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Validity of Customized Branched Tissue Engineered Vascular Graft in a Porcine Model

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ABSTRACT

BACKGROUND Patient-specific, 3-dimensional printed, tissue engineered vascular grafts (3DTEVGs) are manufactured to optimize hemodynamic performance and to accommodate growth. We evaluate growth outcomes of 3DTEVGs compared with standard grafts for pulmonary artery reconstruction in porcine models.

METHODS Magnetic resonance imaging (MRI) with 4-dimensional flow data was acquired in porcine models (n = 8). 3DTEVGs guided in design by computational flow dynamics were implanted (n = 4), with polytetrafluorethylene grafts used as controls (n = 4). Postoperative MRI and histologic features of explanted grafts were evaluated after 10 weeks.

RESULTS All pigs survived, with evidence of patent grafts on postoperative MRI. Graft inner diameter changes were 0.47 ± 2.31 mm in 3DTEVGs and −4.61 ± 2.15 mm in controls ($P = .018$). Mean main pulmonary artery wall shear stress was significantly lower in 3DTEVGs (7.12 ± 4.21 Pa) than in controls (18.15 ± 8.37 Pa; $P = .0396$). Histologic evaluation of 3DTEVGs showed a single layer of endothelial cells, an organized smooth muscle layer, and collagen deposition with a remaining scaffold area of 21.37% ± 20.46%.

CONCLUSIONS Our patient-specific 3DTEVGs demonstrated optimal anatomic fit while maintaining ideal flow dynamics and promoting appropriate neovessel formation.

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Complex vascular reconstruction in children with congenital heart defects remains a challenge because of smaller vasculature, complicated anatomy, and growth inevitability.[1] Current practice is limited because standard grafts, like homografts or polytetrafluoroethylene (PTFE) grafts, do not accommodate growth and pose risk of thrombosis.[2] Consequently, children with congenital heart defects often undergo multiple high-risk open heart surgical procedures under cardiopulmonary bypass to replace

IN SHORT

- Patient-specific grafts demonstrate optimal anatomic fit in porcine central pulmonary artery reconstruction models.
- Graft design guided by computational fluid dynamics helps maintain ideal flow parameters with animal growth.
- Tissue engineered vascular grafts help promote appropriate neovessel formation for pulmonary artery reconstruction.

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Abbreviations and Acronyms

3D = 3-dimensional
3DTEVG = 3-dimensional printed, tissue engineered vascular graft
4D = 4-dimensional
CFD = computational fluid dynamics
LPA = left pulmonary artery
MPA = main pulmonary artery
MRI = magnetic resonance imaging
PA = pulmonary artery
PTFE = polytetrafluoroethylene
RPA = right pulmonary artery
WSS = wall shear stress

grafts, with greater risk of infection, neurologic deficits, and death.[3,4]

Our team developed patient-specific, 3-dimensional printed, tissue engineered vascular grafts (3DTEVGs) aimed at optimization of hemodynamic performance and accommodation of growth. Previous work in silico revealed that 3DTEVGs outperform standard grafts in terms of hemodynamics.[5] In vivo studies in sheep[6] and porcine[7] models also validated feasibility of patient-specific 3DTEVGs to promote cellular proliferation. However, there are no studies comparing growth outcomes of patient-specific 3DTEVGs and standard PTFE grafts (GORE-TEX; Gore Medical).

This study evaluated the growth outcomes of customized 3DTEVGs compared with PTFE grafts for pulmonary artery (PA) reconstruction in porcine models. We hypothesized that 3DTEVGs will grow and perform like native tissue and outperform PTFE grafts with regard to 4-dimensional (4D) flow magnetic resonance imaging (MRI)-acquired hemodynamics and histologic composition.

MATERIAL AND METHODS

PREOPERATIVE PLANNING. Four weeks before the surgical procedure, eight 14-week-old mixed Yorkshire and Landrace pigs underwent MRI on a 3.0T magnet (Philips Ingenia), with acquisition parameters stated in previous work.[8] Each pig was then randomly assigned a group: 3DTEVG or control. For the 3DTEVG cohort, patient-specific grafts were designed through magnetic resonance image segmentation, computer-aided design guided by computational fluid dynamics (CFD), and 3D-printed mold-based electrospinning of 1:1 polyglycolic acid and poly-L-lactide-co-ε-caprolactone, a process described in previous work.[5-8] Anatomically feasible designs with ideal wall shear stress (WSS) (1-10 Pa[9]) and flow distributions to the branch PAs (50/50 split ± 15%[10]) were chosen (Supplemental Figure 1). Controls received straight PTFE conduits fitted on the basis of main PA (MPA) size (Figure 1).

