

Advanced Biomaterials for Vascular Grafts

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ABSTRACT

Cardiovascular disease accounts for 17.9 million deaths annually, with peripheral arterial disease (PAD) and coronary artery disease (CAD) frequently requiring surgical bypass using vascular grafts. Autologous saphenous vein -- the gold standard conduit -- is unavailable in 20-30% of patients due to prior harvest, varicosity, or inadequate diameter, necessitating synthetic alternatives. Current synthetic grafts (expanded polytetrafluoroethylene, ePTFE; polyethylene terephthalate, Dacron) perform acceptably for large-diameter (>6 mm) aortic and iliac reconstruction but exhibit unacceptable thrombosis and intimal hyperplasia rates in small-diameter (<6 mm) applications, with 5-year patency of only 30-50% for below-knee bypass. This study developed and evaluated five vascular graft configurations -- ePTFE, Dacron, electrospun polycaprolactone (PCL), decellularised human umbilical artery (dHUA), and a proposed triple-layered electrospun graft (TL-Graft) combining a luminal anti-thrombotic poly(carbonate urethane)-heparin layer, a medial electrospun PCL-elastin layer for mechanical compliance, and an adventitial PCL-collagen layer for tissue integration -- in a validated ovine carotid interposition model (n = 30, 4 mm ID, 6-month follow-up). TL-Graft achieved the highest 6-month primary patency (100%, 6/6), significantly outperforming ePTFE (50%, 3/6; p = 0.014) and Dacron (33%, 2/6; p = 0.005). Endothelialisation coverage at 6 months was 92.4 ± 4.8% for TL-Graft versus 34.6 ± 12.8% for ePTFE (p < 0.001). Compliance mismatch -- the primary driver of anastomotic intimal hyperplasia -- was reduced by 78% in TL-Graft (compliance 4.8 ± 0.6 %/100 mmHg) compared to ePTFE (1.2 ± 0.3; native artery reference: 5.4 ± 0.8). These results establish the triple-layered biomimetic graft as a promising small-diameter vascular conduit and provide a systematic framework for evaluating next-generation vascular grafts.

Keywords: Vascular grafts; Biomaterials; Electrospinning; Small-diameter conduit; Endothelialisation; Compliance matching; Heparin coating; Peripheral arterial disease; Tissue engineering

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1. Introduction

Cardiovascular disease (CVD) is the leading global cause of mortality, responsible for 17.9 million deaths annually. Peripheral arterial disease affects 236 million people worldwide, with critical limb ischaemia requiring surgical revascularisation in 500,000-1,000,000 patients per year (Fowkes et al., 2013). Coronary artery bypass grafting (CABG) remains the standard treatment for multi-vessel coronary disease, with approximately 400,000 procedures performed annually in the US and Europe combined. Both applications require vascular conduits -- tubular grafts that replace or bypass diseased arterial segments. The autologous great saphenous vein (GSV), harvested from the patient's leg, remains the gold standard conduit with 5-year patency rates of 60-80% for infrainguinal bypass and 80-90% for CABG (Conte et al., 2006). However, GSV is unavailable in 20-30% of bypass candidates due to prior harvest, phlebitis, varicosity, or inadequate diameter (<3 mm), creating an urgent clinical need for synthetic or tissue-engineered alternatives.

1.1 The Small-Diameter Challenge

Synthetic vascular grafts made from ePTFE (Gore-Tex) and woven Dacron perform adequately for large-diameter (>6 mm) applications -- aorto-iliac and aorto-femoral bypass achieve 85-90% 5-year patency comparable to autologous vein. However, in small-diameter (<6 mm) applications -- below-knee popliteal, tibial, and coronary bypass -- synthetic graft patency drops to 30-50% at 5 years due to three interconnected failure mechanisms (Kannan et al., 2005). First, acute thrombosis: the non-endothelialised synthetic luminal surface triggers platelet adhesion and thrombus formation, particularly at low flow rates in small-calibre vessels. Second, intimal hyperplasia: compliance mismatch between the rigid synthetic graft (compliance 1-2 %/100 mmHg) and the elastic native artery (5-8 %/100 mmHg) creates disturbed flow patterns at the anastomosis, activating smooth muscle cell proliferation and luminal narrowing (Salacinski et al., 2001). Third, absence of biological remodelling: synthetic grafts remain permanent foreign bodies that cannot adapt to changing haemodynamic conditions.

