



Article

Biofunctionalized Vascular Access Graft Improves Patency and Endothelialization in a Porcine Arteriovenous Model

Aurora Battistella ¹, Morgan Linger ², Meredith Overton ², Unimunkh Uriyanghai ³, Christine Wai ³, Gang Xi ³, Prabir Roy-Chaudhury ^{3,4} and Wei Tan ^{1,2,*}

¹ Department of Mechanical Engineering, University of Colorado at Boulder, Boulder, CO 80309, USA; aurora.battistella@colorado.edu

² Department of Biomedical Engineering, University of Colorado at Boulder, Boulder, CO 80309, USA; morgan.linger@colorado.edu (M.L.)

³ UNC Kidney Center, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA; gang_xi@med.unc.edu (G.X.)

⁴ WG (Bill) Hefner Salisbury VA Medical Center, Salisbury, NC 28144, USA

* Correspondence: wtan@colorado.edu

Abstract

Reliable vascular access remains a major clinical challenge for hemodialysis patients, as expanded polytetrafluoroethylene (PTFE) grafts exhibit poor patency and frequent complications driven by thrombosis and neointimal hyperplasia. Tissue-engineered vascular grafts offer a regenerative alternative but often lack the mechanical resilience required for high-flow arteriovenous (AV) environments. Here, we developed a reinforced, biofunctionalized coaxial electrospun graft comprising a poly(ϵ -caprolactone) mechanical core and a norbornene-functionalized poly(ethylene glycol) sheath incorporating pro-endothelialization cues. Circumferential PTFE rings were added to improve kink resistance. Grafts were implanted in a porcine AV configuration that recapitulates clinical hemodynamic conditions. Mechanical characterization included compliance, burst pressure, and kink resistance; host remodeling was assessed using histology, immunofluorescence, and multiphoton imaging at 4 weeks. Ring-reinforced electrospun grafts demonstrated a kink radius of 0.187 cm, compliance of $1.04 \pm 0.29\%/100$ mmHg, and burst pressure of 1505 ± 565 mmHg, values all comparable to Gore-Tex PTFE and within industrial performance standards. In vivo, the electrospun grafts showed extensive host cell infiltration, collagen deposition, and formation of smooth muscle-like tissue, whereas PTFE controls remained largely acellular. Immunofluorescence confirmed intramural α -SMA⁺ and CD31⁺ cell populations, and multiphoton microscopy revealed significantly greater collagen and elastin content compared with PTFE ($p < 0.05$). Collectively, these findings demonstrate that the reinforced electrospun graft maintains mechanical integrity under physiological AV loading while supporting in situ endothelialization and extracellular matrix remodeling in a clinically relevant, large animal model. This work provides one of the first demonstrations of functional tissue regeneration within a fully synthetic, acellular scaffold in a porcine hemodialysis model and advances the translational development of durable, regenerative vascular access grafts that couple mechanical resilience with bioactive healing capacity.

Keywords: tissue-engineered vascular grafts (TEVGs); arteriovenous (AV) access; porcine model; biofunctionalized scaffold; endothelialization



Academic Editor: Ilia Fishbein

Received: 9 December 2025

Revised: 20 January 2026

Accepted: 22 January 2026

Published: 27 January 2026

Copyright: © 2026 by the authors.

Licensee MDPI, Basel, Switzerland.

This article is an open access article

distributed under the terms and

conditions of the [Creative Commons](#)

[Attribution \(CC BY\)](#) license.

1. Introduction

The global prevalence of end-stage kidney disease (ESKD) continues to rise, driven by the growing incidence of diabetes, hypertension, and an aging population [1]. In the United States alone, more than 130,000 new cases were reported in 2022, with nearly 83% of patients initiating hemodialysis [2]. Reliable vascular access is essential for hemodialysis. Although autogenous arteriovenous fistulas are preferred due to superior long-term patency, they frequently fail to mature in patients with significant comorbidities [3,4]. Consequently, arteriovenous grafts (AVGs), most commonly constructed from expanded polytetrafluoroethylene (ePTFE), are widely used when fistulas are not feasible. However, AVGs exhibit poor one-year primary patency rates (approximately 50%) [5] and high complication rates, most often due to venous anastomotic stenosis from neointimal hyperplasia [6].

The failure of current synthetic grafts arises from both mechanical and biological limitations [7]. Mechanically, ePTFE grafts are prone to thrombosis, particularly when repeatedly cannulated [8]. Biologically, they are bioinert, presenting non-physiologic surfaces that provoke inflammatory and fibrotic responses and lacking the regenerative capacity needed to heal after needle access during each hemodialysis session. These combined shortcomings drive neointimal hyperplasia and graft stenosis, the leading causes of AVG failure [9]. Collectively, these challenges underscore the need for vascular grafts that mitigate mechanical complications while promoting rapid endothelialization, tissue integration, and long-term patency.

Tissue-engineered vascular grafts (TEVGs) offer a promising solution. Unlike inert synthetics, TEVGs are designed to integrate with host tissue by permitting cell infiltration, extracellular matrix (ECM) deposition, and remodeling in situ into a functional “neovessel” [10]. Approaches have included cell-seeded scaffolds, acellular biodegradable polymers, and decellularized natural matrices [7]. Cell-seeded constructs, while effective in preclinical models, are limited by cost, regulatory hurdles, and risks of infection or immune rejection [11]. Decellularized scaffolds provide a natural ECM environment but suffer from donor variability and limited availability [12,13]. Emerging approaches such as Humacyte’s human acellular vessels (HAVs) demonstrate the potential of lab-grown, decellularized extracellular matrix conduits, though their production remains time-intensive and costly [14]. Consequently, acellular synthetic scaffolds fabricated from bioabsorbable polymers, such as poly(ϵ -caprolactone) (PCL) or poly(l-lactide-co- ϵ -caprolactone) (PLCL), have gained interest due to their reproducibility, tunable degradation, and scalability [15].

Electrospinning is among the most versatile methods for TEVG fabrication, generating nanofibrous architectures that mimic native ECM organization [16]. Electrospun fibers provide high surface area for protein adsorption and cellular attachment, while tunable porosity can regulate cell infiltration and nutrient transport [17]. The choice of polymer is central to achieving appropriate mechanical, biological, and degradation profiles [18]. PCL provides robust mechanical stability and ease of processing but degrades slowly, potentially prolonging inflammatory responses. To address these limitations, hybrid approaches combining PCL with bioactive components have been explored [19].

Surface biofunctionalization provides an additional design axis for improving hemocompatibility and promoting regeneration. Poly(ethylene glycol) (PEG) resists nonspecific protein adsorption and thrombosis, but can be chemically modified to present biological cues [20]. Norbornene-functionalized PEG enables orthogonal thiol–ene “click” chemistry for conjugation of peptides and growth factors [21]. Incorporation of integrin-binding motifs (e.g., RGD sequences) can promote endothelial adhesion, while immobilization of vascular endothelial growth factor (VEGF) can accelerate endothelialization. Combining PEG’s antifouling properties with targeted bioactivity offers a path to overcoming the thrombotic and hyperplastic responses associated with conventional grafts [22].

Despite these advances, most reported TEVG strategies address mechanical performance and biological function in isolation rather than through an integrated design. Many electrospun grafts rely on single-polymer systems without spatial control of bioactivity, while others incorporate biologically derived matrices or growth factors that can compromise mechanical stability or reproducibility. Reinforcement strategies, when employed, are often embedded within the graft wall, which may improve kink resistance but can restrict cellular infiltration and limit regenerative remodeling. In contrast, the present approach uniquely integrates (i) coaxial electrospinning to achieve compartmentalized scaffold architecture, (ii) PEG–norbornene chemistry to enable stable, spatially controlled covalent immobilization of pro-regenerative ligands, and (iii) independent external PTFE ring reinforcement that enhances kink resistance without mechanically constraining the bioactive scaffold. This combined mechanical and biofunctional strategy is designed to preserve surgical handling and durability while promoting in situ tissue integration, capabilities that have remained difficult to achieve simultaneously in existing TEVG platforms.

Nevertheless, many promising TEVGs have failed to perform in large animal or clinical settings, where higher flow rates, arterial pressures, and extended implantation times reveal vulnerabilities not evident in small-animal models. Inadequate kink resistance and delayed endothelialization remain key translational barriers [23]. This underscores the importance of validating TEVGs in clinically relevant large animal models to ensure TEVG performance under realistic physiological hemodynamics.

Our group previously developed an acellular coaxial electrospun TEVG composed of a PCL core for mechanical support and a sheath of norbornene-functionalized PEG to enhance hemocompatibility and host cell integration. In a rat aorta interposition model, this design demonstrated improved patency, reduced thrombosis, and early evidence of neotissue formation and endothelialization [24]. Building on these findings, we further optimized the graft by incorporating circumferential reinforcement rings to enhance kink resistance, a critical mechanical improvement for surgical handling and long-term function.