IN VIVO IMPLANTATION. All procedures were performed at the University of Chicago Medical Center and approved by the Institutional Animal Care and Use Committee (72605; approved October 24, 2019). One month after preoperative MRI, each pig underwent an implantation surgical procedure through left thoracotomy under general anesthesia and cardiopulmonary bypass. For 3DTEVGs, MPA and branch PA were removed, and grafts were implanted with proximal and distal end-to-end anastomosis. For controls, MPA was removed, branch PAs were sutured together posteriorly, and straight PTFE conduits were directly sutured anteriorly to the MPA and branch PA. All pigs survived and were extubated in the operating room.

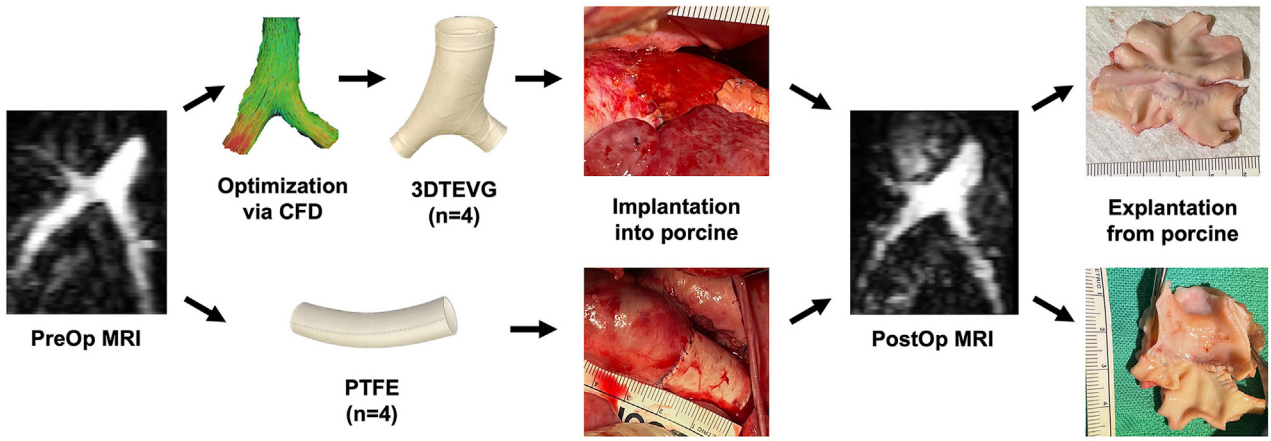

FIGURE 1 Three-dimensional printed, tissue engineered vascular graft (3DTEVG) workflow. Pigs underwent magnetic resonance imaging (MRI) 4 weeks before and 10 weeks after the surgical procedure. The 3DTEVG group (n = 4) then received customized grafts created with computational fluid dynamics (CFD) optimization and 3-dimensional printed electrospinning. Controls (n = 4) received standard polytetrafluoroethylene (PTFE) grafts. All grafts were inserted through thoracotomy and explanted 10 weeks after the surgical procedure (PreOp, preoperative; PostOp, postoperative).

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POSTOPERATIVE ANALYSIS. Each porcine model underwent postoperative 4D flow MRI 10 weeks after the surgical procedure. Flow rates and WSS were measured across the whole vessel using iTFlow (Cardio Flow Design), with standard landmarks distinguishing MPA, right PA (RPA), and left PA (LPA) stated in previous work.[8] Branch PA flow was determined by %RPA, or percentage of flow in the RPA compared with total flow (RPA + LPA).

Subsequently, grafts were explanted, fixed in 10% formalin for 24 hours, and embedded in paraffin. Standard histologic analysis was completed with hematoxylin and eosin, Masson trichrome, von Kossa, and picrosirius red staining. Primary antibodies for immunohistochemistry included von Willebrand factor and smooth muscle actin. To measure remaining scaffold area, polarized microscopic images were obtained from hematoxylin and eosin-stained tissues, filtered, and photomerged (Adobe). Pixels were calculated and averaged using ImageJ (National Institutes of Health).