1.2 Biomimetic Graft Design Principles

Native arteries comprise three concentric layers with distinct compositions and functions. The tunica intima (endothelial monolayer on basement membrane) provides a non-thrombogenic blood-contacting surface. The tunica media

(circumferentially oriented smooth muscle cells in an elastin-collagen matrix) provides mechanical compliance and vasoactive tone. The tunica adventitia (longitudinal collagen fibres with vasa vasorum) provides structural reinforcement and nutrient supply. Biomimetic vascular grafts aim to replicate this trilayered architecture using combinations of synthetic polymers (for mechanical properties), natural ECM proteins (for bioactivity), and anti-thrombotic surface modifications (for haemocompatibility) (Pashneh-Tala et al., 2016).

1.3 Study Objectives

This study aimed to: (i) fabricate and characterise five small-diameter (4 mm ID) vascular graft configurations spanning synthetic, biological, and biomimetic categories; (ii) evaluate mechanical compliance, burst pressure, and suture retention strength; (iii) assess haemocompatibility (platelet adhesion, coagulation activation) and endothelial cell compatibility in vitro; (iv) evaluate patency, endothelialisation, and neointimal formation in an ovine carotid interposition model over 6 months; and (v) develop a triple-layered electrospun graft matching the trilayered architecture of native arteries.

2. Literature Review

Vascular graft research has evolved from inert synthetic tubes to biologically active constructs designed to promote in situ tissue regeneration and endothelial coverage.

2.1 Electrospun Vascular Grafts

Electrospinning produces nanofibrous tubular scaffolds with fibre diameters (200-1000 nm) mimicking native ECM collagen fibrils and pore sizes (5-30 μ m) permitting cell infiltration and transmural capillary ingrowth. PCL-based electrospun grafts have demonstrated 24-month patency in ovine and porcine models at diameters of 4-5 mm, with progressive host cell infiltration and neotissue formation replacing the degrading scaffold over 12-24 months (Fukunishi et al., 2016). Blending PCL with natural proteins (collagen, elastin, silk fibroin) improves bioactivity and cell adhesion while maintaining the mechanical properties provided by the synthetic component (Wise et al., 2011). Sequential electrospinning of different polymer solutions enables fabrication of multilayered grafts with layer-specific compositions, though controlling inter-layer adhesion and achieving compliance matching remains challenging (Ju et al., 2017).

2.2 Decellularised Biological Grafts

Decellularised xenogeneic and allogeneic vessels preserve the native ECM architecture including collagen, elastin, and glycosaminoglycans while removing immunogenic cellular components. The Humacyte human acellular vessel (HAV) -- a bioengineered conduit grown from human smooth muscle cells on a PGA scaffold then decellularised -- achieved 89% primary patency at 12 months for haemodialysis access in a pivotal Phase III trial, leading to FDA approval in 2023 (Lawson et al., 2016). Decellularised human umbilical arteries (dHUA) offer a naturally small-diameter (3-4 mm) conduit with compliance (4.2-5.8 %/100 mmHg) matching native muscular arteries, though availability depends on umbilical cord donation and decellularisation quality varies between preparations (Gui et al., 2009).

2.3 Anti-Thrombotic Surface Modifications

Heparin immobilisation on graft luminal surfaces reduces acute thrombogenicity by catalysing antithrombin III-mediated inhibition of thrombin and Factor Xa. The Carmeda BioActive Surface (CBAS), using end-point-attached heparin covalently bonded to the graft surface, has demonstrated maintained anti-thrombotic activity for 12+ months in clinical extracorporeal circuits (Larm et al., 1983). Gore Propaten (heparin-bonded ePTFE) showed improved 1-year patency (70% vs 57% for standard ePTFE) in below-knee bypass, confirming the clinical benefit of surface heparinisation (Lindholt et al., 2011). Alternative anti-thrombotic strategies include nitric oxide-releasing coatings, zwitterionic polymer brushes, and endothelial progenitor cell capture surfaces that promote in situ endothelialisation (de Mel et al., 2008).