In this study, we evaluated this reinforced TEVG in a porcine AVG model, which more closely replicates the clinical hemodynamic environment. We comprehensively assessed mechanical properties, surgical feasibility, and in vivo remodeling using histology, immunofluorescence, and multiphoton microscopy. This work represents an important translational step toward the clinical application of biofunctional vascular access grafts that integrate mechanical resilience with regenerative healing potential for improved outcomes in hemodialysis patients.

2. Materials and Methods

2.1. Materials

Polycaprolactone (PCL, Mw = 70,000–90,000 Da, Sigma-Aldrich, St. Louis, MO, USA), poly(ethylene glycol)-norbornene (PEG-NB, Mw = 5000 Da, Biopharma PEG Scientific Inc., Watertown, MA, USA), and polyethylene glycol dithiol (PEG-SH, Mw = 1000 Da, Sigma-Aldrich) were used to fabricate coaxial electrospun vascular grafts. Poly(ethylene oxide) (PEO, Mw = 400,000 Da, Sigma-Aldrich) was included in the sheath formulation to support fiber formation. The photoinitiator used for crosslinking was Irgacure 2959 (Ciba Specialty Chemicals, Basel, Switzerland), prepared as a stock solution at 18 mg in 3.7 mL of Hexafluoroisopropanol (HFIP, Cova Chem LLC, Loves Park, IL, USA). All solvents, including HFIP, were used as received.

2.2. Graft Fabrication

Reinforced vascular grafts were fabricated using coaxial electrospinning onto a 5.5 mm diameter stainless steel mandrel. The core solution consisted of 5% *w/v* PCL dissolved in

HFIP, with a total volume of 8 mL. The sheath solution contained 0.8% *w/v* PEO, 5.268% *w/v* PEG-NB, and 1.75% *w/v* PEG-DI in HFIP, with a total volume of 10 mL. Solutions were stirred overnight until completely dissolved. Immediately before electrospinning, 1.75 mL of Irgacure 2959 photoinitiator stock solution was added to the sheath solution, which was thereafter protected from light.

Biological moieties were incorporated at this stage: diallyl trisulfide (DATS) was added to the core solution, while CGRGDS and Biotin–QK–VEGF-express peptides (GenScript, Piscataway, NJ, USA) were added to the sheath solution. CGRGDS was added at 3.32 mg/mL, and biotin–QK–VEGF at 10 $\mu\text{L/mL}$ from a 100 μM stock solution. DATS was introduced at 78.86 $\mu\text{L/mL}$ from a 100 μM stock solution. Peptide immobilization was enabled by thiol–norbornene photochemistry between cysteine residues on the peptides and norbornene groups of the 4-arm PEG-NB backbone, with PEG dithiol serving as the crosslinker to form a covalently bound network.

Electrospinning was performed under controlled ambient conditions (22 °C, 30–40% relative humidity) using a coaxial spinneret connected to two independently controlled syringe pumps (Pump 11 Plus, Harvard Apparatus, Boston, MA, USA). The core and sheath solutions were extruded at 0.8 mL h^{−1} and 1.0 mL h^{−1}, respectively, under an applied voltage of 12–15 kV from a high-voltage power supply (ES30P 10 W, Gamma High Voltage Research, Ormond Beach, FL, USA). The spinneret–mandrel distance was maintained at approximately 10 cm. Fiber collection was continued until the desired wall thickness was achieved (~10 h).

During fabrication, grafts were intermittently crosslinked by ultraviolet (UV) exposure (365 nm, 10 mW cm^{−2}) for 1 min every 5 min to initiate thiol–ene reactions within the PEG–NB sheath. Following deposition, grafts were further crosslinked under continuous UV exposure for 8 h. The grafts were then immersed in sterile phosphate-buffered saline (PBS) overnight, carefully removed from the mandrel, and stored at −80 °C until freeze-drying. Lyophilized grafts were subsequently stored at 4 °C.

2.3. Post-Production Processing

Grafts were trimmed to a final length of 3 cm and sterilized by immersion in 70% ethanol for 30 min, followed by multiple rinses in sterile PBS. To improve anti-kinking performance, reinforcement strategies were employed. Initially, circumferential suture loops were placed at 2 mm intervals. Subsequently, external PTFE reinforcement rings were adopted to improve independence and uniformity. Individual rings were manually positioned along the graft and secured using a medical-grade cyanoacrylate adhesive (LOCTITE® 4013, Henkel, Düsseldorf, Germany). A small volume of adhesive was applied at the interface between each ring and the graft sheath, and rings were placed sequentially by hand to ensure uniform spacing and circumferential contact.

To prevent delamination and improve handling during implantation, the graft ends were folded and bonded using the same biocompatible adhesive. Bond integrity between the PTFE rings and the polymer sheath was verified qualitatively by visual inspection and gentle manual manipulation prior to implantation, ensuring stable attachment without ring displacement (Figure 1).

Dimensional measurements (inner diameter, outer diameter, wall thickness) were conducted on hydrated samples using digital calipers and brightfield microscopy (*n* = 3 per condition). All grafts were hydrated in PBS for 24 h at room temperature before mechanical testing or implantation.

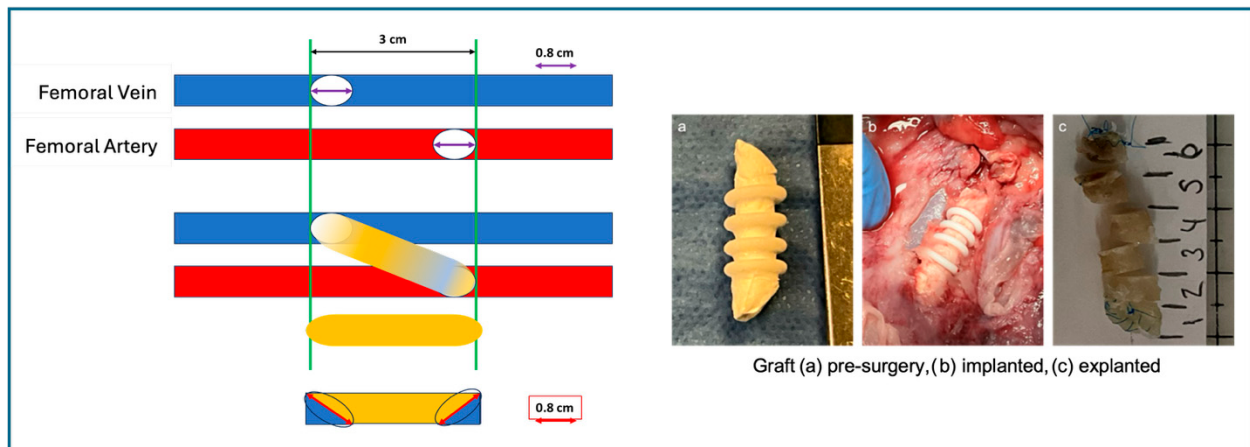


Figure 1. Schematic representation of end-to-end anastomosis and graft dimension (**left**) and images of electrospun TEVG (**a**) pre-surgery, (**b**) implanted, and (**c**) explanted.

The chemical composition, surface chemistry, and micro-morphology of the electrospun scaffold used in this study have been previously characterized in our earlier work [25]. Multiple scaffold formulations were analyzed in that study, and the formulation used here corresponds to one of those previously reported groups (PCL-PEGNB FD). X-ray photoelectron spectroscopy (XPS) confirmed the expected polymer chemistry and network formation, while scanning electron microscopy (SEM) demonstrated uniform fiber morphology and consistent fiber diameters and porosity. As the scaffold formulation and fabrication parameters were identical and no new chemical modifications or processing steps were introduced, additional chemical or morphological characterization was not repeated in the present study.

2.4. Mechanical Testing

Mechanical performance of the grafts was evaluated by measuring compliance, burst pressure, and kink resistance.

Kink radius was compared across five graft groups (PTFE, PTFE with rings, electrospun graft, electrospun graft with suture reinforcement, and electrospun graft with ring reinforcement) using one-way ANOVA followed by post hoc multiple-comparison testing.

Compliance and burst pressure were evaluated only for PTFE and electrospun grafts to assess intrinsic material properties without the confounding effects of external reinforcement. These comparisons were analyzed using an unpaired *t*-test, with significance set at $p < 0.05$.

