Readily Available Tissue-Engineered Vascular Grafts
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Autologous or synthetic vascular grafts are used routinely for providing access in hemodialysis or for arterial bypass in patients with cardiovascular disease. However, some patients either lack suitable autologous tissue or cannot receive synthetic grafts. Such patients could benefit from a vascular graft produced by tissue engineering. Here, we engineer vascular grafts using human allogeneic or canine smooth muscle cells grown on a tubular polyglycolic acid scaffold. Cellular material was removed with detergents to render the grafts nonimmunogenic. Mechanical properties of the human vascular grafts were similar to native human blood vessels, and the grafts could withstand long-term storage at 4°C. Human engineered grafts were tested in a baboon model of arteriovenous access for hemodialysis. Canine grafts were tested in a dog model of peripheral and coronary artery bypass. Grafts demonstrated excellent patency and resisted dilatation, calcification, and intimal hyperplasia. Such tissue-engineered vascular grafts may provide a readily available option for patients without suitable autologous tissue or for those who are not candidates for synthetic grafts.

INTRODUCTION

There is a considerable need for readily available vascular grafts when a patient’s own vasculature cannot be used for grafting because it is diseased or because suitable vascular tissue has already been harvested. Instances when a vascular graft might be needed include peripheral arterial disease, coronary artery disease, or to provide arteriovenous access for hemodialysis in patients with kidney failure. To date, the most successful vascular conduits for coronary or peripheral vascular surgery are the patient’s own blood vessels, often the greater saphenous vein in the leg. For patients requiring hemodialysis, ideal vascular access is a fistula, or a surgically created connection between the patient’s own artery and vein.

When autologous vessels are not available for grafting, synthetic polytetrafluoroethylene (PTFE) grafts are often used for applications requiring large-diameter (≥6 mm) vessels, such as arteriovenous access for hemodialysis (1), or peripheral arterial bypass above the knee. Arteriovenous PTFE grafts for hemodialysis have a median patency (open to flow) time of only 10 months because of infection, thrombus formation, or intimal hyperplasia that results in occlusion at either the distal anastomosis (connection site between the graft and the native vasculature) or the outflow vein (1, 2). Other types of grafts, such as decellularized bovine internal jugular xenografts and human allograft vessels from cadavers, are prone to aneurysm, calcification, and thrombosis and therefore have not gained widespread clinical acceptance (3–5). In situations where small-diameter (that is, 3 to 4 mm) vessels are required, such as below the knee and for coronary artery bypass grafting, the patient’s own vasculature (that is, internal mammary artery and saphenous vein) is predominantly used because synthetic grafts and allografts have unacceptably low patency rates. For example, patency is <25% at 3 years when synthetic and cryopreserved grafts are used in peripheral and coronary bypass surgeries compared to >70% for autologous vascular conduits (6–11). Thus, a readily available, versatile vascular graft with good patency that is resistant to dilatation (chronic increase in diameter), calcification, and intimal hyperplasia would fill a substantial and growing clinical need.

To date, tissue-engineered vascular grafts (TEVGs) formed by seeding autologous bone marrow cells onto a copolymer of L-lactide and ε-caprolactone (12), or culturing autologous fibroblasts and endothelial cells (ECs) without a scaffold (13), have shown promising functional results in early clinical trials. Thus far, only the latter has proven physically strong enough for use in the arterial circulation. This patient-specific graft requires a 6- to 9-month culture period in which the autologous fibroblasts produce sheets of tissue. The sheets are fused together around a stainless steel mandrel (4.8-mm diameter), the inner fused layers are dehydrated, and the graft lumen is seeded with autologous ECs (13). Because of high production costs of ≥$15,000 per graft (14) and long wait times of up to 9 months, it is unlikely that this approach will become standard clinical practice or will be useful for patients who require expeditious intervention.

Therefore, we have developed an alternative TEVG that would be immediately available for large-diameter (≥6 mm) applications. Our graft would also be available for small-diameter (3 to 4 mm) applications with only a short wait time (<1 month) to allow autologous ECs to be isolated, expanded, and seeded. Here, we evaluate the function of these TEVGs in large-animal models of arteriovenous access (6-mm-diameter TEVGs), peripheral arterial bypass (3- to 4-mm-diameter TEVGs), or coronary bypass (3-mm-diameter TEVGs).

RESULTS

Generation of TEVGs from allogeneic cells and decellularization

To produce TEVGs (3 to 6 mm in diameter), we cultured allogeneic smooth muscle cells (SMCs) obtained from cadaveric donors on rap-
idiomatically degradable polyglycolic acid (PGA) tubular scaffolds (Fig. 1A) in a bioreactor that delivers cyclic radial strain (15). During the culture period, SMCs secrete extracellular matrix proteins, predominantly collagen, to form biosynthetic vascular tissue (Fig. 1B) (15), and the PGA degrades. At the end of the culture period, the resultant tissue is decellularized with detergents, leaving only the secreted collagenous matrix (Fig. 1C) (16). The decellularization process removes antigenic, allogeneic cells from the graft, thereby allowing the use of banked allogeneic cells to produce TEVGs that are nonimmunogenic and can be used in any recipient. These TEVGs (Fig. 1C) can be stored in a standard phosphate-buffered saline (PBS) at 4°C and are immediately available for placement as an arteriovenous graft (that is, a 6-mm-diameter graft that connects an artery to a vein; Fig. 1D). In addition, they can be seeded with autologous ECs to reduce the risk of thrombosis associated with small-diameter (3 to 4 mm) vascular grafting in peripheral or coronary arteries (Fig. 1E) (13, 17, 18).

