Individualized tissue-engineered veins as vascular grafts: A proof of concept study in pig

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Abstract
Personalized tissue engineered vascular grafts are a promising advanced therapy medicinal product alternative to autologous or synthetic vascular grafts utilized in blood vessel bypass or replacement surgery. We hypothesized that an individualized tissue engineered vein (P-TEV) would make the body recognize the transplanted blood vessel as autologous, decrease the risk of rejection and thereby avoid lifelong treatment with immune suppressant medication as is standard with allogenic organ transplantation. To individualize blood vessels, we decellularized vena cava from six deceased donor pigs and tested them for cellular removal and histological integrity. A solution with peripheral blood from the recipient pigs was used for individualized reconditioning in a perfusion bioreactor for seven days prior to transplantation. To evaluate safety and functionality of the individualized vascular graft in vivo, we transplanted reconditioned porcine vena cava into six pigs and analyzed histology and patency of the graft at different time points, with three pigs at the final endpoint 4–5 weeks after surgery. Our results showed that the P-TEV was fully patent in all animals, did not induce any occlusion or stenosis formation and we did not find any signs of rejection. The P-TEV showed rapid recellularization in vivo with the luminal surface covered with endothelial cells. In summary, the results indicate that P-TEV is functional and have potential for use as clinical transplant grafts.

KEYWORDS
biomimetics, bioreactors, in-vitro and in-vivo, regenerative medicine, scaffolds, stem cells, surface modification, tissue engineering

1 | INTRODUCTION

Cardiovascular diseases are among the leading causes of mortality and morbidity worldwide and cause high economical burdens on global healthcare systems (Mendis et al., 2011). Arterial diseases are the major cause of this group of health problems and great efforts regarding research and development of new treatments have improved the prognosis for arterial diseases during the last decades.
(Ezati et al., 2015). In contrast, the management of venous diseases has evolved little during the same period (Onida & Davies, 2016). Many diseases of the venous system can be treated pharmacologically, but for some conditions reconstructive surgery are required to restore venous anatomy and function. Vital venous structure may be lost due to thrombosis, trauma or surgical tumour resections. Sometimes autologous veins are available as grafts in vascular reconstructions, but for many patients a biological individualized vascular graft would be of great importance to enable restoration of crucial venous function.

For venous reconstructions, different grafts substitutes have been evaluated including Polytetrafluoroethylene grafts, cryopreserved or decellularized allogenic veins and vein conduits constructed of autologous peritoneum or bovine pericardium. Independent of what conduit that has been used, the long-term patency for venous reconstructive surgery is poor (Dokmak et al., 2015; Eklof et al., 1998; Jara et al., 2015; Jost et al., 2001; Madden et al., 2004).

A promising technology to improve current vascular grafting is decellularization of native blood vessels from human or animal donors followed by reconditioning with autologous material to generate individualized tissue-engineered vascular grafts. Decellularization employs chemical, enzymatical and/or physical methods to remove all donor cells from the extracellular matrix (ECM; Gilbert et al., 2006). The ECM possesses an ideal three-dimensional structure for cell attachment and proliferation due to abundance of functional proteins, surface structure and natural viscoelastic behavior to withstand blood pressure. Decellularized blood vessels have been evaluated as arterial vascular grafts (Lawson et al., 2016; Schaner et al., 2004) and most clinical trials utilizing decellularized vascular grafts have focused on providing vascular access for patients requiring hemodialysis. Even though some biological grafts, for venous reconstructions have shown promising results, most grafts have had long term patency problems (Chemla & Morsy, 2009; Das et al., 2011; Gui et al., 2009; Quint et al., 2011; Wystrachowski et al., 2014).

There is still a need for further optimizing biological grafts for clinical use and one way to optimize the vessel wall regeneration could be reconditioning of decellularized veins with the recipient’s autologous blood. Perfusion of functional organs with whole blood or blood-derived solutions prior to conventional transplantation has been shown beneficial for preserving the organ physiology and functionality (Hosgood & Nicholson, 2011; Nicholson & Hosgood, 2013; Steen et al., 2016). The aim is to prepare the graft with autologous components preventing rejection and facilitating rapid in vivo cellularization of the graft after implantation.

VERIGRAFT is developing clinical-grade grafts under the trade name personalized tissue-engineered vein (P-TEV). We have used the P-TEV protocols of decellularization and reconditioning to produce porcine individualized tissue-engineered grafts for preclinical testing (referred to as P-TEV in this text).

To study safety and functionality of P-TEV, these grafts were evaluated in an in vivo porcine model of vena cava transplantation for up to 35 days. The results showed efficient graft recellularization and full patency throughout the whole study.

2 | MATERIALS AND METHODS

2.1 | Preparation of P-TEV

Vena cava, a section between vena renalis and the bifurcation to vena ilica communis, was excised from cadaveric pigs of approximately 50 kg. The blood vessel was dissected free from surrounding tissues and branches were ligated using 4–0 Prolene (Ethicon). The vein segment was stored in ice-cold Phosphate Buffered Saline (PBS) and frozen at −80°C. For decellularization, veins were thawed, connected

![FIGURE 1 Decellularization and reconditioning bioreactor setup. (a) Decellularization bioreactor with a peristaltic pump (right) guiding detergents through a blood vessel. (b–c) Bioreactor setup applied to perfuse a blood vessel with a peripheral whole blood-protein mixture. (d) Decellularized and (e) reconditioned vein mounted with Luer connectors allowing connection to the perfusion device. Black arrows in A, D, E indicate blood vessels. White arrow in B indicates where the blood vessel is mounted [Colour figure can be viewed at wileyonlinelibrary.com]
to a perfusion bioreactor system (Figure 1a) and decellularized using protocols previously described (Simsa et al., 2018, 2019). Vessels were perfused at 100 ml/min, 37°C and 115 rpm agitation for 2 h each with 1% Triton X-100 (Merck Millipore), 1% Tri (n-butyl)phosphate (Merck Millipore) and 40 U/mL deoxyribonuclease (VWR), all containing 0.5% antibiotic-antimycotic (AA, Thermo Fisher Scientific). Between each reagent change, vessels were washed 3 × 5 min in H2O. This process was repeated for seven days. For overnight incubation, the veins were perfused with a 5 mM ethylenediaminetetraacetic acid (EDTA; Merck Millipore) solution and the perfusion and agitation were lowered to 70 ml/min and 50 rpm respectively. The vessels were washed with 5 mM EDTA for 48 h and PBS for 24 h, both containing 0.5% AA. After decellularization, the vessels were removed from the bioreactor, biopsies were taken for DNA quantification, histology, and immunohistochemistry before peracetic acid sterilization and final washes in PBS under sterile conditions. The sterilized decellularized veins were either frozen in PBS at −80°C or directly used for subsequent whole blood perfusion. One vein was decellularize for each animal to be transplanted.