2.4.1. Compliance Testing

Compliance was measured on hydrated grafts ($n = 5$) after 24 h equilibration in PBS, which allowed the hydrogel to reach equilibrium swelling. The samples were mounted on a custom flow loop equipped with Luer lock barbs and a pressure gauge and perfused with deionized (DI) water at 1 mL min^{-1} . The pressure was increased stepwise from 0 to 120 mmHg, and outer diameters at 80 mmHg (D_{80}) and 120 mmHg (D_{120}) were extracted from video recordings analyzed using FIJI (ImageJ, Version 2.16.0/1.54p). Compliance ($\%/100 \text{ mmHg}$) was calculated as:

$$\text{Compliance}(\frac{\%}{100\text{mmHg}}) = \frac{D_{120} - D_{80}}{(D_{80} \times \Delta P)} \times 10^4$$

where D_{80} and D_{120} are the inner diameters of the graft at 80 mmHg and 120 mmHg, respectively, and ΔP is 40 mmHg, the difference between 120 and 80 mmHg [26].

2.4.2. Burst Pressure

Immediately after compliance testing, the same grafts were measured for burst pressure. A latex lining was inserted and sealed to Luer lock barbs with Parafilm and sealing tape. Grafts were then pressurized with DI water at 20 mL/min under closed-end conditions until rupture, and the maximum pressure prior to rupture was recorded as the burst pressure ($n = 5$) [27].

2.4.3. Kink Resistance

Kink resistance was assessed following ISO 7198:2016 (A.5.8 e) [28]. Briefly, a cylindrical mandrel was used to determine the kink radius. Grafts were looped around cylindrical mandrels of decreasing diameter until kinking occurred. The corresponding mandrel diameter was recorded as the kink diameter. Images were captured and analyzed in ImageJ to calculate the kink radius.

2.5. Surgical Implantation

All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of North Carolina at Chapel Hill. Six male Yorkshire swine (45–50 kg; sourced from Wesley Looper Farm, NC, USA) were used to establish the model. The animals were housed under standard laboratory conditions and maintained in accordance with the Public Health Service Policy on Humane Care and Use of Laboratory Animals.

2.5.1. Anesthesia and Surgical Procedure

On the day of surgery, the animals were sedated with intramuscular ketamine (15 mg/kg) and midazolam (0.2 mg/kg). An ear vein catheter was placed, and anesthesia was induced with intravenous propofol (2–6 mg/kg to effect). Following endotracheal intubation, anesthesia was maintained with 1–3% isoflurane in 100% oxygen.

A longitudinal incision was made over the femoral triangle to expose the femoral artery and femoral vein. In four pigs, our graft (CO-Graft) with a 5.5 mm inner diameter was implanted, while in another three pigs, a GORE-TEX® PTFE graft with a 6 mm inner diameter was used. End-to-side anastomoses to the femoral artery and vein were constructed using continuous 6-0 Prolene sutures (Ethicon, Raritan, NJ, USA). Grafts were positioned within a subcutaneous pocket, with meticulous care taken to prevent kinking. Layered closure of the fascia and skin was performed using 2-0 Dexon sutures.

2.5.2. Anticoagulation and Postoperative Care

A single intraoperative dose of heparin (100 IU/kg) was administered immediately before vessel clamping. Enteric-coated aspirin (325 mg, oral) was initiated one day before surgery (Day−1) and continued daily until euthanasia. Buprenorphine was provided as needed for postoperative analgesia.

Graft patency was confirmed intraoperatively by direct visualization and palpation of graft flow. The animals were assessed every three days by auscultation and weekly with Doppler ultrasound to evaluate both stenosis and volume flow.

2.5.3. Euthanasia and Tissue Harvest

At Day 28 post-implantation, or on Day 18 in one animal with graft occlusion, grafts were excised en bloc with adjacent vessel segments and fixed in 10% neutral-buffered formalin.

A total of seven animals were included in the study: four animals received electrospun grafts, and three animals received PTFE control grafts. Among the electrospun grafts, one was terminated at 18 days due to graft occlusion. Of the remaining three animals that

completed the 28-day study, all grafts were pulsatile at Day 21, but two were occluded by Day 28. Explanted grafts were collected en bloc with adjacent vessel segments for morphometric analysis.

A diagrammatic representation of the graft–femoral artery anastomosis is provided (Figure 2), illustrating the precise locations where tissue sections were obtained every 0.5 cm. Sampling was performed along the length of the graft, from the arterial graft anastomosis to the venous graft anastomosis.

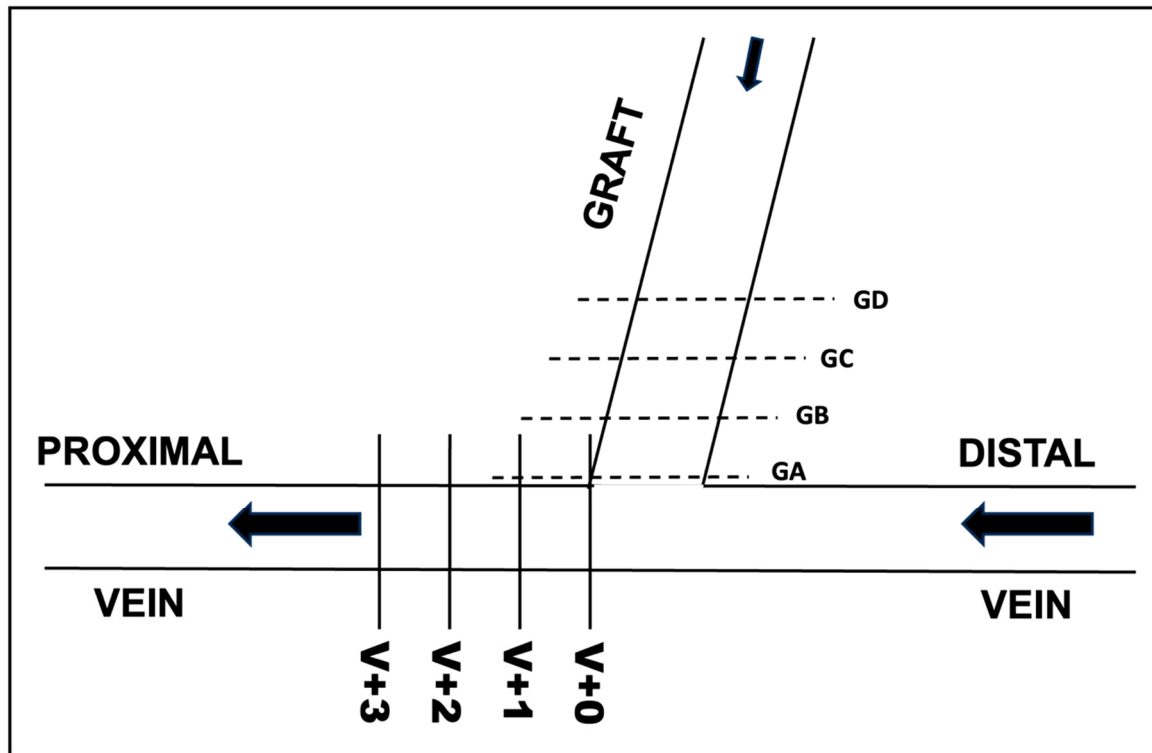


Figure 2. A diagrammatic representation of the graft–femoral vein anastomosis is provided, illustrating the precise locations where tissue sections were obtained at 0.5 cm intervals. Samples were collected along the vascular graft conduit, spanning from the venous graft anastomosis to the arterial graft anastomosis. Arrows indicate the blood flow.

2.6. Histological Analysis

Explanted grafts were fixed in 10% neutral-buffered formalin for 24–48 h, then processed through graded alcohols and paraffin-embedded for sectioning. Each graft was transversely sectioned into six evenly spaced segments along its length, including both anastomotic sites and four midgraft regions. Sections were cut at 5 µm thickness using a microtome and mounted on glass slides.

Histological staining included hematoxylin and eosin (H&E) for general tissue morphology and cellular infiltration, and Masson’s Trichrome for evaluation of collagen deposition and ECM organization. Hematoxylin and eosin (Richard Allan # 7221 and #7111, Kalamazoo, MI, USA) were performed following the Thermo Scientific Histology Staining Guidelines with minor modification (Hematoxylin1 staining time of 2.5 min and Eosin-Y staining time of 20 s). Masson’s Trichrome Stain (KTMTR StatLab, McKinney, TX, USA) was performed following the kit’s protocol.

All slides were imaged using light microscopy (Nikon Eclipse Ti) equipped with a digital camera. Images were captured at multiple magnifications (4×, 10×, 20×, and 40×) and analyzed using ImageJ software.

Qualitative assessments included evaluation of neotissue formation, inflammatory response, and presence of thrombus or stenosis. Collagen-rich regions were identified by blue staining in Masson's trichrome images. Comparative analysis across the proximal, midgraft, and distal regions was performed to assess regional remodeling. Results were compared to the PTFE graft.