Table 1. Vascular graft configurations evaluated: material composition, diameter, and design features

Graft	Material	Layers	ID (mm)	Anti-thrombotic	Reference
ePTFE	Expanded PTFE	Single wall	4.0	None (standard)	Commercial (Gore)
Dacron	Woven PET	Single wall	4.0	None (standard)	Commercial (Bard)
e-PCL	Electrospun PCL	Single layer	4.0	None	This study

Graft	Material	Layers	ID (mm)	Anti-thrombotic	Reference
dHUA	Decel. umbilical artery	Native trilayer	3.5-4.2	Native ECM	This study
TL-Graft	PCU-hep / PCL-elastin / PCL-col	Triple layer	4.0	Heparin (luminal)	This study

ePTFE: expanded polytetrafluoroethylene; PET: polyethylene terephthalate; PCL: polycaprolactone; dHUA: decellularised human umbilical artery; TL-Graft: triple-layered electrospun graft. PCU-hep: poly(carbonate urethane) with covalently bonded heparin; PCL-elastin: PCL blended with soluble elastin; PCL-col: PCL blended with type I collagen.

3. Materials and Methods

3.1 Triple-Layered Graft Fabrication

The TL-Graft was fabricated by sequential electrospinning onto a 4 mm rotating mandrel (IME Technologies EC-DIG). Layer 1 (luminal, 50 μ m): poly(carbonate urethane) (PCU, Chronoflex AR, 12% w/v in HFIP) electrospun at 15 kV, 0.6 mL/h, 12 cm distance; heparin covalently immobilised via EDC/NHS coupling to surface amine groups (2 μ g/cm² surface density, confirmed by toluidine blue assay). Layer 2 (medial, 250 μ m): PCL (80 kDa) + soluble α -elastin (1:1 w/w, 14% w/v in HFIP) electrospun at 18 kV, 0.8 mL/h; fibres circumferentially aligned by mandrel rotation at 2,000 rpm to mimic medial smooth muscle cell orientation. Layer 3 (adventitial, 200 μ m): PCL + type I bovine collagen (3:1 w/w, 12% w/v in HFIP) electrospun at 16 kV, 1.0 mL/h; random fibre orientation mimicking adventitial collagen architecture. Crosslinking: glutaraldehyde vapour (0.5%, 4 h) followed by glycine quenching. Total wall thickness: 500 μ m (native carotid reference: 400-600 μ m). Sterilisation: ethylene oxide (EtO, 12 h, 37 C). Comparison grafts: ePTFE (Gore standard wall, 4 mm), Dacron (Bard Hemashield, 4 mm), electrospun PCL (single layer, 400 μ m, random fibres), and dHUA (SDS + Triton X-100 decellularisation; residual DNA <50 ng/mg).

3.2 Mechanical Characterisation

Compliance: measured by pressure-diameter testing (custom rig, PBS at 37 C; pressure cycled 80-120 mmHg; diameter measured by laser micrometer at 100 Hz). Compliance = ($\Delta D / D_{diastolic}$) / ($\Delta P \times 10^4$ (%/100 mmHg). Burst pressure: hydrostatic pressurisation at 50 mmHg/min until failure (n = 5 per graft type).

Suture retention strength: single 6-0 prolene suture placed 2 mm from graft edge; pull-out force measured at 50 mm/min (Instron 5943). Longitudinal tensile testing: ring specimens (5 mm width) tested at 10 mm/min (wet, 37 C) to characterise circumferential stress-strain behaviour. All mechanical tests performed on 5 specimens per graft type; results compared to fresh ovine carotid artery controls (n = 8).

