-fold lower than synthetic grafts. Similarly, allografts or chemically treated xenografts have been shown to be resistant to infection, although these grafts trigger inflammatory and immune responses. Not surprisingly, cadaver-sourced homografts have been associated with tissue degradation and mechanical instability linked to immune responses. In addition, the use of allogeneic tissue for hemodialysis access has been shown to trigger immunosensitization that can later preclude patients from transplantation. However, a devitalized, tissue-engineered graft is unlikely to be recognized as a foreign material for two principal reasons:

First, the laboratory-grown graft is a simple tissue made from human extracellular matrix (ECM) and fibroblasts. It lacks endothelial cells or other cells and structures prone to trigger a specific immune system response, and the literature supports the concept that allogeneic fibroblast-based constructs are well tolerated by the immune system.

Second, the graft does not undergo any chemical modifications or denaturing steps that would damage the ECM and initiate degradation through a nonspecific immune system response. The lack of structural degradation, the stable levels of lymphocyte, and the negative PRA results up to 24 weeks after implantation confirmed the innocuity of the allogeneic Lifeline graft. This is in contrast to homografts that can raise PRA levels by as much as 80% at 3 months.

One concern with the use of a devitalized graft, however, is its ability to withstand significant mechanical loads during the repopulation of the implant by the host’s cells. The nonliving graft used in this study withstood the harsh environment of the AV shunt, without signs of aneurysm, for up to 11 months. The absence of cells may, however, have adversely affected graft performance; for example, delayed extravasation with slight bleeding into the surrounding tissue at the site of previous needle placement was noted. Although this type of complication is common with synthetic grafts, it was not a significant observation with the autologous, living Lifeline graft used previously. The lack of living cells in the graft wall may slow the repair process at the puncture site. However, the size and complexity of this patient population is not suitable for the determination of graft performance.

Although most efforts to produce nonliving transplant tissues use detergent-based decellularization processes, this study used a simple dehydration process. Because fibroblasts in culture do not express major histocompatibility complex class II antigens and no immunologically active or xenogeneic cells are present, the rationale for decellularization is clearly reduced. Moreover, that detergent-based processing damages the ECM has been well established. In previous human and animal studies performed with a live graft, a dehydrated tissue layer sandwiched between endothelial cells and a living adventitia layer proved to be extremely durable in vivo. This suggests that this devitalization process did not compromise the mechanical strength and preserved the natural structure of the ECM.

The presence of an antithrombogenic lining plays an important role in preventing occlusive failures in low-flow indications such as lower limb bypass or coronary bypass. However, in the high-flow setting of AV access, an endothelium may not be required to maintain the patency of a biologic graft. Although thrombogenic failures were noted at 3 months in patient Allo 3 and at 5 months in patient Allo 1, these longer-term complications appeared to be secondary to stenosis.

CONCLUSIONS

The lack of graft degradation or immunosensitization in all patients suggests that this allogeneic graft is safe from an immune perspective. Taken together, this clinical study suggests that neither the transition to allogeneic cells nor the change to a nonliving graft adversely affected the mechanical stability of the graft.

We gratefully acknowledge the critical roles of Dr Zbigniew Darocha and Dr Dariusz Klein in managing these hemodialysis patients at their respective nephrology clinics. We thank Dr Grzegorz Oczkowski and Dr Przemyslaw Pencak, chief of Interventional Radiology Division, for their assistance in caring for the patients.

REFERENCES


Submitted May 10, 2013; accepted Aug 14, 2013.