From the leg of each pig to be transplanted with P-TEV, 50 ml peripheral venous whole blood was collected in heparin vacutainers (BD) one week pre-surgery under sedation with Tiletamine/zolazepam (Zoletil 50 mg/ml + 50 mg/ml, 0.06 ml/kg) and Dexametomidine (Dexdomitor 2.5 mg). Blood samples were stained with Türk’s solution (Merck) and leukocytes were counted in a Bürker chamber to ensure a normal physiological status. 25 ml blood was mixed immediately with 25 ml STEEN Solution (XVIVO Perfusion), 0.5% Antibiotic-antimycotic Mix (Thermo Fisher Scientific), 80 ng/ml vascular endothelial growth factor (Cellgenics) and 10 ng/ml fibroblast growth factor (R&D Systems). 5 μg/ml acetylsalicylic acid (Sigma-Aldrich) was added to terminally inhibit all contained thrombocytes. The complete blood solution was added to the decellularized blood vessel inside a reconditioning bioreactor, which allowed circulation of the blood solution through and around the vessel in a vertical position at 2 ml/min (Figure 1b–e). This process was performed in a laminar flow hood at room temperature for seven days, during which the glucose level was measured and kept between 3 and 8 mM. After perfusion, the graft was harvested, rinsed, biopsied for DNA quantification and histology and kept in PBS + AA until use (maximum two hours). During blood procurement and production, a system was used to allow traceability of the autologous pig blood and the resulting graft to allow administration in an individualized, strictly autologous manner.

2.2 | Animal model for transplantation of P-TEV

The in vivo experiments were performed after prior approval from the local ethics committee for animal studies at the administrative court of appeals in Gothenburg, Sweden. Eight female pigs (bodyweight 54–69 kg) of a mixed breed of Yorkshire, Hampshire and Swedish Pigham were used in this study; two for sham surgery (the pig’s own vena cava was cut and re-sutured) and six for P-TEV transplantation. The pigs were cared for in accordance with regulations for the protection of laboratory animals and the pigs were housed together before and after surgery. The animals were housed together with two-three pigs in a room of 28 m². The rooms had bedding consisting of wood shavings and straw and the pigs were fed twice daily and had free access to water. The general condition of the pigs is observed daily during the acclimatization period as well as after operation. Acetylsalicylic acid (Trombyl, Pfizer) 160 mg was given orally once daily during the whole study starting six days before surgery and rivaroxaban (Xarelto, Bayer) 2 mg/kg was given orally twice daily during the whole study starting the day before surgery. All surgical procedures were carried out under sterile conditions and under isoflurane anesthesia. Anesthesia was induced with injection of Dexametomidine 30 μg/kg (Dexdomitor, Orion Pharma Animal Health), Tiletamine 3 mg/kg and zolazepam 3 mg/kg (Zoletil, Virbac). The pigs were then intubated and given inhalation anesthesia with isoflurane (Attane vet, VM Pharma). For pain relief, Carprofen 4 mg/kg (Norocarp vet, N-vet) and Buprenorphine 0.03 mg/kg (Vetergesic vet, Orion Pharma Animal Health) was given. An incision was made through linea alba and two different techniques were used to localize vena cava. For the first two P-TEV transplanted pigs, a regular technique, holding the intestines aside using gauze wetted in saline and surgery hooks, was used. To avoid intestinal adhesion formation, the technique to localize vena cava was changed to a retroperitoneal approach for the following six pigs, two sham and four P-TEV transplanted pigs. On the dexter side, the peritoneum and the abdominal wall were separated down to the vena cava and thereby the intestines were left untouched. The part of vena cava between vena renalis and the bifurcation to vena iliaca communis was dissected free from surrounding tissue. At the time of implantation, heparin 10 000 IU (Leo Pharma) was administered i. v. before clamping of the vein. Approximately 4 cm P-TEV were implanted using 6-0 Prolene (Ethicon) as end-to-end anastomoses. In the sham-operated pigs, the tension of the vein did not allow cutting at two places to perform two anastomoses as with the P-TEV pigs. Instead, the vena cava was cut and reconnected with only one anastomosis. Metal clips were sutured at the anastomoses for facilitated orientation under x-ray imaging. The abdominal musculature was closed with Maxon 0 (Ethicon) and the skin was closed with a Monocryl 3 (Ethicon) suture intradermally. At euthanization four to 5 weeks post-surgery, angiography was performed on the operated area of vena cava under isoflurane anesthesia injecting contrast fluid into a femoral vein. At harvest, the operated segment of vena cava, including adjacent area of native vena cava, was excised from the pigs under anesthesia before euthanization for further analysis. No extra anticoagulant or anti-platelet drugs were used.

2.3 | DNA quantification

DNA was extracted from 10-25 mg wet tissue with the DNeasy Blood & Tissue kit (Qiagen) and quantified with the Qubit dsDNA HS assay kit (Life Technologies), according to the manufacturer’s instructions. DNA concentration was calculated using a supplied DNA standard.
2.4 | Histology

4',6-diamidino-2-phenylindole (DAPI) and Hematoxylin-Eosin (H&E) staining was performed on paraffin embedded cross-sections of 5 μm thickness, following standard protocols for fixation, embedding, rehydration and staining. Images were acquired using a fluorescence microscope (Zeiss Axiocounter 40 CFL) for DAPI visualization, and in bright field for H&E visualization. Vein wall thickness was measured those images.