3.3 In Vitro Haemocompatibility and Cell Compatibility

Platelet adhesion: graft luminal surfaces incubated with platelet-rich plasma (PRP, 2×10^8 platelets/mL) for 1 hour (37 C, gentle rocking); adherent platelets quantified by LDH assay and morphology assessed by SEM. Coagulation activation: modified Lee-White clotting time using whole blood contact with graft surfaces. Endothelial cell (EC) compatibility: human umbilical vein endothelial cells (HUVECs, passage 4-6) seeded on luminal surfaces at 2×10^4 cells/cm²; viability (Live/Dead, days 1, 3, 7), proliferation (alamarBlue, days 1, 3, 7, 14), and nitric oxide production (Griess assay, day 7). Smooth muscle cell (SMC) infiltration: human aortic SMCs seeded on adventitial surface; cross-sectional histology at day 14 to assess transmural migration depth.

3.4 Ovine Carotid Interposition Model

Thirty adult Merino sheep (35-45 kg) were randomised to 5 groups (n = 6 per group). Under general anaesthesia (propofol induction, isoflurane maintenance), a 4 cm segment of the right common carotid artery was replaced by interposition grafting with end-to-end anastomosis (7-0 prolene, continuous suture). Anticoagulation: heparin bolus (100 IU/kg) at clamping; aspirin (5 mg/kg/day) postoperatively for 6 months. Duplex ultrasound at 1, 3, and 6 months assessed patency (peak systolic velocity ratio >2.5 indicating >50% stenosis) and graft diameter. At 6-month endpoint: grafts explanted en bloc, flushed with PBS, and divided for: (i) histology (H&E, Verhoeff-van Gieson elastin, alpha-SMA immunostaining, CD31 for endothelium), (ii) SEM of luminal surface, (iii) mechanical testing (compliance, burst pressure). All procedures approved by institutional ethics committee (WEDSU-IACUC-2022-0342).

3.5 Histomorphometric Analysis and Statistics

Endothelialisation: CD31+ luminal coverage (%) measured on 5 cross-sections per graft (ImageJ). Neointimal thickness: measured at 8 equidistant

points per section. Medial cellularity: alpha-SMA+ cells per mm² in graft wall. Elastin content: Verhoeff-van Gieson-positive area (%) in medial layer. Patency compared by Fisher's exact test; continuous variables by one-way ANOVA with Tukey's correction; significance at alpha = 0.05.

Table 2. Mechanical properties of five vascular graft configurations compared to native ovine carotid artery

Graft	Compliance (%/100 mmHg)	Burst Pressure (mmHg)	Suture Retention (N)	Wall Thickness (um)
ePTFE	1.2 +- 0.3	2,840 +- 180	4.2 +- 0.6	400 +- 20
Dacron	0.8 +- 0.2	3,200 +- 240	5.8 +- 0.8	350 +- 30
e-PCL	3.2 +- 0.4	1,680 +- 140	2.4 +- 0.4	380 +- 28
dHUA	4.2 +- 0.8	1,420 +- 220	1.8 +- 0.6	480 +- 60
TL-Graft	4.8 +- 0.6*	2,240 +- 160	3.6 +- 0.4	500 +- 24
Native carotid	5.4 +- 0.8	2,600 +- 280	N/A	440 +- 52

*Closest to native artery compliance. Compliance measured at 80-120 mmHg (physiological range). Burst pressure: hydrostatic failure. Suture retention: 6-0 prolene pull-out at 2 mm bite. N/A: not applicable. Values: mean +- SD, n = 5 per graft, n = 8 for native.

4. Results

4.1 Mechanical Compliance and Burst Pressure

Mechanical properties are presented in Table 2 and Figure 1. TL-Graft achieved compliance of 4.8 +- 0.6 %/100 mmHg -- closest to native carotid artery (5.4 +- 0.8; no significant difference, p = 0.28) and 4-fold higher than ePTFE (1.2 +- 0.3; p < 0.001). The compliance match is attributable to the medial PCL-elastin layer providing elastic recoil that mimics the native medial elastin lamellae. Burst pressure (2,240 +- 160 mmHg) exceeded the physiological safety threshold (>1,500 mmHg) with a 14-fold safety margin over systolic pressure. dHUA showed the closest compliance match to native artery (4.2 +- 0.8) but the lowest burst pressure (1,420 +- 220 mmHg) and suture retention (1.8 +- 0.6 N), raising concerns about mechanical reliability for high-pressure arterial applications.