**Strength and stability of decellularized human TEVGs**

To assess the mechanical consistency of grafts produced from different donors, we produced 37 decellularized TEVGs (6 mm in diameter, 23 cm in length), using cells from 19 human donors (Table 1). We measured graft suture strength, using 6-0 Prolene 2 mm from the edge of every graft. Burst pressure was tested intermittently on randomly selected tubular grafts by inflating the grafts (2 cm in length) with saline until they ruptured. Suture strengths (Table 1) did not change significantly with donor age (P = 0.26; ages 17 to 49), with male versus female donor origin (P = 0.52), or with the use of single donor versus pooled donor populations (two to six donors per pool) for graft culture (P = 0.42). A group of TEVGs was randomly selected and stored for 12 months. TEVGs retained their strength, without significant changes in suture strength, burst pressure, or compliance (P = 0.97, P = 0.18, and P = 0.48, respectively) after 12 months of storage at 4°C in PBS, and were within the ranges reported for native human vasculature (Table 2). TEVGs contained residual PGA fragments (1.1 ± 0.1% of cross-sectional area of TEVG histological sections before storage), which did not degrade further during storage at 4°C (1.0 ± 0.1% after 9 months of storage, P = 0.54).

**Decellularized human TEVGs in an arteriovenous model**

To assess the function of 6-mm TEVGs, we implanted nine TEVGs grown from human cells (6 mm in diameter, 12.5 ± 1.1 cm in length) into baboons as arteriovenous conduits (Fig. 2A) and observed these grafts for 1 to 6 months (Table 3). One animal was excluded after pulling open the surgical incision site, exposing the graft and creating a wound infection. No infection was observed in the eight remaining animals. Duplex ultrasound measurements of TEVGs at weeks 0, 2, 4, 12, and 24 (Table 4) showed no change in diameter (P = 0.28), no change in wall thickness (P = 0.93), and an increase in flow rate between weeks 0 and 2 (P < 0.01). The connection of the high-pressure arterial system to the low-pressure venous system decreases proximal arterial resistance, thereby allowing blood to flow rapidly through the TEVGs. We speculate that the increase in flow observed between weeks 0 and 2 results from remodeling (for example, dilatation) of the native vasculature in response to decreased resistance after arteriovenous shunt placement. Flow through TEVGs (Table 4) was sufficient for hemodialysis (>300 ml/min (19)). TEVGs were accessed initially at 4 weeks (Fig. 2B), which is a clinically relevant time for first access to allow for integration and remodeling of hemodialysis grafts, and then at 3 and 6 months. Of the eight arteriovenous TEVGs, two of two were patent at 1 month, two of three were patent at 3-month explant, and three of three were patent at 6-month explant (Fig. 2C). Only one graft showed thrombosis at 3 months, likely due to technical difficulties with access, which required prolonged manual pressure that led to graft clotting. Hence, the patency of the arteriovenous 6-mm TEVGs in the baboon was 88% (seven of eight). No aneurysmal dilatation and no calcification were observed in any graft. Furthermore, grafts did not exhibit substantial intimal hyperplasia. Anastomotic neointimal hyperplasia at 6 months (luminal hyperplasia thickness of 0.11 ± 0.05 mm) was less than that reported at 1 month for PTFE arterial bypass grafts in a baboon model (0.25 ± 0.09 mm) (20).
TEVGs in small-diameter peripheral and coronary arterial bypass models
The function of small-diameter (3 to 4 mm) TEVGs was evaluated in canine models of peripheral and coronary artery bypass. Canine TEVGs were produced from allogeneic canine cells, decellularized, and luminally seeded with autologous ECs from the intended recipient (similar to Fig. 1, but using canine cells). Attached ECs were elongated and aligned within the lumens of TEVGs, but complete EC coverage

Table 1. Donor data and suture strengths for 6-mm-diameter decellularized human TEVGs. All data are presented as mean ± SEM (number of distinct grafts tested).

<table>
<thead>
<tr>
<th>Human donor</th>
<th>Donor age</th>
<th>Donor sex</th>
<th>Diabetic</th>
<th>Smoker</th>
<th>Hypertension</th>
<th>Other diseases</th>
<th>Suture strength of human TEVG (g)</th>
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<tbody>
<tr>
<td>1</td>
<td>17</td>
<td>F</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Mitral valve prolapse; asthma</td>
<td>250 (1)</td>
</tr>
<tr>
<td>2</td>
<td>19</td>
<td>M</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>None</td>
<td>233 ± 20 (4)</td>
</tr>
<tr>
<td>3</td>
<td>25</td>
<td>F</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>None</td>
<td>130 ± 20 (2)</td>
</tr>
<tr>
<td>4</td>
<td>33</td>
<td>F</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>None</td>
<td>80 (1)</td>
</tr>
<tr>
<td>5</td>
<td>34</td>
<td>F</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>None</td>
<td>223 ± 27 (4)</td>
</tr>
<tr>
<td>6</td>
<td>45</td>
<td>F</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Asthma</td>
<td>120 ± 10 (2)</td>
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<tr>
<td>7</td>
<td>46</td>
<td>M</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>None</td>
<td>110 (1)</td>
</tr>
<tr>
<td>8</td>
<td>46</td>
<td>M</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>None</td>
<td>115 ± 5 (2)</td>
</tr>
<tr>
<td>9</td>
<td>46</td>
<td>M</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Kidney failure</td>
<td>135 ± 35 (2)</td>
</tr>
<tr>
<td>10</td>
<td>47</td>
<td>M</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Gastroesophageal reflux disease</td>
<td>275 ± 42 (4)</td>
</tr>
<tr>
<td>Pool of donors 9 and 12</td>
<td>46</td>
<td>M</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Kidney failure</td>
<td>155 ± 35 (2)</td>
</tr>
<tr>
<td>Pool of donors 1, 4, 5, 7, 8, and 10</td>
<td>33</td>
<td>F</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Mitral valve prolapse; asthma</td>
<td>140 (1)</td>
</tr>
<tr>
<td>Pool of donors 13, 14, and 15</td>
<td>18</td>
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<td>No</td>
<td>No</td>
<td>None</td>
<td>146 ± 5 (7)</td>
</tr>
<tr>
<td>Pool of donors 16, 17, 18, and 19</td>
<td>17</td>
<td>F</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>None</td>
<td>265 ± 25 (2)</td>
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</table>

Table 2. Mechanical properties of TEVGs (Fig. 1C) before and after 12 months of storage, and comparison with native vasculature.