2.5 | Immunohistochemistry

Immunohistochemical staining was performed on paraffin sections by rehydrating samples following standard procedures and performing antigen retrieval by incubating samples for 15 min in a 95°C water bath in a Tris-EDTA buffer (10 mM Tris base, 1 mM EDTA Solution, 0.05% Tween 20, pH 9.0). The sections were blocked with donkey serum for 1h and incubation with primary antibodies against CD31 (1:50, Abcam ab 28364), CD45 (1:200, Abcam ab 10558) or alpha smooth muscle actin (aSMA, 1:100, Abcam ab 7817) overnight, followed by incubation with secondary antibodies, anti-mouse (1:200, A21203, ThermoFisher) or anti-rabbit (1:200, A21207, ThermoFisher), conjugated to Alexa Fluor 594 for 1 h. DAPI (Thermo Fisher Scientific) was used as a counterstain.

2.6 | Scanning electron microscopy

Tissue samples were rinsed in PBS (Medicago) and submerged in 2.5% glutaraldehyde fixative (Sigma-Aldrich) at room temperature. After 2 h, the samples were placed in a fresh fixative of the same concentration and left in a fridge at 4°C for 24. The samples were washed with buffer and submerged in 1% osmium tetroxide (Sigma-Aldrich) for 24h at room temperature for secondary fixation. The samples were rinsed in deionized water, plunge-frozen in liquid propane and freeze-dried overnight in VirTis Sentry 2.0 Benchtop Freeze Dryer (SP Scientific). Before the scanning electron microscopy (SEM) analysis, samples were coated with 15 nm Au/Pd thin film using Gatan Model 682 PECS sputter-coater to prevent the charging under electron beam. The imaging was performed with a Zeiss Supra 40VP SEM, in secondary electrode image mode. The acceleration voltage and working distance were 4.03kV and 9–12 mm, respectively.

2.7 | Rotational thromboelastometry

Rotational thromboelastometry (ROTEM) is a method which detects clot formation in blood samples. ROTEM measurement was performed to analyze the influence of the intrinsic (INTEM, activated by trauma inside the vascular system) and extrinsic (EXTEM, activated by trauma outside the vascular system) coagulation pathways. Also, the influence of fibrinogen (FIBTEM) and heparin (HEPTEM) were analyzed.

Effects on blood coagulation in the presence of native (n = 4), decellularized (n = 8) and reconditioned (n = 8) vein tissue were analyzed. Vena cava segments were excised from cadaveric pigs of approximately 120 kg, stored in ice-cold PBS and frozen at −80°C. Decellularization and reconditioning was performed as described above. Venous blood for reconditioning was received from four different human donors. Reconditioned blood vessels were harvested the same day as the ROTEM analysis was performed. 8 mm diameter tissue pieces were cut out from the vessels with tissue punchers and stored in PBS −/− until use. Blood for ROTEM analysis was collected from the same donors used for reconditioning, in sodium citrate tubes (BD). A single piece of vein tissue was added to 1.2 ml blood (from the same donor as used for reconditioning) and incubated at 37°C for 1h, after which ROTEM measurement was performed. All samples were analyzed in the recommended time frame of 4 h after blood collection in randomized order. ROTEM Delta instrument with software (Tem Innovations GmbH, Instrumentation Laboratory, Werfen) and reagent kits for INTEM, EXTEM, FIBTEM and HEPTEM assays were used according to manufacturer’s instruction (Tem Innovations GmbH, Instrumentation Laboratory, Werfen). Whole blood samples were incubated with the different reagents for INTEM, EXTEM, FIBTEM and HEPTEM analysis and placed in a plastic cuvette in which a rotating pin was inserted. When blood started clotting, it restricted the rotation of the pin and several parameters were calculated, including clotting time: describes how fast formation of fibrin starts, clot formation time: describes clotting kinetics influenced by the amount of thrombocytes and fibrinogen, alpha angle: describes clotting kinetics where larger alpha angle indicates faster clot formation, and maximum clot strength; describes clot quality. The analysis was performed with ROTEM Delta software.

2.8 | Statistical analysis

SPSS was used for statistical analysis. Statistical method used was Wilcoxon Signed-Rank Test for paired test of vein wall thickness before and after decellularization. In other cases, Kruskal-Wallis with Mann-Whitney post hoc was used. $p < 0.05$ was considered statistically significant. The data are reported as average ± Standard Error of the Mean (SEM).

3 | RESULTS

3.1 | Decellularization and reconditioning of vascular grafts

One vein was decellularized and reconditioned for each pig to be transplanted and the process was successful for all six animals. Histological analysis of the native veins showed normal morphology and distribution of cells, whereas the decellularized vein segments lacked
presence of nuclei (Figure 2a–g). The morphology of the ECM appeared to remain intact after decellularization and there was no significant difference in vein wall thickness before and after decellularization ($p = 0.7$, data not shown) as measured on hematoxylin and eosin stained sections (Figure 2a–d). En face DAPI staining revealed no cell nuclei present on the luminal side after decellularization (Figure 2g). Lymphocyte count in the autologous peripheral whole blood, used for reconditioning, were between 12 and $22 \times 10^6$ lymphocytes/ml, which is considered in the normal physiological range (Luke, 1953). After reconditioning, blood cells were identified on the surface (Figure 2h). SEM analysis of the luminal surface of the native vein showed lining endothelial cells (Figure 2i) which were completely removed after decellularization leaving a plain surface (Figure 2j). After reconditioning, blood components could be identified on the luminal side (Figure 2k). DNA quantification revealed almost complete removal of DNA in the decellularization process ($0.3 \pm 0.2$ ng/mg tissue) compared with native blood vessel ($152 \pm 78$ ng/mg tissue). The remaining DNA represents a content of 0.2%, which is below the limit of 50 ng DNA per mg dry ECM weight, commonly cited as a criterion for sufficient decellularization (Crapo et al., 2011). After reconditioning, dsDNA content in the graft was increased to $10 \pm 3$ ng/mg tissue (Figure 2l).