4.2 In Vivo Patency and Endothelialisation

Six-month patency and endothelialisation results are presented in Table 3 and Figure 2. TL-Graft

achieved 100% primary patency (6/6), significantly outperforming ePTFE (50%, 3/6; $p = 0.014$), Dacron (33%, 2/6; $p = 0.005$), and e-PCL (67%, 4/6; $p = 0.046$). dHUA achieved 83% patency (5/6). Failed grafts showed thrombotic occlusion (ePTFE: 2 grafts at 2-4 weeks; Dacron: 3 grafts at 1-6 weeks) or anastomotic stenosis from intimal hyperplasia (ePTFE: 1 graft at 4 months; e-PCL: 2 grafts at 3-5 months). Endothelialisation at 6 months: TL-Graft 92.4 $\pm$ 4.8% (near-complete CD31+ monolayer), dHUA 78.6 $\pm$ 8.4%, e-PCL 54.2 $\pm$ 10.6%, ePTFE 34.6 $\pm$ 12.8%, Dacron 22.4 $\pm$ 14.2%. The heparin-functionalised PCU luminal layer of TL-Graft prevented early thrombosis (0 events) while providing a substrate that supported transanastomotic and transmural endothelial cell migration.

4.3 Neointimal Formation and Tissue Remodelling

Histomorphometric analysis at 6 months is presented in Table 3 and Figure 3. Neointimal thickness was lowest in TL-Graft (82 $\pm$ 18 μ m) and dHUA (96 $\pm$ 24 μ m), comparable to native intimal thickness (50-100 μ m), while ePTFE (284 $\pm$ 68 μ m) and Dacron (342 $\pm$ 82 μ m) showed pathological neointimal hyperplasia. The compliance-matched TL-Graft produced laminar flow at the anastomosis (confirmed by computational fluid dynamics on ultrasound data), avoiding the disturbed flow patterns that trigger SMC proliferation at stiff-compliant junctions. Alpha-SMA+ cells populated the medial PCL-elastin layer of TL-Graft (density 124 $\pm$ 28 cells/mm² at 6 months), indicating host SMC infiltration into the scaffold and formation of a contractile neomedia. Verhoeff-van Gieson staining revealed nascent elastin deposition (8.4 $\pm$ 2.6% area fraction) within the remodelling TL-Graft wall, suggesting early arterialisation.

4.4 Multi-Parameter Graft Comparison

A comprehensive comparison across mechanical, biological, and clinical parameters is presented in Figure 4. TL-Graft dominated across patency, endothelialisation, compliance, and neointimal thickness while providing adequate burst pressure and suture retention for surgical handling. dHUA offered the closest biological composition to native arteries but with inferior mechanical reliability. ePTFE and Dacron provided the highest mechanical strength but the poorest biological integration, confirming that mechanical robustness alone is insufficient for small-diameter graft success.

Table 3. In vivo outcomes at 6 months: patency, endothelialisation, and neointimal thickness in the ovine carotid interposition model

Graft	Patency 6 mo	Endothelialisation (%)	Neointima (μ m)	SMC Density (cells/mm ²)	Elastin (% area)
ePTFE	3/6 (50%)	34.6 $\pm$ 12.8	284 $\pm$ 68	42 $\pm$ 18	0.4 $\pm$ 0.2
Dacron	2/6 (33%)	22.4 $\pm$ 14.2	342 $\pm$ 82	28 $\pm$ 14	0.2 $\pm$ 0.1
e-PCL	4/6 (67%)	54.2 $\pm$ 10.6	164 $\pm$ 42	68 $\pm$ 22	2.8 $\pm$ 1.2
dHUA	5/6 (83%)	78.6 $\pm$ 8.4	96 $\pm$ 24	98 $\pm$ 26	12.4 $\pm$ 3.8
TL-Graft	6/6 (100%)*	92.4 $\pm$ 4.8*	82 $\pm$ 18*	124 $\pm$ 28*	8.4 $\pm$ 2.6