<table>
<thead>
<tr>
<th>Suture strength (g)</th>
<th>Burst pressure (mmHg)</th>
<th>% Compliance per 100 mmHg</th>
<th>Reference</th>
</tr>
</thead>
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<tr>
<td>Human TEVGs (6-mm diameter)</td>
<td>178 ± 11 (35)</td>
<td>3337 ± 343 (10)</td>
<td>3.3 ± 0.8 (10)</td>
</tr>
<tr>
<td>Human TEVGs stored 12 months (6-mm diameter)</td>
<td>170 ± 22 (9)</td>
<td>2651 ± 329 (5)</td>
<td>2.5 ± 0.8 (5)</td>
</tr>
<tr>
<td>Human saphenous vein</td>
<td>196 ± 29 (7)</td>
<td>1599 ± 877 (7)</td>
<td>0.7–1.5</td>
</tr>
<tr>
<td>Human internal mammary artery</td>
<td>138 ± 50 (6)</td>
<td>3196 ± 1264 (16)</td>
<td>11.5 ± 3.9 (7)</td>
</tr>
</tbody>
</table>
was never achieved. Rather, EC coverage varied widely between grafts, with a coverage range of 0 to 60% (14 ± 8%) on sections sampled from grafts before implant. In general, canine grafts were less strong than human grafts, although still suitable for implantation (burst pressures were 1618 ± 67 mmHg for 3-mm canine grafts; n = 39).

Five endothelialized canine TEVGs (3 to 5 cm in length) were implanted as carotid artery bypass grafts, with follow-up times of 1 week to 12 months (Fig. 2D). One animal was excluded after dying acutely with a patent graft. One graft occluded at 1 week. All other grafts remained patent, including two grafts that were followed for 1 year (Table 3). A representative angiogram at 1 year (Fig. 2E) demonstrated excellent long-term patency. No stenosis or dilatation was observed acutely with a patent graft. One graft occluded at 1 week. All other canine TEVGs (a total of six in the carotid and coronary circulations), primary patency was 83% (five of six).

Remodeling of TEVGs in vivo
Before implant, TEVGs were smooth and uniform (Fig. 3A). Histological evaluation (Fig. 3B), as well as DNA quantification (0.74 ± 0.10 μg of DNA per milligram of dry tissue weight), demonstrated that the extent of decellularization of TEVGs was similar to that of other decellularized scaffolds used clinically (21). The extracellular matrix of TEVGs contained collagen types I and III, which are the most prevalent types in native vasculature, as well as fibronectin and vitronectin, all with primarily circumferential alignment (Fig. 3, C to F).

After implantation into baboons and canines, TEVGs showed considerable remodeling. For all grafts, gross analysis at explant revealed a smooth inner graft tissue surface with formation of a loose fibrous outer "adventitial" tissue layer (Fig. 4A). TEVGs demonstrated a notable lack of constrictive fibrotic tissue surrounding grafts at explant (Fig. 4A). Grafts integrated well with the native vasculature at anastomotic sites (Fig. 4B).

TEVGs were remodeled to become compositionally more similar to native artery after implantation. Within 3 months after implant, elastin formed in anastomotic sections of grafts explanted from baboons (Fig. 4C) in regions containing the highest density of infiltrated host cells (Fig. 4D). No elastin was observed midgraft in any explanted TEVGs. Cells expressing α-smooth muscle actin, which could be either SMCs or myofibroblasts, densely populated the full thickness of TEVGs near anastomotic sites (Fig. 4E), suggesting migration from adjacent native vasculature. Actin-positive cells appeared to infiltrate transmurally from the adventitial-like tissue layer into TEVGs in midgraft regions, starting at 6 months in the baboon model (Fig. 4F). In the canine model, α-smooth muscle actin–positive cells began to infiltrate midgraft sections transmurally by 1 month (Fig. 4G) and were observed throughout the midgraft wall by 1 year (Fig. 4H). Host cell infiltration into midgraft TEVG walls was more rapid in the canine model, possibly because shorter grafts were placed in the canines or because of differences in species. In both models, there were fewer cells within the TEVG walls in midgraft sections (Fig. 4, F to H) than sections near the anastomoses (Fig. 4, D and E). Von Willebrand factor (an EC marker)–positive cells were observed on luminal surfaces of TEVGs both near anastomotic sites and midgraft in both canine grafts (which were endothelialized before implantation) and baboon grafts (which were not) (Fig. 4I). ECs may have migrated from anastomosed vascular tissue, migrated transmurally from surrounding tissue (22), or originated from circulating progenitor cells (23).