Sample length, inner diameter, and wall thickness of tissue samples were recorded. With use of a load cell connected to the tensile load frame, force and displacement data were collected to calculate compliance, circumferential tensile strength, and ultimate tensile strength. For both groups, native MPA tissue proximal to anastomosis was used as an internal control.

Analysis of variance F tests and post hoc Dunnett multiple comparison tests were performed to compare outcomes of native tissue, PTFE control, and 3DTEVG. Statistical significance was determined by $P < .05$ (Prism V8; GraphPad).

RESULTS

All 8 pigs survived throughout the 10-week follow-up period. Their average weight was 28.46 ± 2.58 kg at preoperative MRI, 38.66 ± 4.15 kg at time of the surgical procedure, and 63.71 ± 9.36 kg at postoperative MRI (Supplemental Figure 2).

All 3DTEVGs were designed with emphasis on WSS and flow distribution in CFD simulations. Mean WSS of the MPA was significantly lower in 3DTEVGs (7.12 ± 4.21 Pa) than in controls (18.15 ± 8.37 Pa; $P = .0396$). 3DTEVG WSS values were also comparable to native tissue (8.72 ± 3.74 Pa; $P = .8682$; Figure 2A). Furthermore, control and 3DTEVG groups showed flow distributions to the RPA and LPA similar to those before the surgical procedure. The native vessel preoperatively had a %RPA flow split of 59.61 ± 6.21, whereas control and 3DTEVG groups had %RPA flow splits of 63.47 ± 12.60 and 61.24 ± 5.51, respectively (Figure 2B). Graft inner diameter growth was measured as the inner diameter difference between 10 weeks postoperatively and at time of the surgical procedure. The mean change of the graft inner diameter was 0.47 ± 2.31 mm in 3DTEVGs and −4.61 ± 2.15 mm in controls ($P = .0180$). On the other hand, the mean change in internal diameter of the native PA tissue was 1.56 ± 2.51 mm, comparable to 3DTEVG ($P = .6729$; Figure 2C).

Biocompatibility of 3DTEVGs was evaluated after 10 weeks and compared with the native tissue and control (Supplemental Figure 3). Histologic staining of 3DTEVGs revealed a single layer of endothelial cells, an organized smooth muscle cell layer, and collagen deposition (Figures 3A-3F). That cohort also showed ongoing extracellular matrix formation with no ectopic calcification. There was no graft-related stenosis, dilation, rupture, or clot formation during follow-up. Because of graft integration, the average remaining scaffold of MPA was 21.37% ± 20.46% (Figure 3G). The mean intimal thickness was 230.0 ± 135.27 μm in the 3DTEVG group and 843.53 ± 694.56 μm in the control group ($P < .0001$). Conversely, the native PA tissue had a mean intimal thickness of 78.14 ± 33.86 μm, more aligned with the values from the 3DTEVG group ($P = .3787$; Figure 3H).

Last, mechanical testing revealed that native tissue had the greatest compliance of 11.37 ± 4.32 %/mm Hg,

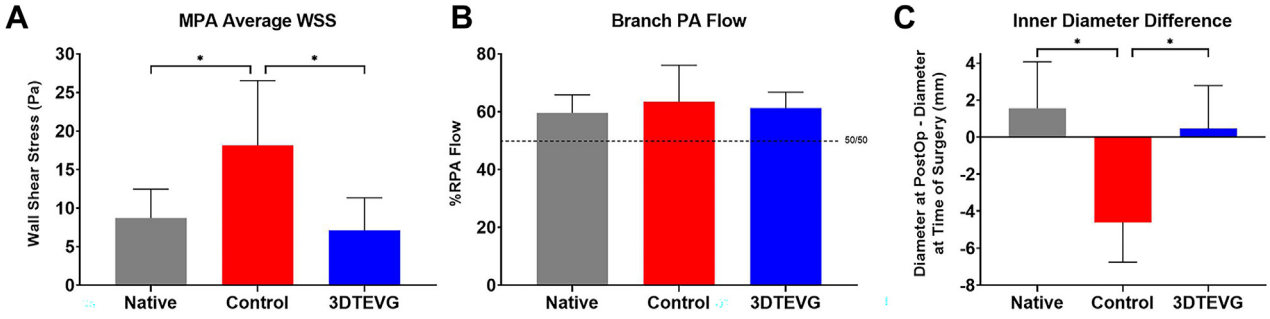

FIGURE 2 Four-dimensional flow analysis for native, polytetrafluoroethylene (PTFE) control, and 3-dimensional printed, tissue engineered vascular graft (3DTEVG) pulmonary arteries, including (A) wall shear stress (WSS), (B) branch pulmonary artery (PA) flow distribution, and (C) inner diameter difference. (MPA, main pulmonary artery; PostOp, postoperative; RPA, right pulmonary artery.)