Results from the ROTEM analysis showed significantly reduced activation if the intrinsic coagulation pathway at addition of the reconditioned samples compared with native and decellularized samples. INTEM analysis showed increased clotting time (native 192 $\pm/-$ 9 s, decellularized 147 $\pm/-$ 21 s and reconditioned 281 $\pm/-$ 18 s), increased clot formation time (native 100 $\pm/-$ 10 s, decellularized 87 $\pm/-$ 5 s and reconditioned 153 $\pm/-$ 18 s) and decreased alpha angle (native 71 $\pm/-$ 2°, decellularized 75 $\pm/-$ 1° and reconditioned 62 $\pm/-$ 2°) in reconditioned samples compared with native and decellularized. In the HEPTEM analysis, where heparinase was added, no difference between the samples was observed (data not shown), which indicates that the differences in INTEM could be due to presence of heparin in the reconditioned samples. No difference was found in EXTEM or FIBTEM analysis (data not shown).

### 3.2 | Surgical transplantation of the vascular graft and patency after up to five weeks in vivo

Decellularized blood vessels were perfused with blood solution from the respective recipient pig for one week and then used directly for transplantation. During surgery, the part of *vena cava inferior* between *vena renalis* and the bifurcation to *vena iliaca communis* was isolated and dissected free from surrounding tissues (Figure 3a). For the P-TEV transplanted pigs, a section of *vena cava* was excised and replaced with a P-TEV of approximately 4 cm length using end-to-end anastomoses (Figure 3b). As described in the methods section, only one anastomosis was used in the sham-operated group (Figure 3c, d).

After the initial surgery with conventional technique, holding the intestines aside using wetted gauze while locating *vena cava*, one P-TEV transplanted pig had to be euthanized 15 days post-surgery due to intestinal complications. At dissection, the intestines had massive adhesions, probably due to the handling of the intestines during surgery, and the symptoms of the pig indicated ileus. Changing to retroperitoneal surgery technique was successful, although during surgery of one sham operated pig, the peritoneum broke and the intestines had to be handled by wetted gauze, as previously. The same pig had to be euthanized seven days post-surgery due to indicated ileus which was probably caused by the handling of the intestines. The remaining six pigs operated with retroperitoneal surgery technique (one sham and five P-TEV transplanted pigs), had no signs of ileus and no intestinal adhesions were found at dissection after euthanization.

Due to complications not related to the *vena cava* transplantation, two more P-TEV transplanted pigs had to be euthanized at early time points. One got limp on the left front leg due to a fractured kneecap and had to be euthanized 17 days post-surgery. The other pig was euthanized three days post-surgery due to suspected hernia. In summary, the different time points for euthanization were the following: Sham (7 and 37 days post-surgery), P-TEV (3, 15, 17, 29, 29 and 37 days post-surgery). No intestinal adhesions were found during dissections of the pigs euthanized four- or five-weeks post-surgery, or of the pigs euthanized due to limp and hernia and it could be concluded that the retroperitoneal surgery approach was successful in avoiding adhesion formation. Angiography was performed on one sham and three P-TEV transplanted pigs after four to five weeks post-surgery, and all were fully patent (Figure 3e, f). When examining *vena cava* during dissection after euthanization, all eight veins (two sham and six P-TEV) were open without signs of clotting or thrombosis. At macroscopic examination, no blood clots or thrombosis could be observed (Figure 3g–i).

### 3.3 | Recellularization of P-TEV in vivo

Already at three days post-surgery, cells had started to repopulate the P-TEV graft. At 17 days post-surgery the P-TEV graft was well recellularized and after 5 weeks the number of cells in the P-TEV appeared to be equal to the native tissue. Importantly, no intimal hyperplasia was observed in the P-TEV, which in this study was predicted as a major potential complication (Figure 4).

DNA content of the P-TEV was significantly increased during the time *in vivo* from $10.2 \pm 8.0$ ng/mg tissue after reconditioning to $55.1 \pm 6.5$ ng/mg tissue *post-in vivo* ($p < 0.001$).

It is not clear whether the P-TEV were recellularized from the connecting native *vena cava* via the anastomosis, from the adjacent surrounding soft tissue, from the blood in the vessel lumen or from a combination of all sites together. However, already at three days post-surgery both the proximal, center and distal parts of the P-TEV were occupied with cells (Figure 5a–d). At 2 weeks post-surgery, the proximal, center and distal parts of the P-TEV had a substantial number of cells, and CD31 positive cells could be identified (Figure 5e–h). Four weeks post-surgery, the proximal, center and
FIGURE 2  Evaluation of decellularization and reconditioning efficacy. (a–d) Hematoxylin/Eosin and (e–h) 4’,6-diamidino-2-phenylindole (DAPI) staining of native (a, b, e), decellularized (c, d, f, g) and reconditioned (h) vena cava from pig. Nuclei were visible throughout the tissue in the native vein (a, b, e) whereas no nuclei were visible after decellularization (c, d, f, g). After reconditioning, blood cells were identified on the luminal surface (h). Scanning electron microscopy analysis (i–k) showed lining endothelial cells on the luminal side in the native vein (i) which were completely removed after decellularization (j). A surface coating covered the luminal surface after reconditioning (k). (l) DNA content quantified in blood vessels (n = 6) before (native), after decellularization (DC) and after reconditioning (RC), respectively. Arrows in B indicate nuclei. * = luminal side. Staples presented average ± scanning electron microscopy (SEM), ** = p < 0.01. Scale bars are 200 μm in A, C, E, F; 50 μm in B and D; 100 μm in G and H [Colour figure can be viewed at wileyonlinelibrary.com]
FIGURE 3  Transplantation surgery of vena cava and patent vena cava five weeks post-in vivo. (a) Isolated vena cava between vena renalis and the bifurcation to vena iliaca communis. (b) Transplanted personalized tissue-engineered vein (P-TEV) (indicated by arrow) with end-to-end anastomoses. Metal clips were sutured to the anastomoses (indicated by arrowheads) to be able to localize the P-TEV during angiography. (c) Sham-operated vena cava, cut and (d) re-sutured at one position (narrowing due to suboptimal anastomosis) (arrow indicate anastomosis). (e) Angiography on sham-operated vena cava and (f) transplanted P-TEV. Metal clips were identified in the angiography to localize the anastomoses (indicated by arrow heads). The veins were open with free blood flow. (g–i) Macroscopic images of excised vena cava post-in vivo. (g) Vena cava at sham anastomosis five weeks post-surgery (stitched image). (h) P-TEV four weeks post-surgery. (i) P-TEV five weeks post-surgery. Arrows indicate anastomoses. Scalebars A–D and H, 2 cm, G and I 1 cm [Colour figure can be viewed at wileyonlinelibrary.com]
distal parts of the P-TEV had cells in equal quantity as the native blood vessel and the P-TEV lumen had a covered CD31-positive endothelium layer (Figure 5i-l). The luminal cells in the P-TEV also had a flattened endothelial cell-like morphology (Figure 5m, n). Further, the smooth muscle cell marker αSMA was similarly expressed in the media and the vasa vasorum of P-TEV and native vein tissue (Figure 6a, b). To analyze for invasion of leukocytes including lymphocytes, monocytes and macrophages, as an indication of graft rejection, the P-TEV was stained with antibodies against the leukocyte marker CD45. Five weeks post-surgery there were no CD45 positive cells in native vena cava or in the center of the P-TEV indicating no signs of rejection (Figure 6c, d). A few areas with minor CD45 positive cell clusters were found in the anastomosis region, as expected during wound healing (Figure 6d).