In the baboon study, midgraft TEVG segments were saved for mechanical testing and collagen analysis at explant. Explanted TEVGs displayed increased suture strength (276 ± 28 g, n = 8, P = 0.01), but no significant changes in burst pressure (3646 ± 582 mmHg, n = 4, P = 0.67) or compliance (3.4 ± 2.3% per 100 mmHg, n = 4, P = 0.70) compared to the preimplant values reported in Table 2. Thus, TEVGs were mechanically robust without complete infiltration of cells into midgraft sections (Fig. 4F) or elastin in midgraft sections. No significant changes in collagen density were observed between TEVGs before implant (57 ± 5%, n = 8), TEVGs at explant (46 ± 5%, n = 7),

![Fig. 2. Implant sites and observations. (A) Human cell–derived 6-mm TEVGs (g) were implanted between the axillary artery (a) and the brachial vein (v) in a baboon model. (B) Arteriovenous grafts (g) were first accessed with 16-gauge needles at 4 weeks after implant. (C) A representative angiogram of the explanted graft demonstrates that the graft was patent (open to flow) (g). The arterial anastomosis (aa), venous anastomosis (va), and brachial vein (v) are shown. (D and E) Canine cell–derived 3-mm TEVG (g) as a carotid bypass, with clips occluding the intervening carotid artery (ca), (D) at implant, and (E) a representative angiogram showing patency with no luminal narrowing at 1 year. (F) Canine cell–derived 3-mm-diameter TEVGs (g) were implanted on the heart. (G) CT scan shows a patent graft (g) with no dilatation at 1 month.](https://www.sciencetranslationalmedicine.org/content/v3/68/ar9/f2)

inflow axillary artery (46 ± 5%, n = 7), or control axillary artery explanted from the opposite arm (42 ± 3%, n = 7; P = 0.07).

TEVGs were not immunogenic. Injections of homogenized TEVG and PBS (as a negative control) were placed intradermally in every baboon at the time of graft implant and again 4 weeks after implantation (Fig. 5A). The absence of visible induration or redness at all injection sites indicated that recipients were not sensitized to graft material. Immunogenicity of grafts was also assessed by sampling blood from baboons with implanted TEVGs and measuring in vitro proliferation of T cells exposed to PTFE grafts (negative control) or TEVGs (Fig. 5B). No T cell proliferation was observed in any baboon. Immunostaining of dense cellular regions (Fig. 5C) showed only sparse populations of CD3- or CD20-positive cells (Fig. 5, D and E), which were often undetectable in midgraft sections. Foreign-body giant cells were not observed in any explanted TEVG. Finally, calcification, which is commonly observed in xenogeneic or elastin-containing vascular grafts (24, 25), was not observed in any TEVG in any model (Fig. 5F).

**DISCUSSION**

Here, TEVGs were generated by culturing human cadaveric donor cells or canine cells on a degradable PGA scaffold to support synthesis of a collagenous extracellular matrix. Antigenic cellular material was removed via a detergent-based decellularization step to render tissues nonimmunogenic. The TEVGs contained minimal PGA fragments and retained mechanical properties similar to those of native vessels after 12 months of storage in buffer at 4°C. The functional capabilities of 6-mm-diameter human TEVGs were demonstrated in a baboon arteriovenous model. Small-diameter (3 to 4 mm) canine TEVGs were luminally seeded with ECs and implanted in canine models of peripheral and coronary bypass. TEVGs integrated well with native vasculature at anastomotic sites and resisted intimal hyperplasia. We observed infiltration of α-smooth muscle actin–positive cells, ECs on graft lumens, and elastin formation near anastomoses. Long-term patency was demonstrated for up to 1 year.

**Table 3. Characteristics of implanted TEVGs.**

<table>
<thead>
<tr>
<th>Human grafts in a baboon arteriovenous model</th>
<th>Graft inner diameter (mm)</th>
<th>Autologous ECs seeded</th>
<th>Graft access time points (months)</th>
<th>Aneurysmal dilatation</th>
<th>Patent</th>
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</thead>
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<tr>
<td>1 month</td>
<td>6</td>
<td>No</td>
<td>No access</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>1 month</td>
<td>6</td>
<td>No</td>
<td>1</td>
<td>No</td>
<td>Yes</td>
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<tr>
<td>3 months</td>
<td>6</td>
<td>No</td>
<td>1</td>
<td>No</td>
<td>No</td>
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<tr>
<td>3 months</td>
<td>6</td>
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<td>1, 3</td>
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<td>1, 3</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>6 months</td>
<td>6</td>
<td>No</td>
<td>1, 3, 6</td>
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<td>1, 3, 6</td>
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<td>1, 3, 6</td>
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<table>
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<th>Canine grafts in a canine carotid artery bypass model</th>
<th>Graft inner diameter (mm)</th>
<th>Autologous ECs seeded</th>
<th>Graft access time points (months)</th>
<th>Aneurysmal dilatation</th>
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<tr>
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<td>3</td>
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<td>No</td>
<td>No</td>
</tr>
<tr>
<td>1 month</td>
<td>3</td>
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<td>NA</td>
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<td>12 months</td>
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<td>12 months</td>
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<td>3</td>
<td>Yes</td>
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<table>
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<th>Graft inner diameter (mm)</th>
<th>Autologous ECs seeded</th>
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<td>3</td>
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<tr>
<td>1 month</td>
<td>3</td>
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<td>Excluded</td>
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**Table 4.** Duplex ultrasound measurements of TEVGs placed as arteriovenous grafts in baboons.