2.7. Immunofluorescence (DAPI, CD31, SMA) and Cell Counting

Immunofluorescence staining was performed according to standard protocols. Fluorescent detection was performed using species-specific secondary antibodies conjugated to Alexa Fluor dyes (1:500, Invitrogen, A-11070 and A-11019, Eugene, OR, USA). Endothelial cells were identified using anti-CD31 antibodies detected with Alexa Fluor 488-conjugated secondary antibodies (green), while smooth muscle cells were identified using anti- α -smooth muscle actin (α -SMA) antibodies detected with Alexa Fluor 568-conjugated secondary antibodies (red). Nuclei were counterstained with DAPI (SouthernBiotech, OB010020, Birmingham, AL, USA). The slides were mounted with antifade medium and imaged using a fluorescence microscope. The images were taken on a Nikon Eclipse Ti microscope in SPOT basic 5.4.

Unstained sections were used for fluorescence imaging to evaluate tissue integration and material degradation, as described in our previous work [25]. Briefly, synthetic material and cell nuclei were visualized using maleimide and DAPI staining, respectively. Maleimide labeling exploits thiol–maleimide chemistry to localize thiol-containing polymer components, enabling visualization of cellular infiltration within the scaffold. The samples were stained with maleimide (100 μ M in PBS), followed by DAPI, mounted with antifade medium, and stored at -20°C prior to imaging.

For quantitative analysis, twenty images were selected for each group. In particular, the selection was performed from three different sections for each animal, and the material area must be visible in these images. Two images from the end sections and three slides from the middle section were imaged and analyzed.

Cell quantification was performed using a custom MATLAB R2024a script as described in our previous work [25]. Briefly, DAPI and maleimide channels were processed separately. Nuclei were segmented from DAPI images using binary masking with a minimum object size threshold and standard morphological operations, and cells were counted from the resulting masks. The material-associated signal was quantified from the maleimide channel using the same masking approach. Cell density was determined within user-defined regions corresponding to the material area and normalized to the analyzed area.

2.8. Multiphoton Imaging and ECM Quantification

Multiphoton microscopy was performed on paraffin-embedded, unstained sections following the approach described in our previous work [25,29]. Briefly, sections were deparaffinized and rehydrated before imaging. Second harmonic generation (SHG) and two-photon excitation fluorescence (TPEF) were used to visualize collagen and elastin, respectively. The images were acquired on a multiphoton microscope using near-infrared excitation, and selected fields were analyzed to quantify native tissue distribution.

Quantitative analysis was performed using MATLAB. Twenty images per group were analyzed (two from each end section and three from the mid-section). Histogram-based thresholds for collagen (red) and elastin (green) were determined per image; pixels exceeding threshold values within the material area mask were counted. ECM content (collagen/elastin density) was normalized to total analyzed area for inter-sample comparison.

2.9. Data Statistical Analysis

All quantitative results were exported to an Excel file and analyzed in Origin Pro 2025. Statistical analyses on kinking radius, compliance, burst pressure, and cell counting results were performed in Origin Pro. Statistical significance between the two groups was assessed using a two-tailed unpaired *t*-test ($p < 0.05$). The error bars represent standard deviations (SD), which capture sample-to-sample variability.

3. Results

3.1. Mechanical Testing

The mechanical properties of the reinforced electrospun graft were compared with PTFE controls and additional reinforcement strategies.

Kinking resistance was first evaluated across five graft types: PTFE, electrospun graft, PTFE with external rings, electrospun graft reinforced with sutures, and electrospun graft with external rings (Figure 3A). The kink radius values were 1.24, 1.28, 0.105, 0.120, and 0.187 cm, respectively. Both ring-reinforced grafts (PTFE and electrospun) showed significantly reduced kink radius compared with their non-reinforced counterparts, demonstrating the effectiveness of circumferential reinforcement in preventing lumen collapse. Among the electrospun designs, both the ring-reinforced and suture-reinforced grafts exhibited significantly greater kink resistance than the unreinforced graft ($p < 0.05$). No significant difference was observed between the two reinforced configurations. Although the suture-reinforced graft showed a trend toward lower kink radius (i.e., higher kink resistance), the circumferential independence and uniformity provided by the ring reinforcement are expected to offer practical advantages for structural stability.

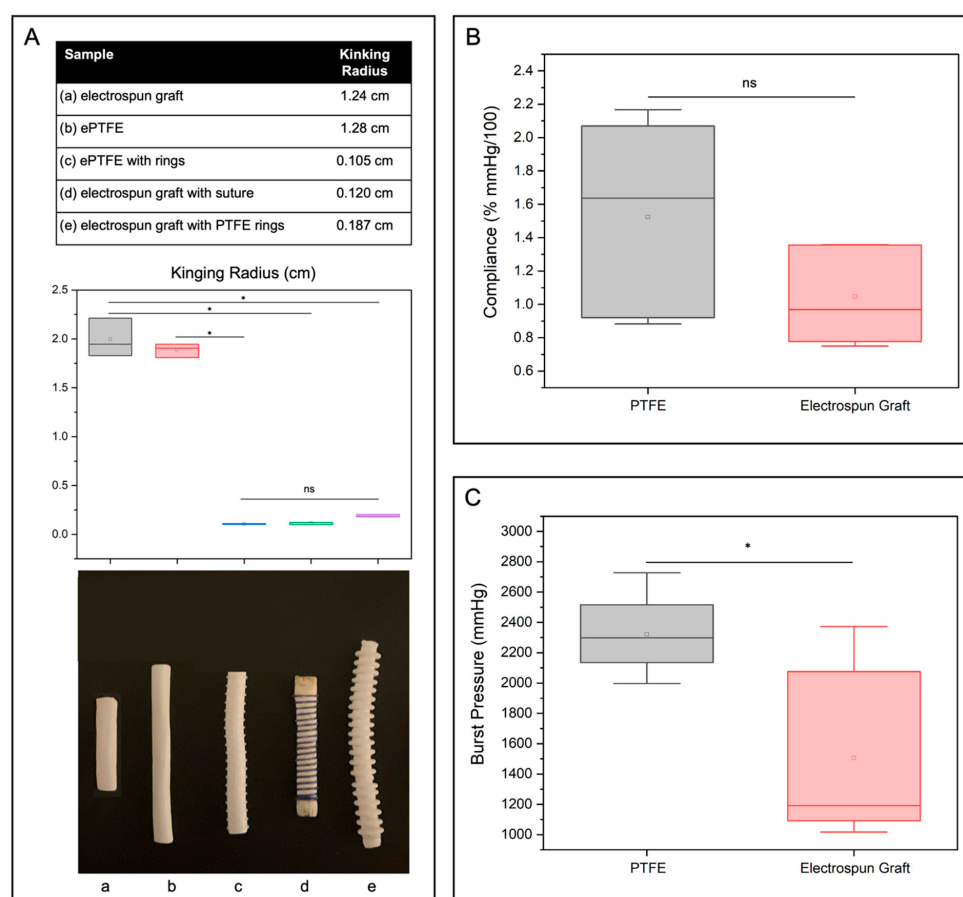


Figure 3. Mechanical testing. (A) Mean kink radii (top) and representative samples (bottom). (B) Compliance and (C) burst pressure. (*) statistical difference $p < 0.05$. (ns) not significant.

Compliance and burst pressure were assessed on the unreinforced electrospun graft and on PTFE to characterize the intrinsic material properties without the influence of external reinforcement. Compliance was calculated from pressure–diameter data between 80 and 120 mmHg using the standard formula. The compliance of the electrospun graft with rings was $1.04 \pm 0.29\%/100 \text{ mmHg}$, compared with $1.50 \pm 0.58\%/100 \text{ mmHg}$ for PTFE with rings (Figure 3B).

The burst pressure of the electrospun graft with rings was $1505 \pm 565 \text{ mmHg}$, while PTFE with rings exceeded the upper limit of the test apparatus ($>2000 \text{ mmHg}$) (Figure 3C). These values are within the physiological range required for surgical implantation and comparable to literature reports for native saphenous vein grafts [30].

Together, these data demonstrate that ring reinforcement significantly improved kink resistance of electrospun grafts while maintaining clinically relevant compliance and burst pressure suitable for vascular access applications.

3.2. Histological Evaluation

Histological analysis of explanted grafts using H&E and Masson's Trichrome staining revealed clear differences in cellular infiltration and tissue remodeling between experimental electrospun grafts and PTFE controls (Figure 4). For each graft type, three representative cross-sections from a single animal were selected, corresponding to proximal (arterial), mid-graft, and distal (venous) regions (left to right).