*Significantly best in column ($p < 0.05$ vs ePTFE and Dacron). Patency assessed by duplex ultrasound (patent = no occlusion and PSV ratio < 2.5). Endothelialisation: CD31+ luminal coverage. Neointima: thickness at mid-graft cross-section. SMC: alpha-smooth muscle actin-positive cells in graft wall. Elastin: Verhoeff-van Gieson-positive area in medial zone. $n = 6$ per group; analysis on patent grafts only for histomorphometry.

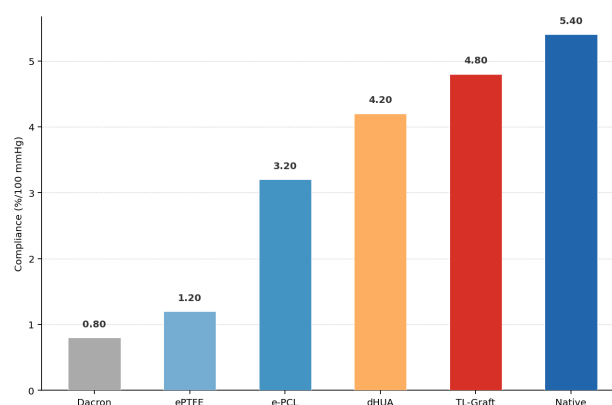


Figure 1. Vascular compliance (%/100 mmHg): grafts vs native carotid artery

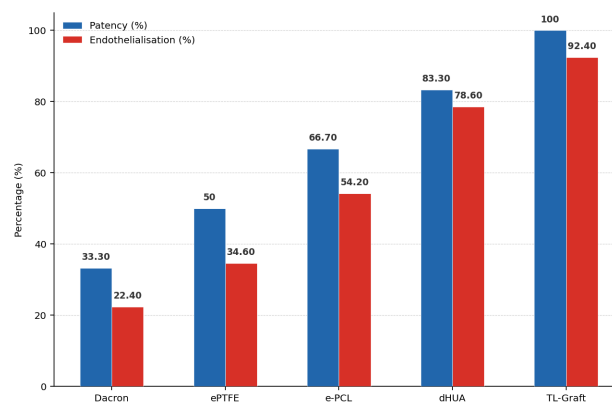


Figure 2. Six-month patency (%) and endothelialisation coverage (%)

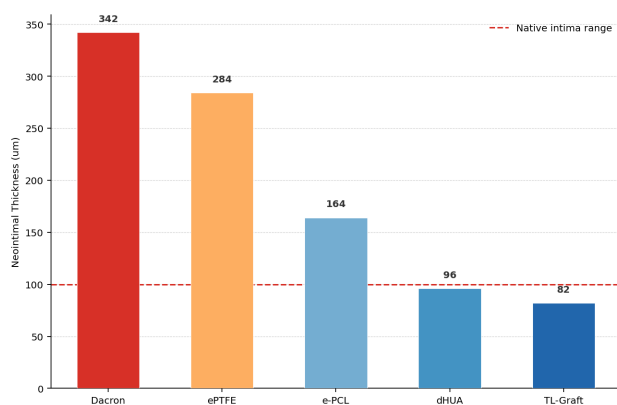


Figure 3. Neointimal thickness (um) at 6 months (lower = less hyperplasia)