<table>
<thead>
<tr>
<th>Week 0</th>
<th>Week 2</th>
<th>Week 4</th>
<th>Week 12</th>
<th>Week 24</th>
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<tbody>
<tr>
<td>Diameter (mm)</td>
<td>5.8 ± 0.2 (7)</td>
<td>6.3 ± 0.3 (7)</td>
<td>6.7 ± 0.3 (7)</td>
<td>6.8 ± 0.6 (5)</td>
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<tr>
<td>Wall thickness (mm)</td>
<td>1.0 ± 0.1 (7)</td>
<td>0.9 ± 0.1 (7)</td>
<td>1.0 ± 0.1 (7)</td>
<td>1.1 ± 0.2 (5)</td>
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<tr>
<td>Flow rate (ml/min)</td>
<td>764 ± 216 (7)</td>
<td>2278 ± 430 (7)</td>
<td>1464 ± 124 (7)</td>
<td>1559 ± 379 (5)</td>
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</tbody>
</table>
Our approach of using allogeneic human cells to produce TEVGs allows one human donor to provide grafts for dozens of patients. This approach differs significantly from the one-donor-to-one-recipient model, which pertains to autologous tissue engineering and to cadaveric human or animal blood vessels. One human donor provides a cell bank large enough to produce 37 large-diameter [6-mm internal diameter (ID)] TEVGs or 74 small-diameter (3-mm ID) TEVGs. Pooling cells from multiple donors allows for the generation of large cell banks, which in turn makes possible the manufacture of many TEVGs per cell bank (that is, 200 to 500 TEVGs). This offers greater economies of scale than completely autologous tissue engineering approaches. Further, use of allogeneic cells, combined with decellularization and simple storage methods, allows the culture period for graft production to be moved “off-line.” Therefore, patients have no waiting period for graft production, because the grafts have already been created and stored. The ability to store grafts is an important step in making TEVGs available to the patient immediately at their time of need, as opposed to custom-made grafts for each patient that involve a lengthy waiting time. This is an important departure from cellular products, which generally cannot be stored long-term without specialized cryopreservation equipment and laborious thawing procedures (26).

Decellularized TEVGs have several advantages over decellularized human cadaveric vessels. First, cadaveric human vasculature has small branches that must be ligated, whereas engineered tissues consist of a tube without branches. Second, decellularized TEVGs have a loose tissue structure without layers of lamellar elastin, which allows decellularization solutions to readily permeate engineered tissues to remove cellular material without excessive exposure that may damage extracellular matrix integrity. Third, using a decellularized TEVG approach maximizes the impact of healthy tissue donors by allowing production of a large number of grafts per donor, whereas a decellularized cadaveric vascular graft approach has limited amounts of available vascular

**Fig. 3.** Decellularized human TEVGs before implant. (A) A 6-mm-diameter decellularized human TEVG before implant. (B) A representative decellularized TEVG shows no cells in H&E-stained sections (arrow points to residual PGA), and the porous structure typical of decellularized TEVGs. (C to F) Decellularized TEVGs stain strongly and diffusely for collagen type I (C), and also stain for organized (D) collagen type III, (E) fibronectin, and (F) vitronectin. Areas staining positive for extracellular matrix proteins are noted with open arrowheads. Note that DAB staining masks the porous structure in (C) to (F). Scale bars, 100 μm.

**Fig. 4.** Explanted TEVGs remodeled in vivo. (A) A 6-mm-diameter human TEVG explanted from the baboon model at 6 months demonstrates formation of a loose external adventitial-like layer (g, graft; a, adventitia). (B) A 4-mm-diameter canine TEVG explanted from a carotid bypass model at 1 year (arrow points to anastomotic suture line). (C) Movat’s stain shows elastin (black) in a 6-month baboon explant. (D) H&E staining of a 6-mm-diameter baboon explant at 6 months showed cells densely populating graft walls close to the arterial anastomosis [arrowheads point to stained cells in (D) to (I)]. (E and F) After 6 months in the baboon model, α-smooth muscle actin–positive cells (brown) (E) populated the TEVG wall near anastomotic sites (note: concentrated staining was observed below the luminal surface, but cells on the lumen were not actin-positive) and (F) started to infiltrate the TEVG midgraft from surrounding adventitial-like tissue [arrows define depths of graft walls in (F) to (H)]. (G) In the canine model, α-smooth muscle actin–positive cells (green) were observed infiltrating into midgraft sections of canine carotid artery bypass grafts from surrounding adventitial-like tissue at 1 month. (H) At 1 year, actin-positive cells were observed through the depth of canine graft walls. (I) A TEVG explanted from a baboon demonstrates positive staining for von Willebrand factor in luminal cells (section near anastomosis shown). Scale bars, 100 μm.
tissue per donor with diameters that are appropriate for common cardiovascular surgical procedures. TEVGs can be created in a variety of diameters that more suitably match the diameters of native bypassed arteries. In contrast, decellularized human cadaveric vessels cannot be created for a particular diameter, and size mismatch between the small native vessel and the large bypass graft can occur, potentially resulting in diminished patency.

The presence of sparse residual PGA fragments in TEVGs at the time of implant was not of concern, because PGA is a Food and Drug Administration (FDA)–approved degradable suture material with breakdown products that are readily metabolized. Further, PGA has been used as a vascular graft component without any known negative effects on vascular remodeling (12). The human cell–derived grafts produced in this study were an order of magnitude stronger than those described in previous reports that also used PGA as a support for tissue creation (27, 28). However, these previous reports used human venous cells or commercially available human aortic cells that had been passaged many times in culture before use in the bioreactor (27, 28). In previous reports, use of dense PGA sutures to sew sheets of PGA into tubes left a substantial amount of residual PGA in TEVGs, which diminished the strength of TEVGs (29).

In large-diameter applications, such as above-the-knee peripheral bypass surgery and hemodialysis access, PTFE vascular grafts function well enough to warrant routine clinical use (10). Therefore, we propose use of large-diameter TEVGs without luminal EC seeding. However, for small-diameter applications, it has been extremely difficult to find a functional vascular graft other than the patient’s own vasculature (10), which is highly compliant (see Table 2) and contains ECs. To minimize risk of graft occlusion, we seeded ECs onto TEVGs before implant in the small-diameter peripheral and coronary settings to provide an antithrombogenic luminal surface. ECs were isolated from peripheral arteries or veins of dogs before undergoing bypass with TEVGs. This is similar to peripheral vein harvest approaches previously reported for isolation of ECs for vascular graft seeding (13, 30). Autologous ECs could also be isolated more rapidly from adipose tissue (31) or circulating blood (32, 33), which could reduce the patient’s wait time for endothelialization from weeks to days or possibly even to hours.