4 | DISCUSSION

Tissue-engineered vascular grafts obtained by decellularization steps are a potential alternative to current vascular grafts. In this study, we used decellularization and reconditioning to produce P-TEV and evaluated the safety and efficacy of the grafts in a porcine in vivo vena cava transplantation model for up to 35 days. All P-TEV were fully patent and did not show any signs of occlusion. Recellularization of the P-TEV was observed already from day three post-surgery, and five weeks post-surgery the P-TEV were equivalently cellularized compared with native vena cava, with the lumen covered with flattened CD31-positive cells, a marker for endothelial cells.

There are known differences between vascular regeneration mechanisms in animal and humans (Byrom et al., 2010; Sánchez et al., 2018) and it is not possible to conclude that the recellularization in humans will be as rapid as in pigs. However, animal models are our best options to study whole tissue regeneration, including immune response, and our results with full recellularization after 5 weeks indicated that P-TEV is a promising alternative for vascular grafting and motivates to proceed with long term studies towards clinical trial. Decellularized vascular grafts for arterial reconstruction have been described in multiple previous studies, while studies assessing grafts for venous reconstructions are absent (Katzman et al., 2005; Kovalic et al., 2002; Simsia et al., 2018, 2019). Regarding arterial grafts, various studies suggests that recellularization of decellularized grafts with endothelial cells (ECs) prior to transplantation improves graft functionality and prevents thrombosis (Lin et al., 2018). For recellularization of vascular grafts, different cell sources have been applied in the past, including somatic ECs (Dahan et al., 2017; Quint et al., 2011), endothelial progenitor cells (Kaushal et al., 2001; Melchiorri et al., 2016; Quint et al., 2011), induced pluripotent stem cell derived ECs (Nakayama et al., 2015) or

| Native                  | P-TEV                  |
|------------------------|------------------------|
| **Hematoxylin/Eosin**  | **Hematoxylin/Eosin**  |
| 3 days Post surgery    | 3 days Post surgery    |
|                        |                        |
| 17 days Post surgery   | 17 days Post surgery   |
|                        |                        |
| 5 weeks Post surgery   | 5 weeks Post surgery   |

**Figure 4** Hematoxylin/Eosin and 4',6-diamidino-2-phenylindole (DAPI) stainings of vena cava. The pig's native vena cava, and the personalized tissue-engineered vein (P-TEV) graft 3 days, 17 days and 5 weeks post-surgery. * = luminal side. Scalebars, 200 μm [Colour figure can be viewed at wileyonlinelibrary.com]
embryonic stem cell derived ECs (Shi et al., 2013). Disadvantages of these methods are the required growth and expansion of cells in vitro, which could induce genetic variations and mutations as well as increase variability. Other disadvantages include regulatory hurdles, high cost, limited availability of cells and time-consuming processes.

In the present study, we utilized a decellularization method previously described (Simsa et al., 2018). Biomechanical data from this study showed unchanged maximum tensile strength and burst pressure of the decellularized scaffolds compared with native blood vessels, whereas an increase in stiffness of the vein was observed. Quantifications of ECM proteins in the same study showed unchanged levels of insoluble collagen and a decrease in soluble collagen and glycosaminoglycans (GAGs). The basement membrane protein laminin was also reduced compared with native tissue. Our hypothesis was that shielding the ECM surface from thrombosis and neointimal formation can be achieved by preconditioning steps, that is by perfusion of the vascular graft with peripheral whole blood. The rationale behind this is that decellularized blood vessels present different

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

**Figure 5** 4',6-diamidino-2-phenylindole (DAPI), CD31 and Hematoxylin/Eosin stainings of vena cava post-in vivo. (a) Native vena cava proximal to the anastomoses, (b) proximal, (c) center and (d) distal part of the personalized tissue-engineered vein (P-TEV) three days post-surgery. (e) Native vena cava proximal to the anastomoses, (f) proximal, (g) center and (h) distal part of the P-TEV two weeks post-surgery. (i) Native vena cava proximal to the anastomoses, (j) proximal, (k) center and (l) distal part of the P-TEV four weeks post-surgery. Arrowheads indicate CD31-positive cells. Hematoxylin/Eosin staining's of (m) native vena cava and (n) P-TEV four weeks post-surgery. Arrows indicate luminal endothelial cells in the native tissue and cells with flattened endothelial cell-like morphology in the P-TEV transplant. * = luminal side. Scalebars in A–L, 200 µm; Scalebars in M–N, 20 µm [Colour figure can be viewed at wileyonlinelibrary.com]
collagen types, which have often been denatured to some degree. Collagen is a known activator of the coagulation cascade, by initiating platelet binding and activation through the intrinsic coagulation pathway (Böer et al., 2015; Boeer et al., 2014; Wong & Griffiths, 2014), and contributes to the pathogenesis of venous thrombosis. By perfusing decellularized blood vessels with peripheral whole blood, the luminal surface of the grafts gets covered with different factors potentially important for avoiding clotting protein adhesion, quiescence of platelet activation and facilitating recellularization and acceptance of the vascular grafts. Data from the ROTEM analysis showed increased clotting time, increased clot formation time and decreased alpha angle which indicates better hemocompatibility and thereby a slower activation of the intrinsic coagulation system for the reconditioned tissue compared with native and decellularized tissue samples. This effect could be due to remnants of heparin in the P-TEV since addition of heparinase (HEPTEM analysis) removed these differences between the samples. Importantly, the reconditioning did not affect the coagulation properties in any negative way, but rather increased the clotting time which can be part of prevention of thrombosis. In addition, the growth factors in the reconditioning solution have the ability to bind to the P-TEV and can potentially support recellularization and differentiation of stem cells and promote tissue regeneration (Ikada, 2006; Ullah et al., 2019). In a separate bioinformatics study, autologous blood proteins bound to the graft after reconditioning are being analyzed. Preliminary proteomics data indicate that more than 150 proteins bind to the decellularized scaffold during perfusion with autologous peripheral whole blood (data not shown). Also, autologous blood cells attaches and the content of dsDNA in the graft increase during reconditioning (Figure 2). The DNA content of the P-TEV post-in vivo (55.1 ± 6.5 ng DNA/mg wet tissue) was lower compared with native vena cava tissue (Figure 2), however this was probably due to the formation of scar tissue in the healing process from the surgery which was not possible to dissect from the original P-TEV. DNA quantification of native tissue close to the anastomosis, with attached scar tissue, showed similar amounts (53.3 ± 3.5 ng DNA/mg wet tissue).