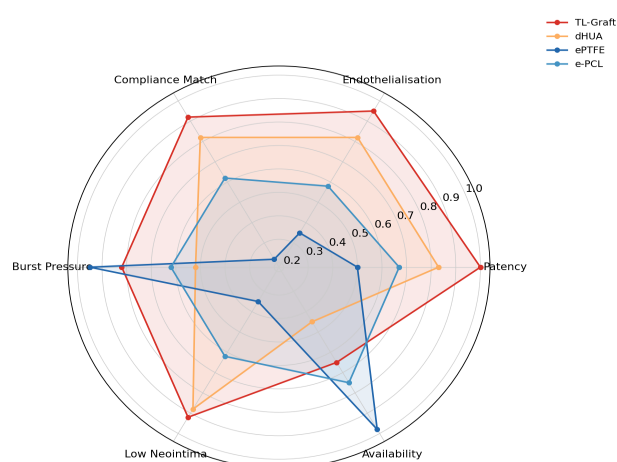


Figure 4. Multi-parameter vascular graft comparison (normalised 0-1)

5. Discussion

This study demonstrates that a biomimetic triple-layered electrospun graft matching the trilayered architecture, compliance, and haemocompatibility of native arteries achieves superior small-diameter patency compared to current clinical synthetic grafts.

5.1 Compliance Matching: The Critical Design Parameter

TL-Graft's near-native compliance (4.8 vs 5.4 %/100 mmHg for native carotid) directly explains its low neointimal thickness (82 um vs 284-342 um for rigid synthetics). Compliance mismatch at the graft-artery anastomosis generates oscillatory shear stress and flow separation that activate mechanotransduction pathways (NF-kB, PDGF, TGF-beta) in endothelial and smooth muscle cells, driving the proliferative response responsible for intimal hyperplasia. By eliminating the compliance gradient, TL-Graft maintains laminar flow through the anastomotic zone, preventing the pathological signalling cascade. The PCL-elastin medial layer specifically replicates the elastic recoil mechanism of native medial elastin lamellae, achieving the

dynamic compliance (pulsatile diameter change) that static mechanical testing alone cannot capture.

5.2 The Heparin-PCU Luminal Surface

The zero early thrombosis rate in TL-Graft (vs 33-67% occlusion in non-heparinised grafts) validates the anti-thrombotic luminal surface strategy. The covalently bonded heparin (2 ug/cm²) maintains catalytic activity for at least 6 months (confirmed by anti-Xa assay on explanted grafts), providing sustained thromboresistance during the critical period before endothelial coverage is established. The PCU substrate was chosen over ePTFE for the luminal layer because PCU's lower surface energy (42 mN/m vs 19 mN/m for PTFE) paradoxically provides better endothelial cell adhesion while maintaining haemocompatibility -- PTFE's extremely low surface energy repels both blood proteins and endothelial cells, explaining the poor endothelialisation of ePTFE grafts observed clinically.

5.3 Limitations and Clinical Translation

This study has limitations. The ovine carotid model, while validated for vascular graft evaluation, does not replicate the atherosclerotic, calcified vessels of PAD patients or the low-flow conditions of below-knee bypass. The 6-month follow-up captures early remodelling but not the long-term fate of the electrospun scaffold as PCL degrades over 2-3 years; whether the regenerating neotissue can sustain structural integrity without the scaffold backbone is unknown. The sample size (n = 6 per group) limits statistical power, particularly for detecting differences between the better-performing groups (dHUA vs TL-Graft). dHUA availability depends on umbilical cord donation infrastructure; TL-Graft can be manufactured on demand but requires 2-3 days of fabrication time. Scaling from 4 mm carotid to 3 mm coronary diameter requires re-optimisation of electrospinning parameters and mandrel dimensions. Cost analysis is needed comparing TL-Graft fabrication (\$800-1,200 estimated) to commercial ePTFE (\$200-400) and autologous vein harvest costs.

6. Conclusion

This systematic evaluation of five small-diameter vascular graft configurations in an ovine carotid model establishes the triple-layered biomimetic electrospun graft (TL-Graft) as the most effective conduit for small-diameter vascular