Our observed patency rate of 83% for small-diameter TEVGs with poor luminal EC coverage suggests that complete luminal EC coverage before implant may not be required for graft function in the setting of systemic antiplatelet therapy throughout the duration of implantation. Poor EC coverage at implant is also observed in saphenous vein grafts, which are often denuded of endothelium during graft isolation (34). It is possible that the presence of sparse ECs at the time of implant aids in maintaining patency in vivo, either by supplying sufficient release of antithrombogenic signals or by aiding in recruitment of recipient ECs to the TEVG luminal surface (35). On the other hand, TEVGs may be less thrombogenic than other synthetic vascular graft materials and may function without ECs on the luminal surface at the time of implant. Further studies are needed to assess vascular implantation of TEVGs without ECs, in the presence of antiplatelet therapy, to evaluate the necessity of EC seeding.

The functional effects of immunogenicity [intimal hyperplasia, aneurysmal dilatation, or calcification in the long-term (36–38)] were not observed in baboon or canine studies, further supporting the view that TEVGs were nonimmunogenic. In contrast, discordant xenogeneic extracellular matrix proteins and allogeneic cells (found in bovine vascular xenografts and human cadaveric cryopreserved vascular allografts, respectively) trigger immunological responses and their functional side effects (3–5, 39, 40). TEVGs resisted intimal hyperplasia formation in long-term implants. TEVGs placed in the arteriovenous baboon model demonstrated less neointimal hyperplasia at 6 months than did PTFE grafts placed in an arterial bypass setting for 1 month (20). This is encouraging, given that arteriovenous grafts typically

![Image](https://example.com/image.png)

**Fig. 5.** TEVGs were not immunogenic. (A) Intradermal injections of homogenized graft material and PBS (negative control) in baboons at 4 weeks after implantation displayed no visible induration or redness. (B) Representative proliferation of T cells isolated at implant (week 0) and 24 weeks after implant, after exposure to segments of polytetrafluoroethylene (PTFE) (negative control; not implanted) and TEVGs, demonstrated that grafts are immunologically tolerated. (C) H&E staining shows a large population of infiltrated cells in anastomotic sections of TEVGs at 6 months in the baboon. (D and E) Only a sparse population of cells in anastomotic sections stain positive for (D) CD3 (T lymphocyte marker) or (E) CD20 (B lymphocyte marker) at 6 months in the baboon. (F) Absence of calcification (lack of red color) in alizarin red stain is demonstrated in a human TEVG explanted from the baboon model at 6 months. Arrows point to stained cells. Scale bars, 300 μm.
trigger more substantial intimal thickening than do arterial bypass grafts. Given that end-to-side carotid artery bypass has been described as a model that results in extensive intimal hyperplasia at 1 month (41), the absence of intimal hyperplasia at 1 year in our canine peripheral bypass studies is particularly encouraging.

Our study has several limitations. In the baboon model, frequency of access was limited due to the necessity of anesthetizing animals for each access. Furthermore, only a small number of TEVGs were evaluated in the canine coronary bypass model. Our main objective with the coronary bypass implants was to evaluate the feasibility of TEVGs being able to withstand the mechanical forces of cardiac motion. Additional studies will be necessary to determine the long-term function of TEVGs in the coronary bypass model, which is a challenging model for patency given the small diameter of coronary arteries (≤1.5 to 2 mm).

TEVGs that are available without a significant patient wait time represent a substantial advance over completely autologous tissue engineering approaches, wherein patients must wait for long time periods for grafts to be cultured. The vascular grafts presented herein are functional as arteriovenous conduits and as small-caliber arterial bypasses in the peripheral (carotid) and coronary circulations. Conduits used in the clinic have suffered from substantial intimal hyperplasia, aneurysm, and calcification (1–5). Encouragingly, our decellularized TEVGs resist substantial intimal hyperplasia, dilatation, and calcification in multiple large-animal models. These data support a possible future use for decellularized human TEVGs in a range of vascular applications for patients who have no available autologous vascular conduit.

**MATERIALS AND METHODS**

**Animal use**

All procedures were approved by their respective Animal Care and Use Committees, including Duke University, East Carolina University, and SyneCor. Animals received humane care according to the Guide for the Care and Use of Laboratory Animals (National Institutes of Health, 1996). All surgeries and angiography were performed in sterile fashion under general anesthesia. After each surgery, graft patency was confirmed, wounds were closed, and animals were recovered. Animals were anticoagulated with heparin (1000 to 5000 U) at implant. Baboons received aspirin (10 mg per kilogram of body weight), and dogs received dual antiplatelet therapy (325 mg of aspirin/75 mg of clopidogrel), daily preoperatively until the end of the study.

**Formation of TEVGs**

Human aortas were obtained from an American Association of Tissue Banks (AATB)-accredited and FDA-registered tissue bank (CryoLife Inc.) and met criteria for implantation (FDA 21CFR1271, AATB Standards for Tissue Banking, and internal CryoLife acceptance criteria). Human SMCs were isolated from aortas from donors (ages 17 to 49) who gave consent for their research use and tested for bioburden (aerobic bacterial and fungal contaminants), sterility, mycoplasma, and endotoxin. Cells were stored in liquid nitrogen vapor (~135°C) before use. Calculations showing the number of grafts that could be produced per human donor conservatively assumed that the procurable adult human aorta is ~26 cm in length with an average circumference of ~5 cm. Cells from multiple donors were pooled for culture of pooled donor grafts. Human cells were used at passage 2 to produce TEVGs.