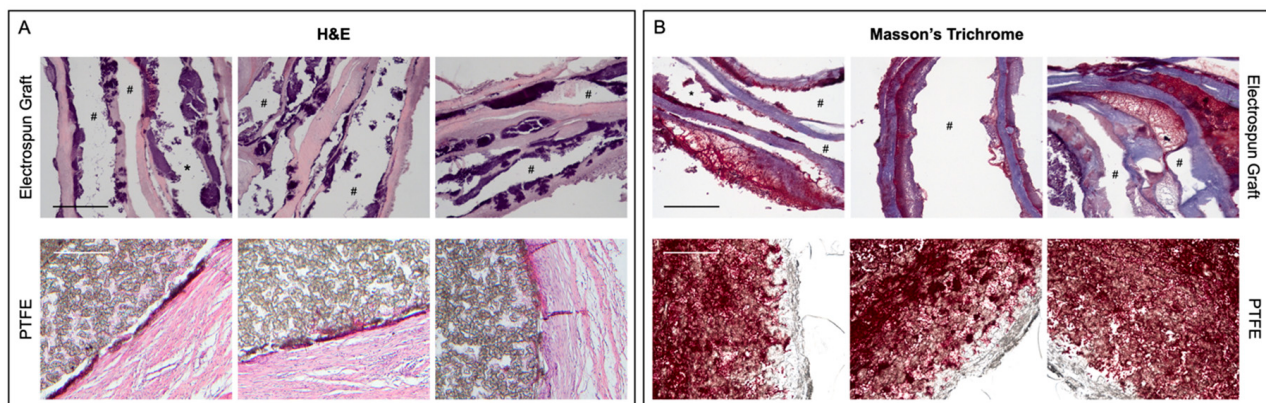


Figure 4. Histological evaluation of explanted vascular grafts. Representative cross-sections of electrospun grafts (**top row**) and PTFE grafts (**bottom row**) stained with H&E (**A**) and Masson's Trichrome (**B**) at $20\times$ magnification. For each graft type, three locations along the graft are shown from left to right: proximal (arterial) region, mid-graft, and distal (venous) region. Electrospun grafts display extensive cellular infiltration, collagen deposition (blue), and muscle-rich tissue (red), while PTFE grafts show minimal infiltration. (#) indicates tissue delamination during handling. Scale bar = $100 \mu\text{m}$.

High-magnification ($20\times$) images of electrospun grafts showed extensive host cell infiltration across all locations, with abundant nucleated cells visible throughout the porous scaffold on H&E staining. Masson's Trichrome staining demonstrated dense collagen deposition (blue) and muscle-rich regions (red), indicating active extracellular matrix remodeling and smooth muscle ingrowth. Regions marked with '#' represent tissue areas that delaminated during histological sectioning.

In contrast, PTFE grafts exhibited minimal cell infiltration across all three regions and showed a sharply defined boundary between the graft material and adjacent tissue, consistent with limited integration. No stenosis or thrombus formation was observed in electrospun grafts.

3.3. Immunofluorescence and Cell Counting

Immunofluorescence analysis revealed pronounced differences in cellularity and cell phenotype between the electrospun and PTFE grafts (Figure 5).

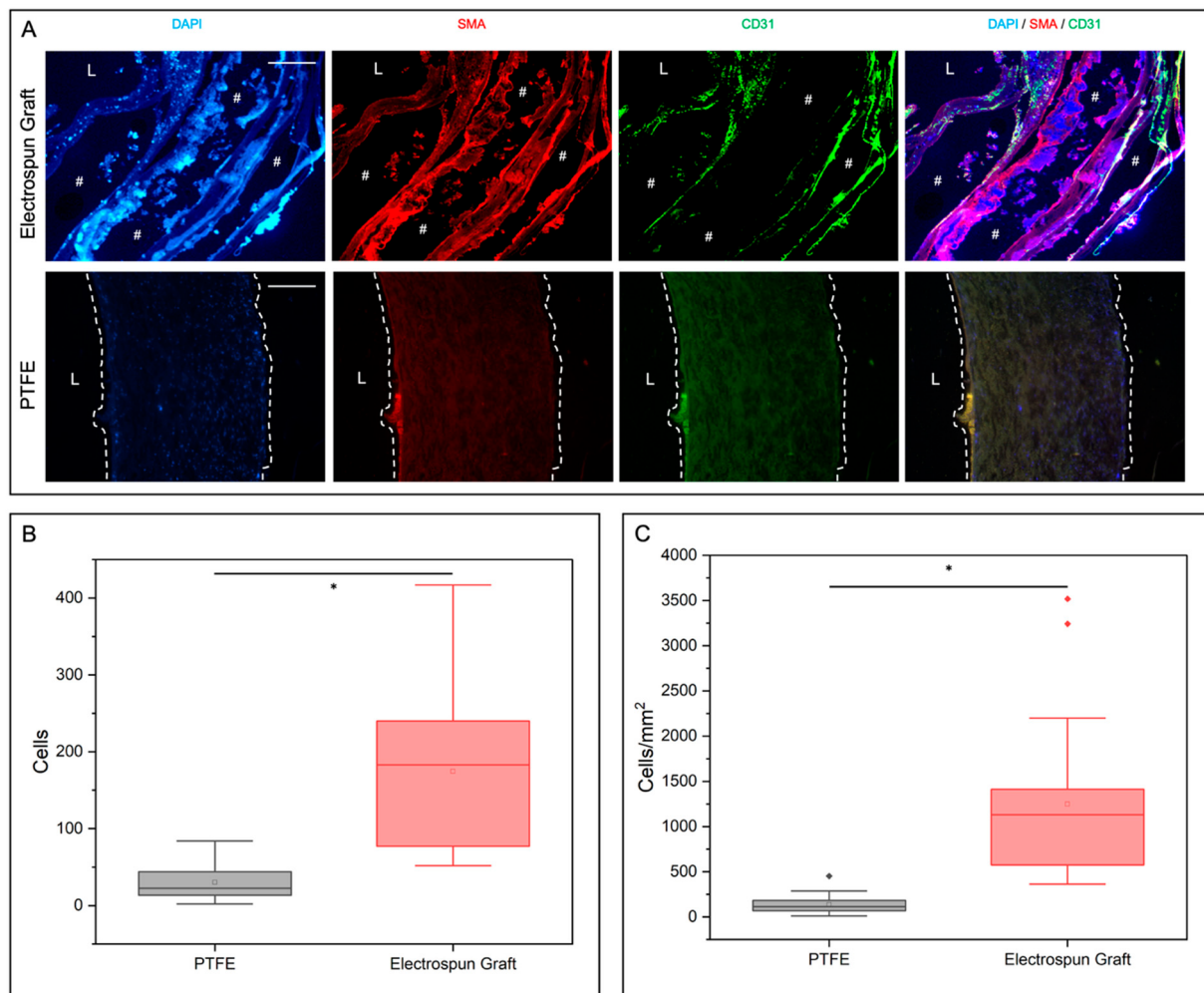


Figure 5. Immunofluorescence and quantitative analysis of cell infiltration in explanted grafts. (A) Representative images of electrospun grafts (top) and PTFE grafts (bottom) stained for nuclei (DAPI, blue), smooth muscle actin (SMA, red), and endothelial marker CD31 (green) at 10× magnification. Merged images highlight the spatial organization of infiltrating cells. Electrospun grafts demonstrated abundant nuclear staining with SMA⁺ infiltrating cells and CD31⁺ endothelial coverage, in contrast to the sparsely populated PTFE grafts. Regions marked with (#) indicate tissue delamination during handling; (L) denotes the luminal surface. The quantification confirms significantly greater DAPI nuclei counts in electrospun grafts versus PTFE controls. Both cell number per field (B) and cell number normalized by area (C) show statistically significant differences (*), $p < 0.05$. Scale bar = 100 μ m.

PTFE grafts exhibited weak DAPI signal, indicating low cellular density, and minimal expression of both smooth muscle α -actin (SMA) and CD31, suggesting a lack of vascular cell integration. In contrast, electrospun grafts showed intense, widespread nuclear staining throughout the graft wall, with distinct SMA⁺ regions corresponding to smooth muscle-like cell infiltration within the medial layer and CD31⁺ staining along the luminal surface, consistent with endothelial cell coverage. Quantitative analysis confirmed significantly greater DAPI⁺ cell number and cell density in electrospun grafts compared with PTFE controls ($p < 0.05$).

These findings corroborate histological evidence of collagen-rich, muscle-positive tissue remodeling in electrospun grafts, demonstrating that electrospun grafts support robust cellular infiltration and vascular-like phenotype development, unlike PTFE grafts, which remain largely acellular.

3.4. Multiphoton Imaging and ECM Quantification

Multiphoton imaging was performed to evaluate ECM composition and organization in the explanted grafts (Figure 6). In electrospun grafts, SHG imaging revealed dense, interwoven fibrillar collagen networks extending through the graft wall, while TPEF demonstrated elastin presence within both luminal and medial regions. Quantitative image analysis confirmed significantly higher collagen and elastin content in electrospun grafts compared to PTFE controls ($p < 0.05$).

