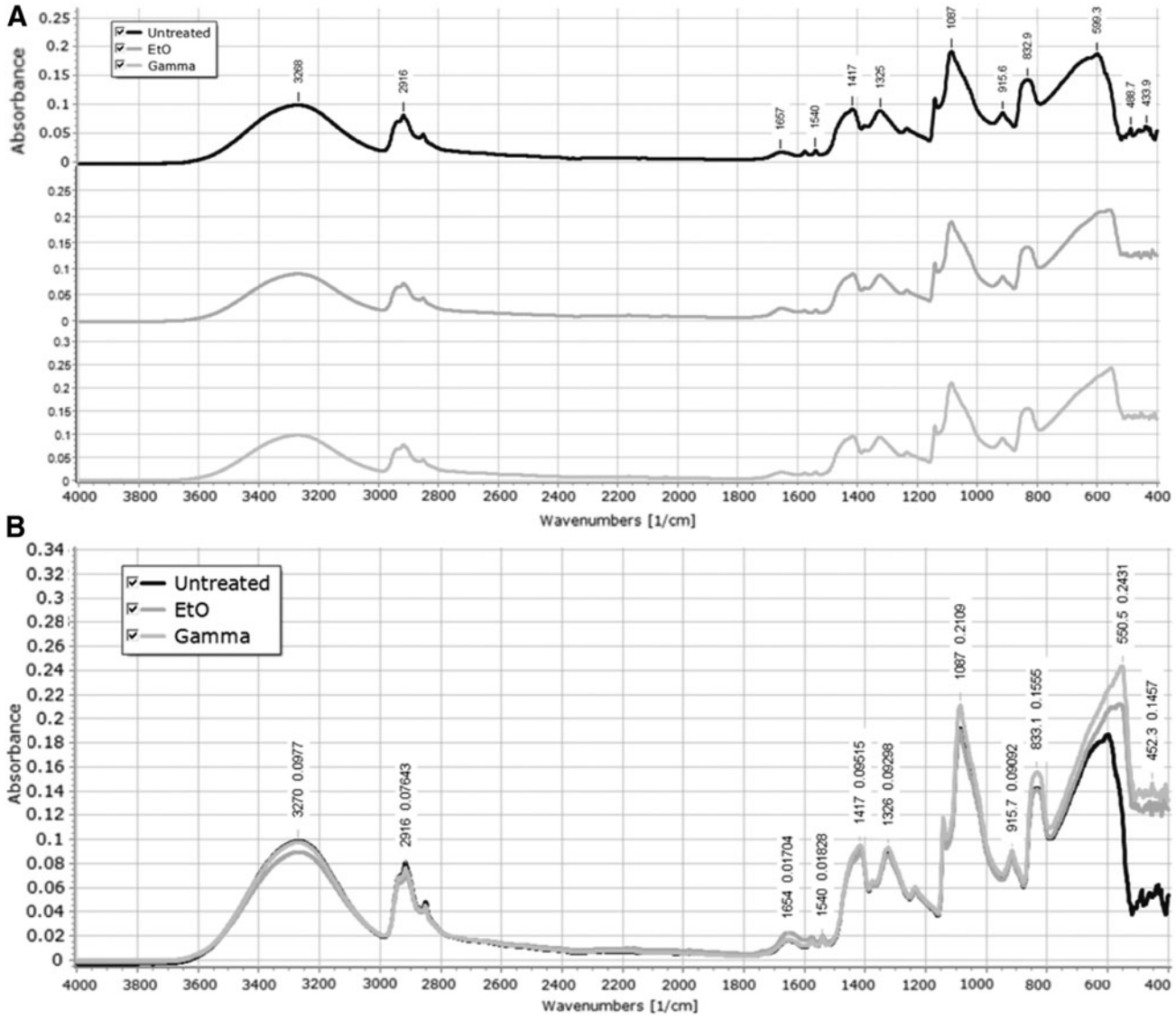
Supplementary Data

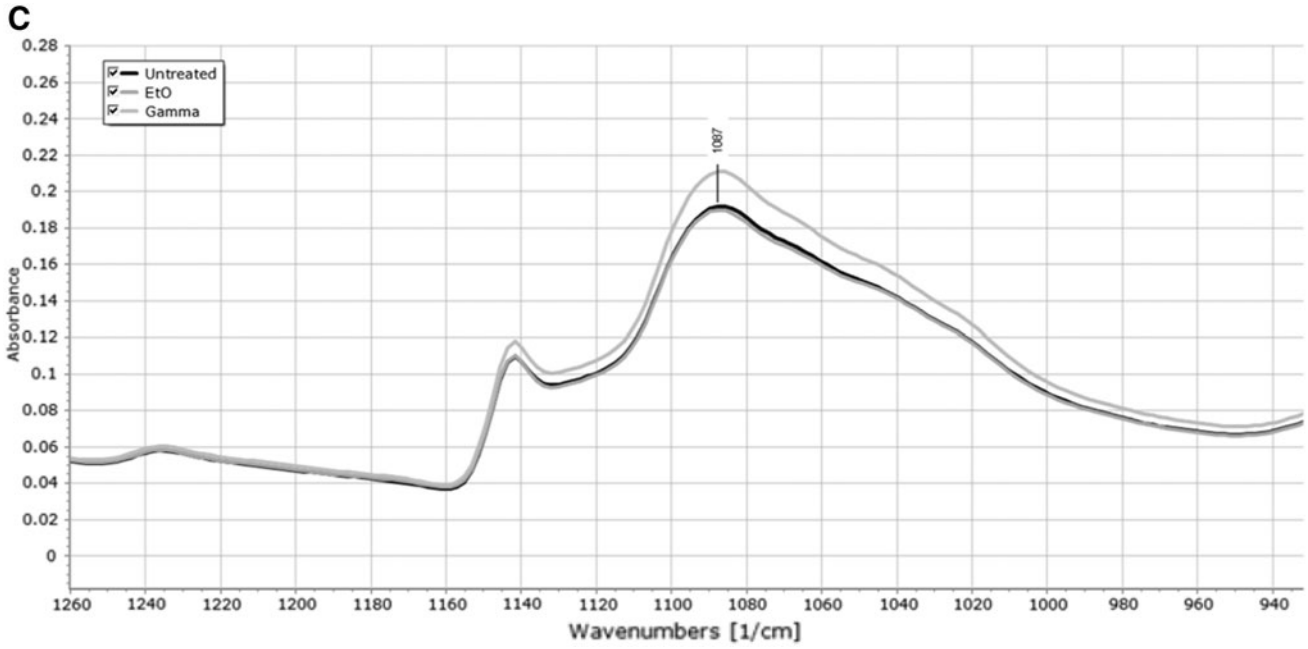
Detailed Materials and Methods

Autoclaving of polyvinyl alcohol solution and crosslinking of polyvinyl alcohol vascular grafts and films

The polyvinyl alcohol (PVA) graft and film fabrications were performed as previously published.[S1] A solution of 10% (w/v) PVA (MW 85,000–124,000 g/mol, 87–89% [w/w] hydrolyzed; Sigma-Aldrich) was autoclaved at 121°C for 20 min as an initial sterilization step and to help in the dissolution of PVA powder in deionized (DI) water, ensure a homogenous solution, and prevent fabrication inconsistencies due to solution inhomogeneity, as is commonly done.[S2–S5] This is not considered part of sterilization because the subsequent solution crosslinking and graft fabrication were performed in a nonsterile environment. All sample groups were made from the same batch of autoclaved 10% (w/v) PVA solution; the only difference between the groups was sterilization treatment, all else being equal. This 10% (w/v) PVA solution was crosslinked using a 15% (w/v) trisodium trimetaphosphate (STMP) (Sigma-Aldrich) and 30% (w/v) sodium hydroxide (NaOH) (Sigma-Aldrich). Cylindrical glass molds, ~1.5 mm in diameter, were air plasma treated at 0.8 Nl h^{-1} and 85 W for 40 s. The