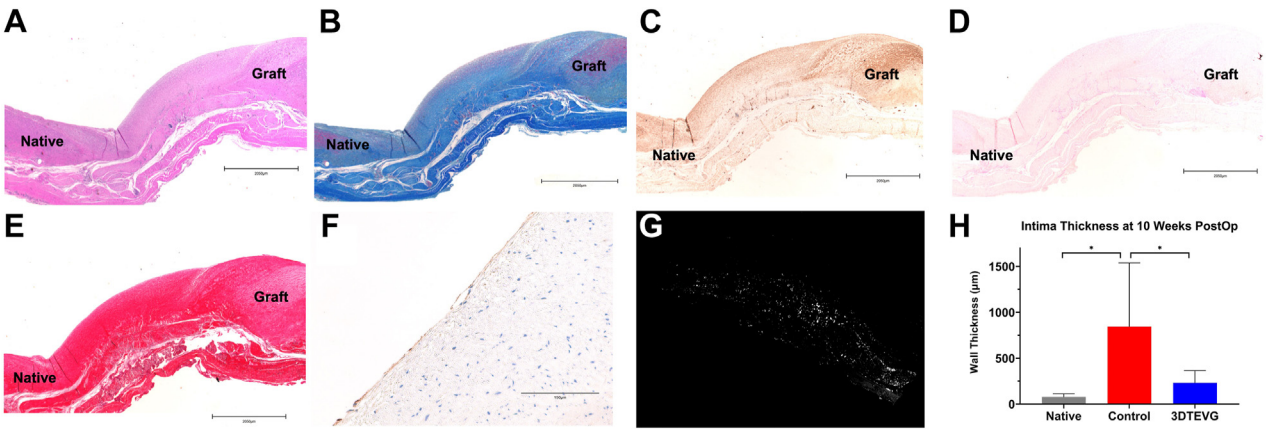

FIGURE 3 Histologic representation of the tissue engineered vascular graft. Staining included (A) hematoxylin and eosin, (B) Masson trichrome, (C) smooth muscle actin, (D) von Kossa, (E) picrosirius red, and (F) von Willebrand factor. (G) Polarized light microscopy revealing the remaining scaffold. (H) Histologic analysis of intima thickness for native tissue, polytetrafluoroethylene control, and 3-dimensional printed, tissue engineered vascular graft (3DTEVG) at 10 weeks after the surgical procedure.

whereas the control and 3DTEVG yielded lower compliances of 0.62 ± 0.64 %/mm Hg and 2.85 ± 1.11 %/mm Hg, respectively (Figure 4A). However, the 3DTEVG acted more similar to the native tissue in terms of tensile strength, although the control graft could withstand much greater stresses. The native vessel had a circumferential and ultimate tensile strength of 0.86 ± 0.37 MPa and 0.96 ± 0.50 MPa, respectively. The 3DTEVG group had a circumferential and ultimate tensile strength of 0.87 ± 0.33 MPa and 1.09 ± 0.40 MPa. Last, the control PTFE graft group had a circumferential and ultimate tensile strength of 8.27 ± 3.81 MPa and 7.25 ± 6.97 MPa (Figures 4B, 4C).

COMMENT

This study validates the benefit of patient-specific 3DTEVG implanted in vivo in a porcine model. All 8 pigs survived an implantation surgical procedure, and all 3DTEVGs revealed graft patency. We found that 3DTEVGs acted similar to native PA vessels by 4D flow and histologic analysis as well as yielded superior results compared with PTFE. Unlike the control, growth of 3DTEVG helped maintain patient-specific shape and attenuate graft stenosis due to lower WSS and milder neointimal thickness.