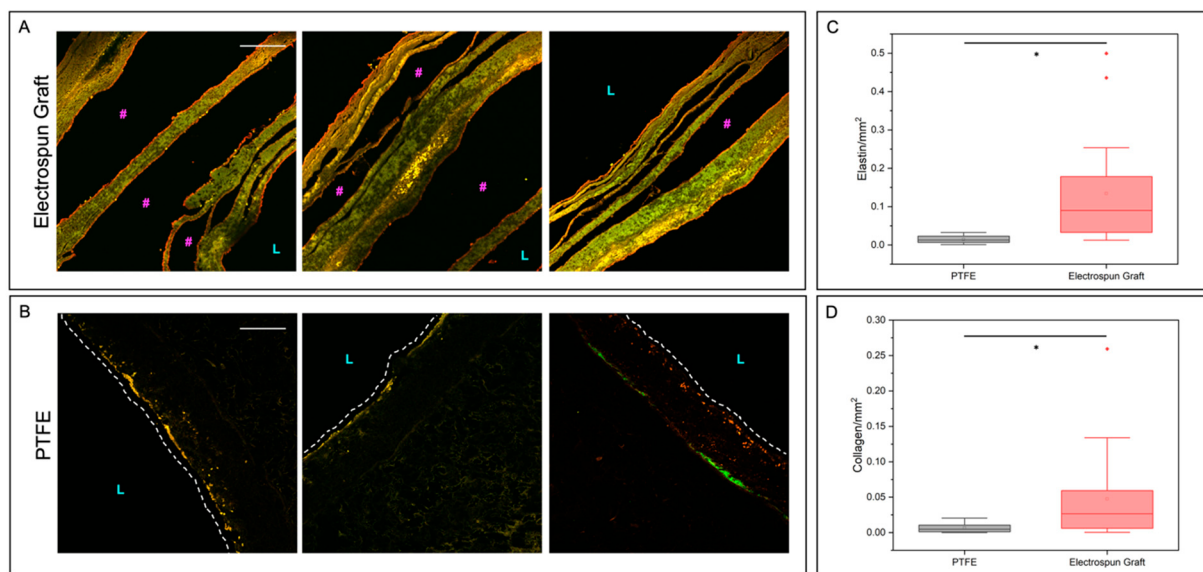


Figure 6. Multiphoton imaging of ECM remodeling in explanted grafts. (A,B) Representative multiphoton images of electrospun (A) and PTFE (B) grafts at 25 $\times$ magnification. Collagen and elastin were visualized using SHG (green) and TPEF (red), respectively. Each panel includes three representative images obtained from explants of three locations along the graft, shown from left to right: proximal (arterial) region, mid-graft, and distal (venous) region. “#” in magenta denotes delamination artifacts from sectioning; “L” in cyan indicates the luminal side of the graft. (C,D) Quantitative analysis of elastin (C) and collagen (D) densities per unit area, demonstrating significantly higher levels of both ECM components in electrospun grafts compared with PTFE controls, where limited collagen and elastin signals were restricted to the luminal surface. (*) statistical difference $p < 0.05$. Scale bar = 50 μm .

These two ECM components—key to vascular elasticity and strength in the native arterial architecture—were well organized, suggesting active, vascular-like matrix remodeling in electrospun grafts. In contrast, PTFE grafts displayed weak SHG and TPEF signals confined mainly to the luminal surface, consistent with limited host remodeling. Overall, these findings reinforce that electrospun grafts not only permit cellular infiltration but also drive the deposition and spatial organization of structural ECM proteins critical for long-term functional vascular integration.

4. Discussion

This study presents a reinforced, biofunctionalized TEVG evaluated in a porcine arteriovenous (AV) graft model, a platform that closely replicates the hemodynamic and

biological complexities of human dialysis access. Although small-diameter TEVGs have been widely investigated, most prior evaluations have relied on rodents [31,32] or ovine carotid interposition models [33,34] or on decellularized biological scaffolds [35,36] rather than fully synthetic materials designed for in situ regeneration. Large animal AV configurations, which are arguably the most clinically relevant context for dialysis access [23], remain notably underexplored. By implementing a porcine AV configuration, this study bridges a critical translational gap and provides one of the few demonstrations of functional host-driven tissue regeneration within a synthetic acellular scaffold exposed to dialysis-relevant high-flow and high-pressure conditions.

A central focus of this study was to engineer a circumferentially reinforced coaxial graft that enhances kink resistance without impeding biological integration. Reinforcement is essential for AV grafts, which undergo repeated bending, cannulation, and substantial pressure fluctuations. However, traditional reinforcement strategies, such as embedded rings or dense weaves, often obstruct cellular infiltration and exacerbate compliance mismatches [33]. Here, external discrete PTFE rings provided mechanical stability while maintaining open inter-ring regions that preserved scaffold porosity and permeability, allowing cell and matrix penetration for tissue integration. This configuration sustained burst pressure within the industry-acceptable range and markedly improved kink resistance, demonstrating that mechanical reinforcement and regenerative potential are not mutually exclusive design requirements. Rather, the findings underscore that reinforcement geometry and spatial distribution, rather than material stiffness alone, are decisive for balancing structural stability and biological performance.

A defining feature of our graft is its ability to promote in situ cellular infiltration and ECM formation without pre-seeding. The hybrid design, combining a durable PCL mechanical core with a PEG-NB sheath, was intended to pair long-term mechanical fidelity with a biofunctional, antifouling interface. PEG-NB chemistry enables incorporation of custom biological motifs while mitigating thrombus formation, whereas the PCL core provides structural stability as the scaffold remodels. The thiol–norbornene crosslinking of the 4-arm PEG-NB network allows stable covalent attachment of RGD and VEGF-mimetic peptides, which provide localized biochemical cues that enhance endothelial and smooth muscle cell infiltration and support tissue integration. Consistent with these design goals, the explanted grafts exhibited substantial host cell infiltration, including α -SMA⁺ smooth muscle-like cells and CD31⁺ endothelial cells, distributed across the graft wall. In contrast, PTFE grafts remained largely acellular and demarcated from surrounding tissue, reinforcing the importance of scaffold chemistry and microarchitecture in directing host response.

Quantitative analyses corroborated these qualitative observations and further revealed a significant increase in total cell density and ECM deposition in coaxial graft compared with ePTFE controls. Multiphoton imaging further identified collagen and elastin fibers interwoven throughout the graft wall, indicating early organization of neotissue resembling native vascular structure. These data confirm that the TEVG is not merely infiltrated, but actively remodeled by host cells, supporting synthesis of structural ECM components critical for long-term mechanical maturation.

The porcine AV graft model was deliberately selected because it more closely mimics human vascular access in vessel caliber, flow rate, coagulation physiology, and hemodynamic stress, compared to other models [37,38]. The observed extensive neotissue formation under standard antiplatelet therapy after a four-week timepoint underscore the hemocompatibility of the PEG-NB-functionalized surface and the regenerative potential of the coaxial architecture. The absence of early thrombosis or perianastomotic stenosis suggests that the graft's antifouling and bioactive features act synergistically to facilitate early integration.

Despite these encouraging findings, several limitations warrant consideration. First, one graft occluded prior to Day 21, and although all grafts remained pulsatile at Day 21, two of the remaining three were occluded by Day 28, reflecting short-term patency challenges that require further optimization. The four-week implantation period provides only a snapshot of early remodeling, limiting assessment of long-term patency, material degradation, and tissue maturation. Additional extended-duration studies will be essential to evaluate whether early ECM deposition and endothelialization progress toward stable, functional neovessels. Second, although the burst pressure and kink resistance met industrial requirements, the graft's compliance remained below that of native vessels or commercial ePTFE grafts, suggesting room to refine fiber alignment, wall thickness, or multilayer design to better match native biomechanics. Third, the sample size was modest, and procedural success rates were influenced by the technical complexity of porcine AV graft implantation, which may introduce variability in local hemodynamics and healing responses. Lastly, only one reinforcement geometry and one biofunctional formulation were evaluated. Systematic exploration of ring spacing, wall architecture, and ligand density could enhance the interplay between mechanical integrity and biological integration.

A clinically significant finding of this study is that a substantial proportion of grafts occluded within 28 days, including one early occlusion at 18 days. For hemodialysis vascular access, patency is the primary determinant of clinical utility, and these occlusions therefore represent a critical outcome rather than a minor technical limitation. The porcine AV configuration subjects grafts to an extreme hemodynamic environment, characterized by high volumetric flow, elevated wall shear stress, and complex, turbulent flow at the anastomotic regions, all of which are known to accelerate thrombosis and neointimal hyperplasia in both experimental and clinical settings. In this context, the observed occlusions underscore both the stringency of the model and the challenges inherent to developing durable AV grafts, even with advanced biofunctional and mechanically optimized designs. While commercial ePTFE grafts also exhibit high early failure rates, these findings highlight the need for further refinement of graft architecture, surface bioactivity, and reinforcement strategy, as well as longer-term evaluation, to improve patency under clinically relevant loading conditions.