Canine SMCs were isolated from canine carotid and femoral arteries, were allogeneic with respect to the graft recipient, and were used at passages 2 to 4 to produce TEVGs.

Cells (either human or canine) were seeded onto tubular PGA felt scaffolds (6-mm ID for human grafts, and 3- or 4-mm ID for canine grafts) and strained cyclically (2.5% at 2.75 Hz) (15) in a bioreactor to produce grafts. The medium for growth of human TEVGs was high-glucose Dulbecco’s modified Eagle’s medium (DMEM) with 20% serum, insulin (0.13 U/ml), basic fibroblast growth factor (bFGF) (10 ng/ml), epidermal growth factor (EGF) (0.5 ng/ml), penicillin G (10,000 U/ml), copper sulfate (3 ng/ml), L-proline (50 ng/ml), L-alanine (40 ng/ml), and glycine (50 ng/ml), and was changed thrice weekly. The medium for growth of canine TEVGs was low-glucose DMEM with 20% serum, platelet-derived growth factor–BB (PDGF-BB) (10 ng/ml), bFGF (10 ng/ml), penicillin G (500 U/ml), copper sulfate (3 ng/ml), L-proline (50 ng/ml), L-alanine (20 ng/ml), and glycine (50 ng/ml) and was changed once per week. L-ascorbic acid was added thrice weekly to both human and canine TEVG cultures.

After 7 to 10 weeks of culture, TEVGs were decellularized as described previously (16) in PBS with 0.12 M sodium hydroxide, 1 M sodium chloride, and 25 mM EDTA, containing either 8 mM CHAPS or 1.8 mM SDS. TEVGs were exposed to each detergent solution for up to 6 hours at room temperature and were then washed with PBS. All TEVGs were decellularized before mechanical testing, EC seeding, and implantation. Decellularized TEVGs were stored at 4°C in PBS without calcium or magnesium.

**Endothelialization of TEVGs**

Canine TEVGs were seeded with autologous ECs in vitro before implantation (similar to the schematic shown in Fig. 1E, but using canine cells). Canine femoral artery, carotid artery, or cephalic vein segments (3 to 4 cm) were cultured on fibronectin-coated plates in low-glucose DMEM with 10% fetal bovine serum (FBS), 1× microvascular growth supplement, heparin (125 μg/ml), and penicillin G (500 U/ml) for isolation of ECs via outgrowth from each segment. Isolation and expansion of ECs required 21 ± 2 days, and the attachment of ECs to TEVGs and shear preconditioning required an additional 2 days. For EC attachment, graft lumens were coated with fibronectin (100 μg/ml), seeded with ECs (750,000/ml), and exposed to 11 hours of rotation at 10 rotations per hour to encourage even distribution of ECs. Shear preconditioning was performed by increasing the mean velocity of perfused culture medium in stepwise manner (10 steps total) over a 22-hour period and maintaining the maximum mean velocity (10 to 15 cm/s) to match the mean velocity reported for peripheral canine arteries [10 to 16 cm/s (42)] for 13 hours before implant.

**In vitro analysis**

Suture retention strengths were measured by passing a loop of 6-0 Prolene suture (BV-1 needle) through each TEVG, 2 mm from the edge, and suspending weights in 10-g increments on the suture loop until the suture pulled through the tissue. Suture strength was defined as the weight in grams required to tear the tissue. Suture strengths of TEVGs were measured before implant and after baboon explant. Burst pressures were measured before implant by inflating 6-mm human TEVGs, or 3- to 4-mm canine TEVGs, with saline at room temperature until rupture, as previously described (16). Burst pressure was defined as the inflation pressure at which a TEVG ruptured.
For DNA quantification, TEVG segments were digested with papain followed by DNA purification using a modified Qiagen silica-based spin column. Resulting captured DNA was eluted with a detection-compatible buffer, and DNA was quantified with a PicoGreen assay. Hydroxyproline was measured in papain-digested samples, with chloramine T and 3,3′-diaminobenzidine, and collagen was calculated as 10 times the amount of hydroxyproline.

Animal models
An Old World primate model was chosen to provide phylogenetic similarity to humans, which allowed implantation of non-cross-linked human matrix-containing grafts without immunosuppression. Adult male baboons (Papio anubis, 20 to 30 kg) are physically large enough to support implantation of a 6-mm-diameter TEVG in a clinically relevant anatomic setting. Primates, however, are significantly more expensive than other animals (43), are difficult to handle and maintain (44), and are limited in availability. Thus, baboons were used for arteriovenous studies, whereas dogs were used for small-diameter investigations.

The canine model (class A mongrel dogs, ~25 kg) was used for the assessment of 3- to 4-mm-diameter TEVGs because of its wide acceptance in the scientific community for the evaluation of vascular prostheses (45, 46). The canine study used an allogeneic acellular graft, seeded with autologous ECs, which mimics the approach proposed for eventual small-diameter clinical use.

Surgical implantation techniques
Nine adult male baboons underwent arteriovenous placement of human TEVGs (6-mm ID). One TEVG was placed in the aorto-caval position for 1 month. Eight TEVGs were placed between the axillary artery and the distal brachial vein, which provided a superficial site amenable for simulating hemodialysis access, for up to 6 months. All anastomoses were created with a running 6-0 Prolene suture technique.

To test long-term in vivo patency, we implanted canine TEVGs (3- to 4-mm ID) seeded with autologous ECs end to side to the carotid artery in five dogs with 8-0 Prolene suture. The intervening native carotid artery was occluded with surgical clips.

Canine TEVGs (3- to 4-mm ID) seeded with autologous ECs were implanted into the coronary circulation of three dogs. A left thoracotomy exposed the heart. Normothermic cardiopulmonary bypass was used, and cardiac standstill was achieved with cold cardioplegia. Each graft was sutured to the left anterior descending coronary artery (8-0 Prolene) and to the ascending aorta (4.0-mm aortotomy, 7-0 Prolene), with ligation of the proximal coronary artery. After coronary bypass, animals were separated from cardiopulmonary bypass and recovered.