A novelty of 3DTEVG is its ability to accommodate growth, which is not possible in standard grafts. We saw that the inner diameter of the 3DTEVG increased, suggesting appropriate neotissue formation and remodeling. Conversely, controls experienced a reduction in inner diameter, revealing material limitations of PTFE. Controls also produced >3 times the intima thickness as the native and 3DTEVG groups. Because intimal thickening and subsequent pulmonary vascular remodeling can cause stenosis, our study elucidates that growth of 3DTEVG may attenuate graft stenosis because of lower neointimal thickness.

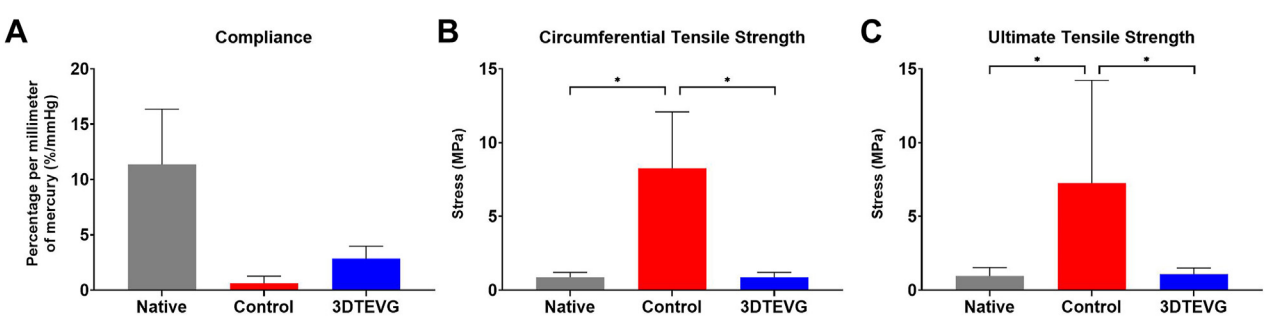

FIGURE 4 Mechanical analysis of (A) compliance, (B) circumferential tensile strength, and (C) ultimate tensile strength for native tissue, polytetrafluoroethylene control, and 3-dimensional printed, tissue engineered vascular graft (3DTEVG).

Our histologic and mechanical analysis demonstrates the effectiveness of 3DTEVG to accommodate healthy vascular growth, with a single layer of endothelial cells, an organized smooth muscle cell layer, and collagen deposition. The presence of collagen and marginal remaining scaffold area suggests successful degradation and neotissue formation, probably contributing to increased compliance seen in mechanical testing. In addition, another goal of 3DTEVG is to minimize WSS and balance branch PA flow to maintain optimal ventilation and perfusion balance.[10] Using 4D flow, our study discovered that WSS of the 3DTEVG was significantly lower than that of the PTFE control. 3DTEVG helped maintain physiologic values of WSS in venous flow (1-10 Pa),[9] similar to WSS in native tissue, whereas PTFE WSS was >20 Pa. Branch PA flow remained consistent between the 3 groups.

However, there remain limitations in this work. This study used only 8 pigs and 2 MRI checkpoints. Future work should increase sample size and incorporate serial MRI to track growth over time. Moreover, we used straight instead of bifurcated grafts that may better mimic 3DTEVG shape and hemodynamics. However, a straight PTFE conduit sutured to native tissue has higher patency in current central PA reconstruction practice as it helps aid in vessel growth. Last, decompressed structures during cardiopulmonary bypass surgical procedure complicate the surgeon's ability to mimic CFD simulations.[8] Ensuring greater precision during cardiac procedures, using augmented reality or virtual surgical procedure software,[1] can help guide implantation.

Overall, our patient-specific 3DTEVG accommodated growth in porcine models and demonstrated optimal anatomic fit while maintaining ideal flow dynamics that attenuated neointimal hyperplasia and promoted appropriate neovessel formation. This study demonstrates the feasibility of patient-specific 3DTEVGs to replace current PTFE grafts for complex PA reconstruction.

Supplemental Figures can be viewed in the online version of this article [https://doi.org/10.1016/j.atssr.2023.05.018] on http://www.annalsthoracicsurgery.org.

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DISCLOSURES

Jed Johnson reports a relationship with Nanofiber Solutions that includes: equity or stocks. Narutoshi Hibino, Jed Johnson, and Axel Krieger has patent Patient-Specific Tissue Engineered Vascular Graft Utilizing Electrospinning issued to WO/2017/035500Al.

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