In addition, although the porcine AV model provides a highly relevant anatomical and hemodynamic environment for vascular access research, important physiological and healing differences exist between pigs and human hemodialysis patients. Species-specific variations in immune response, vascular remodeling kinetics, and coagulation may influence graft integration and patency. Moreover, patients with end-stage kidney disease commonly present with comorbidities such as uremia, diabetes, and vascular calcification, as well as repeated cannulation injury, all of which can substantially alter inflammatory responses, endothelialization, and long-term tissue remodeling. Consequently, the extent of cellular infiltration and extracellular matrix deposition observed in this model may not directly predict clinical outcomes. These factors represent an important limitation for direct translation and motivate future studies incorporating longer implantation periods and disease-relevant or chronic-use models.

Overall, this study demonstrates that mechanical reinforcement and regenerative performance can be successfully integrated within a single scaffold architecture. The externally reinforced coaxial TEVG maintained mechanical integrity under physiological loading while enabling host-driven regeneration characterized by endothelialization, smooth muscle infiltration, and ECM deposition. These findings establish a foundation for longer-term studies to investigate sustained patency, progressive remodeling, and material degradation. Further optimization of the reinforcement pattern and biofunctionalization chemistry may enhance compliance matching and accelerate vascular maturation.

Collectively, this work provides compelling evidence that rationally engineered electrospun grafts with biofunctional interfaces can overcome the dual mechanical and biological limitations of conventional synthetic vascular grafts, advancing the field toward durable, regenerative solutions for hemodialysis access.

5. Conclusions

This study demonstrates the successful development and evaluation of a biofunctional, mechanically reinforced electrospun vascular graft designed for hemodialysis access applications. The grafts supported robust host integration, characterized by cellular infiltration, endothelial coverage, and ECM deposition of both collagen and elastin—hallmarks of early vascular remodeling. Compared with commercial PTFE grafts, the electrospun scaffolds exhibited superior tissue ingrowth, reduced acellularity, and clear evidence of vascular-like neotissue formation, highlighting their potential to improve long-term patency.

Future efforts will focus on extended-duration preclinical studies to evaluate graft durability, remodeling kinetics, and sustained patency, alongside comprehensive mechanical characterization under cyclic loading and fatigue. Parallel optimization of scaffold design, including multilayer structures, tunable reinforcement spacing, and targeted bioactive coatings, will aim to refine the balance between mechanical resilience and biological performance. Together, these efforts will advance the translational readiness of electrospun grafts and lay the groundwork for their eventual clinical application as next-generation vascular access grafts capable of uniting structural durability with regenerative healing potential.

Author Contributions: Conceptualization, W.T. and P.R.-C.; methodology, A.B., U.U., and C.W.; software, A.B., M.L., and M.O.; validation, A.B., W.T., P.R.-C., and U.U.; formal analysis, A.B., M.L., and M.O.; investigation, A.B., U.U., C.W.; resources, W.T. and P.R.-C.; data curation, A.B., M.L., M.O., and C.W.; writing—original draft preparation, A.B., U.U., and C.W.; writing—review and editing, W.T., P.R.-C., and G.X.; visualization, A.B.; supervision, W.T. and P.R.-C.; project administration, W.T.; funding acquisition, W.T. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the National Institutes of Health (Grant: R01 HL119371) and the Microscope Core Facility (Faculty Voucher Program) at the University of Colorado–Boulder.

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of University of North Carolina at Chapel Hill, Institutional Animal Care and Use Committee (IACUC) (protocol code ID 25-047.0 and 28 March 2025).

Informed Consent Statement: Not applicable.

Data Availability Statement: The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding author.

Acknowledgments: This work was supported by the National Institutes of Health and the Microscope Core Facility (Faculty Voucher Program) at the University of Colorado Boulder. The authors thank the anonymous reviewers for their thoughtful feedback, which helped improve the quality of this manuscript. We are grateful to the veterinary staff at the University of North Carolina for their care and support during the in vivo studies. The authors also acknowledge the animals used in this research.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Hoogeveen, E.K. The Epidemiology of Diabetic Kidney Disease. *Kidney Dial.* **2022**, *2*, 433–442. [CrossRef]
2. Johansen, K.L.; Chertow, G.M.; Gilbertson, D.T.; Ishani, A.; Israni, A.; Ku, E.; Li, S.; Li, S.; Liu, J.; Obrador, G.T.; et al. US Renal Data System 2022 Annual Data Report: Epidemiology of Kidney Disease in the United States. *Am. J. Kidney Dis.* **2023**, *81*, A8–A11. [CrossRef]