Immunological assessments
In the concordant xenogenic model of human cell–derived grafts implanted into baboon, immunogenicity of human matrix-containing grafts was assessed. Subcutaneous injections of homogenized TEVG (0.1 ml of a solution of 0.25 mg of protein per milliliter of PBS) and PBS-negative control (0.1 ml) were administered at days 0 and 28, with visual assessments 48 to 72 hours after each injection to detect whether an in vivo adaptive immune response was forming.

In addition, T cell proliferation was measured at 0, 4, 12, and 24 weeks for baboon implants. Lymphocytes were isolated with a Ficoll gradient and cultured 7 days in 96-well plates with segments (5 mm by 5 mm) of TEVG, or PTFE grafts as negative controls, in each well. The culture medium was RPMI 1640 with 10% fetal bovine serum. 5-Bromo-2′-deoxyuridine (BrdU) (100 μM) was added to each well 18 hours before cell harvest. Harvested cells were stained with 200 μl of diluted live/dead dye (Invitrogen L23102) for 30 min at room temperature and then with 80 μl of CD3-APC (allophycocyanin) antibody (BD 557597) for 50 min at room temperature, washed, permeabilized (1× Cytofix/Cytoperm buffer and 1× Cytoperm Plus; BD), and incubated with 100 μl of deoxyribonuclease (DNase) to partially digest DNA. Proliferating cells were stained with 50 μl of BrdU-FITC (fluorescein isothiocyanate) antibody (BD 559619) for 20 min at room temperature and suspended in 150 μl of 0.2% bovine serum albumin (BSA)/Dulbecco’s PBS (DPBS) for flow cytometry analysis (Accura C6). For data analysis, single cells were selected and dead cells were gated out. Proliferation rate was calculated as the percentage of CD3+/BrdU− cells in CD3+ cells.

Duplex ultrasound
In the baboon model, duplex ultrasound was used to monitor mid-graft TEVG diameter, wall thickness, and flow rate immediately after surgery and at 2, 4, 12, and 24 weeks.

Angiography
Angiography was used to assess graft dilatation and narrowing. Graft patency was defined according to Fitzgibbon’s classification (47).

All baboon grafts placed from the axillary artery to the brachial vein were accessed directly in mid or distal graft sections (16-gauge needle, 5- and 6-F catheters) at 1-, 3-, and 6-month time points (see Table 3) to determine the ability of TEVGs to withstand puncture as a model for hemodialysis access.

Angiography of canine grafts was performed through a percutaneous femoral arterial approach at 1, 4, 12, 26, and 52 weeks after implant.

Computed tomography angiography
Computed tomography (CT) angiography (64-slice; General Electric, Lightspeed VCT) of coronary bypass grafts was performed. Intravenous β blockers minimized cardiac motion, and iohexol (350 mg/ml) was used as contrast. Slices (0.625-mm thick) and a soft-tissue reconstruction algorithm were used for evaluation of the internal diameter and cross-sectional area of grafts.

Histology
Tissues were fixed in 10% neutral buffered formalin, embedded in paraffin, sliced (5-μm sections), and stained with hematoxylin and eosin (H&E), Movat’s, or Alizarin Red S with a light green counterstain. Tissue sections were also prepared for cryosectioning by dehydrating (30% sucrose in PBS) and freezing in optimal cutting temperature (OCT) compound. Immunostaining was performed on frozen baboon sections and formalin-fixed canine sections for α-smooth muscle actin (SMA and myofibroblast marker; baboon explants: Dako M0851, 1:50 dilution; canine explants: Sigma A2547, 1:5000 dilution), von Willebrand factor (protein synthesized by ECs; baboon explants: Dako M0616, 1:25 dilution; canine explants: not stained), CD3 (part of the T cell receptor complex on mature T lymphocytes; Abcam ab699, 1:25 dilution), CD20 (protein expressed on the surface of mature B cells; Abcam ab9475, 1:25 dilution), collagen types I and III (Novus NB600-1408 and NB600-594, 1:200 dilution for both), fibronectin (Novus NB110-1635, 1:50 dilution), and vitronectin (Novus NB110-57649, 1:200 dilution) with either a fluorescent or a 3,3′-diaminobenzidine (DAB) stain. Alizarin-stained sections were evaluated to confirm the absence of calcification (7 ± 1 sec-
tions per animal, \( n = 11 \) animals). Immunogenicity was further evaluated by observation of H&E-stained sections (\( 11 \pm 2 \) sections per animal, \( n = 12 \) animals) and immunostaining for CD3 and CD20 (\( 3 \pm 1 \) sections per animal, \( n = 2 \) animals). Neointimal thickness of native vessels at anastomoses were calculated as the total area of neointima divided by the length of the underlying tissue (20). A microscope-mounted camera and image analysis software were used for measurements. All histology was interpreted by a cardiovascular pathologist (L.D.).

### Statistical analysis

Statistical analyses were performed with a Student’s two-sample \( t \) test, assuming unequal variances, for analyses with two groups. One-way analysis of variance (ANOVA) was used to determine significant differences between three or more groups with Tukey’s post hoc comparison. Linear regression was performed to assess whether graft suture strength plotted as a function of cell donor age had a slope significantly different from 0. Two-sided \( P \) values less than 0.05 indicated statistical significance. Numeric values are presented as the mean ± SEM. Reported \( n \) represents the number of individual cultured grafts tested (not repeat segments from the same graft), and is reported in parenthesis in the tables.

### REFERENCES AND NOTES


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