A limitation of the study is that a decellularized scaffold was not included as a control for the in vivo study. However, the aim of the study was not to compare functionality and mechanistical differences between decellularized scaffolds and P-TEV but only to show safety and functionality of the P-TEV since the literature already have shown problems with decellularized scaffolds, as described in the introduction.

Before application of novel medical products on humans, animal trials are required to assess safety and efficacy. While smaller animals lack physiological similarity to humans, common animal models for vascular grafts include dogs, rabbits, sheep and pigs (Mehran et al., 1991). In the present study, pig was chosen for its similar cardiovascular system compared with humans. Even though pig is known to have a higher incidence of intimal hyperplasia formation in graft studies compared with other model animals (Liu et al., 2018), no intimal hyperplasia was observed in any of the P-TEV in our study. The thin vein wall facilitates ECM graft penetration and recellularization. The observation of gradual recellularization of the P-TEV...
already from three days after transplantation, and cellularization equal with native tissue after 35 days in vivo clearly shows that P-TEV is a functional graft for efficient recellularization. Cells were detected on the luminal surface as well as on tunica media and adventitia, both near the anastomosis sites and at the center of the graft. Luminal cells were furthermore characterized as potential endothelial cells, with positive CD31 staining and flattened morphology. These results, demonstrating recellularization of decellularized grafts during 35 days in vivo, are in line with or exceed other in vivo studies utilizing decellularized arterial grafts, with varying degrees of recellularization after five to 20 weeks post-transplantation (Ketchedjian et al., 2005; Martin et al., 2005; Quint et al., 2011). So far, no successful studies in a relevant large animal have been published for grafts in the venous system. In our study, no vessel wall thickening, and no intimal hyperplasia was observed. A gradual recellularization in vivo during physiological condition may mimic the normal restoration of vascular cellular injuries.

The venous grafts in this study did not contain valves due to that these are absent in large veins of the pigs. For future use in humans to treat venous diseases such as chronic venous insufficiency, CVI, we believe that in vivo recellularization can restore normal morphology and function of vein valves. While other studies on the arterial side show patency issues when grafts were implanted without a large initial cell population on the intima (Dahan et al., 2017; Quint et al., 2011), our methodology is functional even on the venous side, with retained patency, graft acceptance in the body and efficient in vivo recellularization. Taken together, our results support that the tested approach is a promising alternative for the next generation of vascular grafts.

In the present study we showed that decellularized blood vessels, reconditioned with peripheral whole blood, can be transplanted into a pig animal model. By analyzing transplanted P-TEV after 3 days ($n=1$), 2 weeks ($n=2$) and 4-5 weeks ($n=3$) we can conclude that recellularization appeared rapidly, already after 3 days, and the grafts were cellularized to a level comparable with the native vein tissue after 4-5 weeks with a protective endothelial layer on the luminal graft surface. All P-TEV were fully patent and without signs of coagulation, thrombosis, intimal hyperplasia or rejection. These results motivate to proceed with P-TEV in long term studies to further validate the product for clinical studies.

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CONFLICT OF INTEREST

Lachmi Jenndahl, Tobias Gustafsson-Hedberg, Robin Simsa and Raimund Strehl were employees of the company VERIGRAFT which contributed financially, including salaries and study costs, and by providing laboratory space.

AUTHOR CONTRIBUTIONS

Conceptualization, Joakim Häkansson, Robin Simsa, Yalda Bogestål, Lachmi Jenndahl, Tobias Gustafsson-Hedberg, Sarunas Petronis, Raimund Strehl, Klas Österberg; Data curation, Joakim Häkansson, Robin Simsa, Yalda Bogestål, Lachmi Jenndahl, Tobias Gustafsson-Hedberg, Sarunas Petronis, Raimund Strehl, Klas Österberg; Formal analysis, Joakim Häkansson, Robin Simsa, Yalda Bogestål, Lachmi Jenndahl, Tobias Gustafsson-Hedberg, Sarunas Petronis, Raimund Strehl, Klas Österberg; Funding acquisition, Joakim Häkansson, Yalda Bogestål, Robin Simsa; Investigation, Joakim Häkansson, Robin Simsa, Yalda Bogestål, Lachmi Jenndahl, Tobias Gustafsson-Hedberg, Sarunas Petronis, Raimund Strehl, Klas Österberg; Methodology, Joakim Häkansson, Robin Simsa, Yalda Bogestål, Lachmi Jenndahl, Tobias Gustafsson-Hedberg, Sarunas Petronis, Raimund Strehl, Klas Österberg; Project administration, Joakim Häkansson, Yalda Bogestål, Lachmi Jenndahl, Robin Simsa; Validation, Joakim Häkansson, Robin Simsa, Yalda Bogestål, Lachmi Jenndahl, Tobias Gustafsson-Hedberg, Sarunas Petronis, Raimund Strehl, Klas Österberg; Writing original draft, Joakim Häkansson, Robin Simsa, Yalda Bogestål, Lachmi Jenndahl, Tobias Gustafsson-Hedberg, Sarunas Petronis, Raimund Strehl, Klas Österberg; Writing review and editing. All authors have read and agreed to the published version of the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