3. Lok, C.E.; Huber, T.S.; Orchanian-Cheff, A.; Rajan, D.K. Arteriovenous Access for Hemodialysis: A Review. *JAMA* **2024**, *331*, 1307. [\[CrossRef\]](#)
4. Roy-Chaudhury, P.; Arend, L.; Zhang, J.; Krishnamoorthy, M.; Wang, Y.; Banerjee, R.; Samaha, A.; Munda, R. Neointimal Hyperplasia in Early Arteriovenous Fistula Failure. *Am. J. Kidney Dis.* **2007**, *50*, 782–790. [\[CrossRef\]](#)
5. Luo, Y.; Bian, X.; Gu, C.; Wu, X.; Bai, L.; Qing, J.; Ye, H.; Dai, C. Impact of Evolving Strategies for Arteriovenous Graft Creation and Management on Patency Outcomes. *Ren. Fail.* **2025**, *47*, 2549776. [\[CrossRef\]](#)
6. Neyra, N.R.; Wazir, S. The Evolving Panorama of Vascular Access in the 21st Century. *Front. Nephrol.* **2022**, *2*, 917265. [\[CrossRef\]](#)
7. Battistella, A.; Linger, M.; Nguyen, A.T.; Madukwe, D.; Roy-Chaudhury, P.; Tan, W. Rebuilding Vascular Access: From the Viewpoint of Mechanics and Materials. *Front. Bioeng. Biotechnol.* **2024**, *12*, 1448186. [\[CrossRef\]](#) [\[PubMed\]](#)
8. Besseling, P.J.; Szymczyk, W.; Teraa, M.; Toorop, R.J.; Bartels, P.A.A.; Arts, B.; Driessen, R.C.H.; Lichauco, A.M.; Bakker, H.C.; Fledderus, J.O.; et al. Evaluation of in Situ Tissue-Engineered Arteriovenous Grafts Suitable for Cannulation in a Large Animal Model. *Commun. Mater.* **2025**, *6*, 151. [\[CrossRef\]](#) [\[PubMed\]](#)
9. Rotmans, J.I.; Velema, E.; Verhagen, H.J.M.; Blankensteijn, J.D.; Kastelein, J.J.P.; De Kleijn, D.P.V.; Yo, M.; Pasterkamp, G.; Stroes, E.S.G. Rapid, Arteriovenous Graft Failure Due to Intimal Hyperplasia: A Porcine, Bilateral, Carotid Arteriovenous Graft Model. *J. Surg. Res.* **2003**, *113*, 161–171. [\[CrossRef\]](#) [\[PubMed\]](#)
10. Gupta, P.; Mandal, B.B. Tissue-Engineered Vascular Grafts: Emerging Trends and Technologies. *Adv. Funct. Mater.* **2021**, *31*, 2100027. [\[CrossRef\]](#)
11. Jouda, H.; Larrea Murillo, L.; Wang, T. Current Progress in Vascular Engineering and Its Clinical Applications. *Cells* **2022**, *11*, 493. [\[CrossRef\]](#) [\[PubMed\]](#)
12. Rajab, T.K.; O'Malley, T.J.; Tchantchaleishvili, V. Decellularized Scaffolds for Tissue Engineering: Current Status and Future Perspective. *Artif. Organs* **2020**, *44*, 1031–1043. [\[CrossRef\]](#)
13. Sun, J.; Yang, M.; Zhou, J.; Jiang, N.; Chen, H. Microstructural Morphology and Mechanical Analysis of Decellularized Ligament Scaffolds. *Eur. Cell Mater.* **2025**, *53*, 28–40. [\[CrossRef\]](#)
14. Lawson, J.H.; Glickman, M.H.; Ilzecki, M.; Jakimowicz, T.; Jaroszynski, A.; Peden, E.K.; Pilgrim, A.J.; Prichard, H.L.; Guzewicz, M.; Przywara, S.; et al. Bioengineered Human Acellular Vessels for Dialysis Access in Patients with End-Stage Renal Disease: Two Phase 2 Single-Arm Trials. *Lancet* **2016**, *387*, 2026–2034. [\[CrossRef\]](#)
15. Zhang, F.; King, M.W. Biodegradable Polymers as the Pivotal Player in the Design of Tissue Engineering Scaffolds. *Adv. Healthc. Mater.* **2020**, *9*, 1901358. [\[CrossRef\]](#)
16. Xu, Y.; Shi, G.; Tang, J.; Cheng, R.; Shen, X.; Gu, Y.; Wu, L.; Xi, K.; Zhao, Y.; Cui, W.; et al. ECM-Inspired Micro/Nanofibers for Modulating Cell Function and Tissue Generation. *Sci. Adv.* **2020**, *6*, eabc2036. [\[CrossRef\]](#)
17. Zhong, S.; Zhang, Y.; Lim, C.T. Fabrication of Large Pores in Electrospun Nanofibrous Scaffolds for Cellular Infiltration: A Review. *Tissue Eng. Part B Rev.* **2012**, *18*, 77–87. [\[CrossRef\]](#)
18. Chen, Y.; Dong, X.; Shafiq, M.; Myles, G.; Radacsi, N.; Mo, X. Recent Advancements on Three-Dimensional Electrospun Nanofiber Scaffolds for Tissue Engineering. *Adv. Fiber Mater.* **2022**, *4*, 959–986. [\[CrossRef\]](#)
19. Kalva, S.N.; Zakaria, Y.; Velasquez, C.A.; Koç, M. Tailoring the Mechanical and Degradation Properties of 3DP PLA/PCL Scaffolds for Biomedical Applications. *Rev. Adv. Mater. Sci.* **2025**, *64*, 20250098. [\[CrossRef\]](#)
20. Lin, P.-H.; Li, B.-R. Antifouling Strategies in Advanced Electrochemical Sensors and Biosensors. *Analyst* **2020**, *145*, 1110–1120. [\[CrossRef\]](#)
21. Jivan, F.; Fabela, N.; Davis, Z.; Alge, D.L. Orthogonal Click Reactions Enable the Synthesis of ECM-Mimetic PEG Hydrogels without Multi-Arm Precursors. *J. Mater. Chem. B* **2018**, *6*, 4929–4936. [\[CrossRef\]](#) [\[PubMed\]](#)
22. Noel, S.; Fortier, C.; Murschel, F.; Belzil, A.; Gaudet, G.; Jolicoeur, M.; De Crescenzo, G. Co-Immobilization of Adhesive Peptides and VEGF within a Dextran-Based Coating for Vascular Applications. *Acta Biomater.* **2016**, *37*, 69–82. [\[CrossRef\]](#) [\[PubMed\]](#)
23. Fang, S.; Ellman, D.G.; Andersen, D.C. Review: Tissue Engineering of Small-Diameter Vascular Grafts and Their In Vivo Evaluation in Large Animals and Humans. *Cells* **2021**, *10*, 713. [\[CrossRef\]](#)
24. Iglesias-Echevarria, M.; Johnson, R.; Rafuse, M.; Ding, Y.; Tan, W. Vascular Grafts with Tailored Stiffness and a Ligand Environment via Multiarmed Polymer Sheath for Expedient Regeneration. *ACS Appl. Bio Mater.* **2021**, *4*, 545–558. [\[CrossRef\]](#)
25. Battistella, A.; Linger, M.; Johnson, R.D.; Sallee, A.; Jain, R.; Antreasian, B.; Ding, Y.; Tan, W. Fabrication of Polymer Blend Vascular Grafts with Enhanced Mechanical Properties and Rapid Cell Infiltration: Influence of Micro/Nanostructure, Polymer Composition, and Post-Processing on Pore Architecture And Bioengineered Environment. *Biomed. Mater.* **2025**, *20*, 065019. [\[CrossRef\]](#)
26. Krammer, J.; Pichlmaier, M.; Stana, J.; Hagl, C.; Peterss, S.; Grab, M.; Grefen, L. Multi-Layered Electrospun Grafts for Surgical Repair: Biomimicking Physiological Ascending Aortic Compliance. *J. Appl. Biomater. Funct. Mater.* **2025**, *23*, 22808000251316728. [\[CrossRef\]](#) [\[PubMed\]](#)
27. Robinson, A.; Duran, J.S.H.; Jiang, D.; Leung, J.; Laude, M.; Nkansah, A.; Guo, L.; Timmins, L.; Cosgriff-Hernandez, E. Design of a Corrugated Vascular Graft with Enhanced Compliance and Kink Resistance. *J. Biomed. Mater. Res.* **2025**, *113*, e37975. [\[CrossRef\]](#)

28. ISO 7198:2016; Cardiovascular implants and extracorporeal systems—Vascular prostheses—Tubular vascular grafts and vascular patches. International Organization for Standardization (ISO): Geneva, Switzerland, 2016.
29. Rafuse, M.; Xu, X.; Stenmark, K.; Neu, C.P.; Yin, X.; Tan, W. Layer-Specific Arterial Micromechanics and Microstructure: Influences of Age, Anatomical Location, and Processing Technique. *J. Biomech.* **2019**, *88*, 113–121. [[CrossRef](#)]
30. Nezarati, R.M.; Eifert, M.B.; Dempsey, D.K.; Cosgriff-Hernandez, E. Electrospun Vascular Grafts with Improved Compliance Matching to Native Vessels. *J. Biomed. Mater. Res.* **2015**, *103*, 313–323. [[CrossRef](#)]
31. Gui, L.; Muto, A.; Chan, S.A.; Breuer, C.K.; Niklason, L.E. Development of Decellularized Human Umbilical Arteries as Small-Diameter Vascular Grafts. *Tissue Eng. Part A* **2009**, *15*, 2665–2676. [[CrossRef](#)]
32. Caldiroli, A.; Pederzani, E.; Pezzotta, M.; Azzollini, N.; Fiori, S.; Tironi, M.; Rizzo, P.; Sangalli, F.; Figliuzzi, M.; Fiore, G.B.; et al. Hybrid Fibroin/Polyurethane Small-Diameter Vascular Grafts: From Fabrication to in Vivo Preliminary Assessment. *Biomed. Mater.* **2022**, *17*, 055015. [[CrossRef](#)]
33. Matsushita, H.; Hayashi, H.; Nurminsky, K.; Dunn, T.; He, Y.; Pitaktong, I.; Koda, Y.; Xu, S.; Nguyen, V.; Inoue, T.; et al. Novel Reinforcement of Corrugated Nanofiber Tissue-Engineered Vascular Graft to Prevent Aneurysm Formation for Arteriovenous Shunts in an Ovine Model. *JVS-Vasc. Sci.* **2022**, *3*, 182–191. [[CrossRef](#)]
34. Ong, C.S.; Fukunishi, T.; Liu, R.H.; Nelson, K.; Zhang, H.; Wiecek, E.; Palmieri, M.; Ueyama, Y.; Ferris, E.; Geist, G.E.; et al. Bilateral Arteriovenous Shunts as a Method for Evaluating Tissue-Engineered Vascular Grafts in Large Animal Models. *Tissue Eng. Part C Methods* **2017**, *23*, 728–735. [[CrossRef](#)] [[PubMed](#)]
35. Chlupac, J.; Frank, J.; Sedmera, D.; Fabian, O.; Simunkova, Z.; Mrazova, I.; Novak, T.; Vanourková, Z.; Benada, O.; Pulda, Z.; et al. External Support of Autologous Internal Jugular Vein Grafts with FRAME Mesh in a Porcine Carotid Artery Model. *Biomedicines* **2024**, *12*, 1335. [[CrossRef](#)] [[PubMed](#)]
36. Aung, O.; Rossi, P.J.; Dyer, M.R.; Stellpflug, A.; Zhai, Y.; Kenneth, A.; Wang, X.; Chang, J.; Chen, Y.; Tefft, B.; et al. Biofabrication of Small Vascular Graft with Acellular Human Amniotic Membrane: A Proof-of-Concept Study in Pig. *Biofabrication* **2024**, *17*, 035003. [[CrossRef](#)] [[PubMed](#)]
37. Ueberrueck, T.; Tautenhahn, J.; Meyer, L.; Kaufmann, O.; Lippert, H.; Gasteringer, I.; Wahlers, T. Comparison of the Ovine and Porcine Animal Models for Biocompatibility Testing of Vascular Prostheses. *J. Surg. Res.* **2005**, *124*, 305–311. [[CrossRef](#)]
38. Vilahur, G.; Padro, T.; Badimon, L. Atherosclerosis and Thrombosis: Insights from Large Animal Models. *BioMed Res. Int.* **2011**, *2011*, 907575. [[CrossRef](#)]

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.