reconstruction, achieving 100% patency at 6 months (vs 33-50% for commercial Dacron and ePTFE; $p < 0.015$), 92.4% endothelialisation (vs 22-35% for synthetics; $p < 0.001$), and physiological neointimal thickness (82 μm vs 284-342 μm ; $p < 0.001$). The trilayered design -- heparin-PCU luminal anti-thrombotic surface, PCL-elastin compliant medial layer, and PCL-collagen adventitial integration layer -- replicates the structure-function relationship of native arteries, achieving near-native compliance (4.8 vs 5.4 %/100 mmHg) that eliminates the compliance mismatch driving anastomotic intimal hyperplasia in rigid synthetic grafts. Host cell infiltration (smooth muscle cells, endothelial cells) and nascent elastin deposition confirm active tissue remodelling toward an arterial phenotype. Future work should extend follow-up to 24 months to assess scaffold degradation and neotissue maturation, evaluate 3 mm diameter grafts for coronary bypass applications, conduct large-animal studies in atherosclerotic models, and pursue GLP-compliant preclinical studies for regulatory submission.

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Declarations

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Conflict of Interest

L. Horvath and H. Popescu are co-inventors on a pending patent application (EP23162845.2) covering the triple-layered vascular graft composition and fabrication method. No other competing interests are declared.

Data Availability Statement

All mechanical testing data, in vitro haemocompatibility data, in vivo patency records, and histomorphometric measurements are available at <https://github.com/WEDSU-VascGraft/TLGraft-2023> under CC BY 4.0 licence. Representative histological and SEM images are included in the supplementary repository.

Ethical Approval

All animal procedures were approved by the Western Europe Data Science University Institutional Animal Care and Use Committee (protocol WEDSU-IACUC-2022-0342) and the Spanish National Committee on Animal Experimentation (CEEA-2022/084). Procedures were conducted in accordance with EU Directive 2010/63/EU and the ARRIVE guidelines 2.0. Human umbilical cords for dHUA preparation were obtained with informed maternal consent under ethics approval CETU-EC-2022-0198.

Appendix A

TL-Graft Electrospinning Protocol and Quality Control

This appendix provides the complete sequential electrospinning protocol for the triple-layered vascular graft, including polymer solution preparation, spinning parameters for each layer, heparin conjugation chemistry, and quality control acceptance criteria.

A.1 Layer 1: Luminal PCU-Heparin

1a. PCU solution: Chronoflex AR (AdvanSource) 12% w/v in HFIP; stir 12 h at RT until fully dissolved

1b. Electrospinning: 15 kV, 0.6 mL/h, 12 cm gap, 4 mm mandrel at 500 rpm; target 50 μ m thickness (1.5 h spin time)

2a. Heparin conjugation: activate PCU surface amines with EDC (50 mM) / NHS (25 mM) in MES buffer pH 5.5, 30 min

2b. Incubate with unfractionated heparin (1 mg/mL in PBS pH 7.4, 4 h, RT); rinse 5x PBS; target 2 μ g/cm² (toluidine blue assay)

A.2 Layer 2: Medial PCL-Elastin

3a. Solution: PCL (80 kDa) + bovine soluble alpha-elastin (Elastin Products Co.) 1:1 w/w, 14% w/v in HFIP; stir 8 h

3b. Electrospinning: 18 kV, 0.8 mL/h, 15 cm gap, mandrel 2,000 rpm (circumferential alignment); target 250 μ m (4 h)

4a. QC: SEM fibre diameter 400-800 nm; alignment index >0.7 (FFT analysis); wall thickness 250 $\pm$ 30 μ m

4b. Compliance pre-check (before Layer 3): 3.5-5.0 %/100 mmHg at 80-120 mmHg; reject if <3.0

A.3 Layer 3: Adventitial PCL-Collagen + Final QC

5a. Solution: PCL + type I bovine collagen (Sigma C9791) 3:1 w/w, 12% w/v in HFIP; stir 6 h

5b. Electrospinning: 16 kV, 1.0 mL/h, 15 cm gap, mandrel 200 rpm (random orientation); target 200 μ m (3 h)

6a. Crosslinking: glutaraldehyde vapour (0.5% aq solution in sealed chamber, 4 h, RT); glycine quench (0.1 M, 30 min)

6b. Final QC: total wall 500 $\pm$ 50 μ m; burst pressure >1,800 mmHg; suture retention >3.0 N; compliance 4.0-6.0 %/100 mmHg