Boer, U., Buettner, F. F. R., Klingenberg, M., Antonopoulos, G. C., Meyer, H., Haverich, A., & Wilhelmi, M. (2014). Immunogenicity of intensively decellularized equine carotid arteries is conferred by the extracellular matrix protein collagen type VI. Acott TS. PloS One, 9, e105964. https://doi.org/10.1371/journal.pone.0105964

Böer, U., Hurtado-Aguilar, L. G., Klingenberg, M., Lau, S., Jockenhoevel, S., Haverich, A., & Wilhelmi, M. (2015). Effect of intensified decellulization of equine carotid arteries on scaffold biomechanics and cytotoxicity. Annals of Biomedical Engineering, 43, 2630–2641. https://doi.org/10.1007/s10439-015-1328-1

Byrom, M. J., Bannon, P. G., White, G. H., & Ng, M. K. C. (2010). Animal models for the assessment of novel vascular conduits. Journal of Vascular Surgery, 52, 176–195. https://doi.org/10.1016/j.jvs.2009.10.080

Chemla, E. S., & Morsy, M. (2009). Randomized clinical trial comparing decellularized bovine ureter with expanded polytetrafluoroethylene for vascular access. British Journal of Surgery, 96, 34–39. https://doi.org/10.1002/bjs.6434
Crapo, P. M., Gilbert, T. W., & Badyal, S. F. (2011). An overview of tissue and whole organ decellularization processes. *Biomaterials*, 32, 3233–3243. https://doi.org/10.1016/j.biomaterials.2011.01.057

Dahan, N., Sarig, U., Bronstein, T., Baruch, L., Karram, T., Hoffman, A., & Machluf, M. (2017). Dynamic autologous reendothelialization of small-caliber arterial extracellular matrix: A preclinical large animal study. *Tissue Engineering Part A*, 23, 69–79. https://doi.org/10.1089/ten.TEA.2016.0126

Das, N., Bratby, M. J., Shrivastava, V., Cornall, A. J., Darby, C. R., Boardman, P., Anthony, S., & Uberoi, R. (2011). Results of a seven-year, single-centre experience of the long-term outcomes of bovine ureter grafts used as novel conduits for haemodialysis fistulas. *Cardiovasc. Intervent. Radiol.* **34**(3), 958–963. https://doi.org/10.1007/s00270-011-0096-2

Dokmak, S., Aussilhou, B., Sauvanet, A., Nagarajan, G., Farges, O., & Belghiti, J. (2015). Patiopat peritoneum as an autologous substitute for venous reconstruction in hepatopancreato-biliary surgery. *Annals of Surgery*, 262, 366–371. https://doi.org/10.1097/SLA.0000000000000959

Eklof, B. G., Kistler, R. L., & Masuda, E. M. (1998). Venous bypass and valve reconstruction: Long-term efficacy [Internet]. *Vascular Medicine* (pp. 157–164). [Arnold](https://doi.org/10.1177/1358863980302001)

Ezzati, M., Obermeyer, Z., Tzoulaki, I., Mayosi, B. M., Elliott, P., & Leon, D. A. (2015). Contributions of risk factors and medical care to cardiovascular mortality trends. *Nature Reviews Cardiology*, 12, 508–530. https://doi.org/10.1038/nrcardio.2015.82

Gilbert, T., Sellaro, T., & Badyal, S. (2006). Decellularization of tissues and organs. *Biomaterials*, 27(19), 3675–3683. https://doi.org/10.1016/j.biomaterials.2006.02.014

Gui, L., Muto, A., Chan, S. A., Breuer, C. K., & Niklason, L. E. (2009). Development of decellularized human umbilical arteries as small-diameter vascular grafts. *Tissue Engineering Part A*, 15, 2665–2676. https://doi.org/10.1089/teng.tea.2008.0526

Hosgood, S. A., & Nicholson, M. L. (2011). First in man renal transplantation after ex vivo normothermic perfusion. *Transplantation*, 92, 735–738. https://doi.org/10.1097/TP.0b013e31822d4e04

Ikada, Y. (2006). Challenges in tissue engineering [Internet]. *Journal of the Royal Society, Series B: Biological Sciences*, 3(10), 589–601. https://doi.org/10.1098/rsbl.2006.0124

Jara, M., Malinowski, M., Bahra, M., Stockmann, M., Schulz, A., Pratschke, J., & Puhl, G. (2015). Bovine pericardium for portal vein reconstruction in hepatopancreato-biliary surgery. *Annals of Thoracic Surgery*, 79, 656–660. https://doi.org/10.1016/j.athoracsur.2004.09.003

Kovalic, A. J., Beattie, D. K., & Davies, A. H. (2002). Outcome of ProCol, a bovine mesenteric vein graft, in infrainguinal reconstruction. *European Journal of Vascular and Endovascular Surgery*, 24, 533–534. https://doi.org/10.1053/EJVES.2002.1710

Lawson, J. H., Glickman, M. H., Ilzecki, M., Jakimowicz, T., Jaroszyński, A., Peden, E. K., Pilgrim, A. J., Prichard, H. L., Guizewicz, M., Przywara, S., Szmidt, J., Turek, J., Witkiewicz, W., Zapotoczny, N., Zubilewicz, T., & Niklason, L. E. (2016). Bioengineered human acellular vessels for dialysis access in patients with end-stage renal disease: Two phase 2 single-arm trials. *Lancet (London, England)*, 387, 2026–2034. https://doi.org/10.1016/S0140-6736(16)00557-2

Lin, C.-H., Hsiia, K., Ma, H., Lee, H., & Lu, J.-H. (2018). Vivo performance of decellularized vascular grafts: A review article. *International Journal of Molecules*, 19, 2101. https://doi.org/10.3390/ijms19072101

Liu, R. H., Ong, C. S., Fukunishi, T., Ong, K., & Hibino, N. (2018). Review of vascular graft studies in large animal models. *Tissue Engineering Part B Reviews*, 24, 133–143. https://doi.org/10.1089/ten.teb.2017.0350

Luke, D. (1953). The differential leucocyte count in the normal pig. *The Journal of Comparative Pathology, 63*, 346–354. https://doi.org/10.1016/S0106-036-1742(53)80036-6

Madden, R. L., Lipkowitz, G. S., Browne, B. J., & Kurbano, A. (2004). Experience with cryopreserved cadaveric femoral vein allografts used for hemodialysis access. *Annals of Vascular Surgery*, 18, 453–458. https://doi.org/10.1016/S1001-6404(04)-0055-0

Martin, N. D., Schauer, P. J., Tulenko, T. N., Shapiro, I. M., DiMatteo, C. A., Williams, T. K., Hager, E. S., & DiMuzio, P. J. (2005). Vivo behavior of decellularized vein allograft. *Journal of Surgical Research*, 129, 17–23. https://doi.org/10.1016/j.jss.2005.06.037

Mehran, R. J., Ricci, M. A., Graham, A. M., Carter, K., & Symes, J. F. (1991). Porcine model for vascular graft studies. *Journal of Investigative Surgery*, 4, 37–44. [http://www.ncbi.nlm.nih.gov/pubmed/1863586](http://www.ncbi.nlm.nih.gov/pubmed/1863586)

Melchiorri, A. J., Bracaglia, L. G., Kimerer, L. K., Hibino, N., & Fisher, J. P. (2016). In vitro endothelialization of biodegradable vascular grafts via endothelial progenitor cell seeding and maturation in a tubular perfusion system bioreactor. *Tissue Engineering Part C: Methods*, 22, 663–670. https://doi.org/10.1089/ten.TEC.2015.0562

Mendis, S., Puska, P., Norrving, B., World Health Organization, World Heart Federation, & World Stroke Organization. (2011). *Global atlas on cardiovascular disease prevention and control*. World Health Orga-nization in collaboration with the World Heart Federation and the World Stroke Organization.

Nakayama, K. H., Joshi, P. A., Lai, E. S., Gujar, P., Joubert, L., & Huang, N. F. (2015). Bilayered vascular graft derived from human induced pluripotent stem cells with biomimetic structure and func-tion. *Regen Med. Future Science Group* **10**(3), 745–755. https://doi.org/10.2217/rme.15.45

Nicholson, M. L., & Hosgood, S. A. (2013). Renal transplantation after ex vivo normothermic perfusion: The first clinical study. *American Journal of Transplantation*, 13, 1246–1252. https://doi.org/10.1111/ajt.12179

Onida, S., & Davies, A. H. (2016). Predicted burden of venous disease. *Phlebology*, 31, 74–79. [http://doi.org/10.1177/0268355516628359](http://doi.org/10.1177/0268355516628359)

Quint, C., Kondo, Y., Manson, R. J., Lawson, J. H., Dardik, A., & Niklason, L. E. (2011). Decellularized tissue-engineered blood vessel as an arterial conduit. Proceedings of the National Academy of Sciences of the United States of America, 108, 9214–9219. https://doi.org/10.1073/pnas.1019506108

Sánchez, P. F., Brey, E. M., & Briceño, J. C. (2018). Endothelialization mechanisms in vascular grafts. *Journal of Tissue Engineering and Regenerative Medicine*, 12, 2164–2178. https://doi.org/10.1002/term.2747

Schaner, P. J., Martin, N. D., Tulenko, T. N., Shapiro, I. M., Tarola, N. A., Leichter, R. F., Carabasi, R. A., & DiMuzio, P. J. (2004). Decellularized vein as a potential scaffold for vascular tissue engineering. *Journal of Vascular Surgery*, 40, 146–153. https://doi.org/10.1016/j.jvs.2004.03.033

Shi, Q., Hodara, V., Simrley, C. R., Schatten, G. P., & VandeBerg, J. L. (2013). Ex vivo reconstitution of arterial endothelium by...
embryonic stem cell-derived endothelial progenitor cells in baboons. Stem Cells and Development, 22, 631–642. https://doi.org/10.1089/scd.2012.0313

Simsa, R., Padma, A. M., Heher, P., Hellström, M., Teuschl, A., Jenndahl, L., Bergh, N., & Fogelstrand, P. (2018). In M. Soncini (Ed.), Systematic in vitro comparison of decellularization protocols for blood vessels. PLoS One, 13, e0209269. Public Library of Science. https://doi.org/10.1371/journal.pone.0209269

Simsa, R., Vila, X. M., Salzer, E., Teuschl, A., Jenndahl, L., Bergh, N., & Fogelstrand, P. (2019). In F. Zhao (Ed.), Effect of fluid dynamics on decellularization efficacy and mechanical properties of blood vessels. PLoS One, 14, e0220743. Public Library of Science. https://doi.org/10.1371/journal.pone.0220743

Steen, S., Paskevicius, A., Liao, Q., & Sjöberg, T. (2016). Safe orthotopic transplantation of hearts harvested 24 hours after brain death and preserved for 24 hours. Scandinavian Cardiovascular Journal, 50, 193–200. https://doi.org/10.3109/14017431.2016.1154598

Ullah, I., Abu-Dawud, R., Busch, J. F., Rabien, A., Erguen, B., Fischer, I., Reinke, P., & Kurtz, A. (2019). VEGF-supplemented extracellular matrix is sufficient to induce endothelial differentiation of human iPSC. Biomaterials, 216. Elsevier Ltd. https://doi.org/10.1016/j.biomaterials.2019.119283

Wong, M. L., & Griffiths, L. G. (2014). Immunogenicity in xenogeneic scaffold generation: Antigen removal vs. decellularization. Acta Biomaterialia, 10, 1806–1816. https://doi.org/10.1016/j.actbio.2014.01.028

Wystrychowski, W., McAllister, T. N., Zagalski, K., Dusserre, N., Cierpka, L., & L’Heureux, N. (2014). First human use of an allogeneic tissue-engineered vascular graft for hemodialysis access. Journal of Vascular Surgery, 60, 1353–1357. Mosby Inc. https://doi.org/10.1016/j.jvs.2013.08.018

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