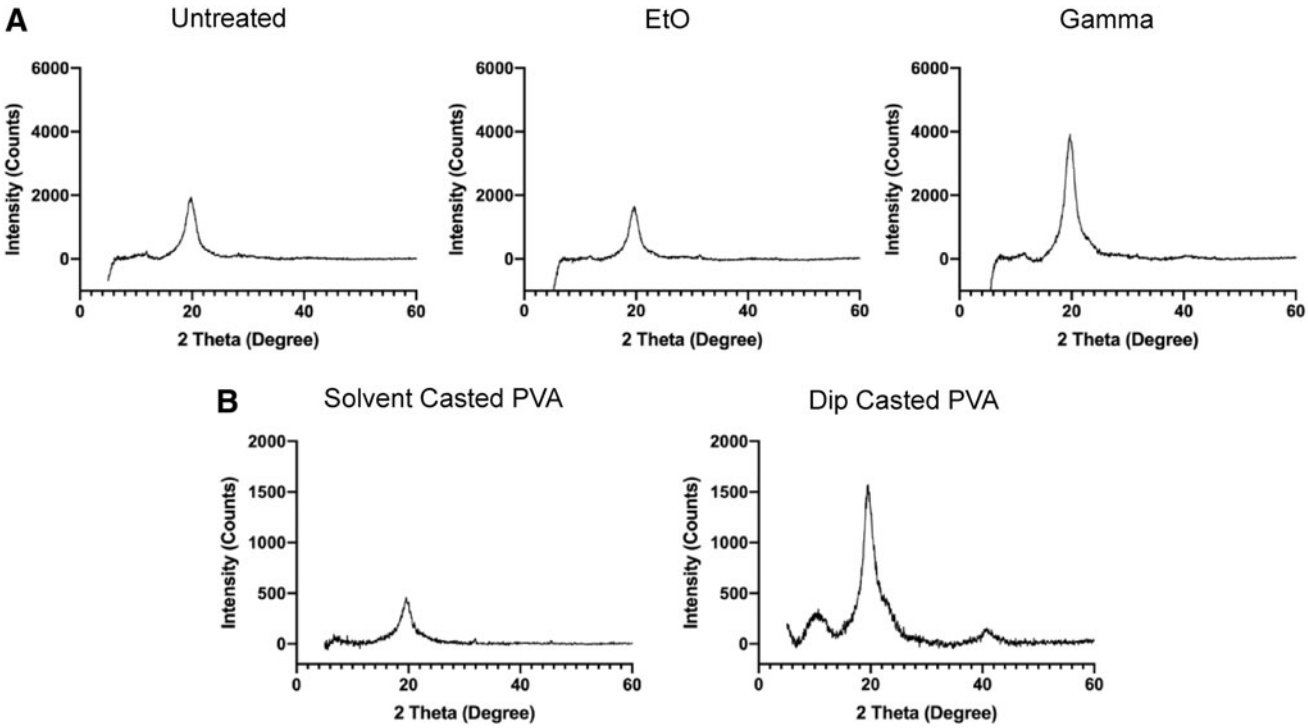


SUPPLEMENTARY FIG. S1. FTIR **(A)** comparison of the spectra indicates that no peaks were formed or lost after either ethylene oxide treatment or γ irradiation and **(B)** overlay of spectra indicates no significant differences in peak heights. **(C)** Overlaid spectra between the ranges of 1200–1000 cm^{-1} indicate slight shifts between treatments, but no statistically significant differences between peak absorbances. The average of $n = 3$ spectra is shown here. FTIR, Fourier-transform infrared spectroscopy.

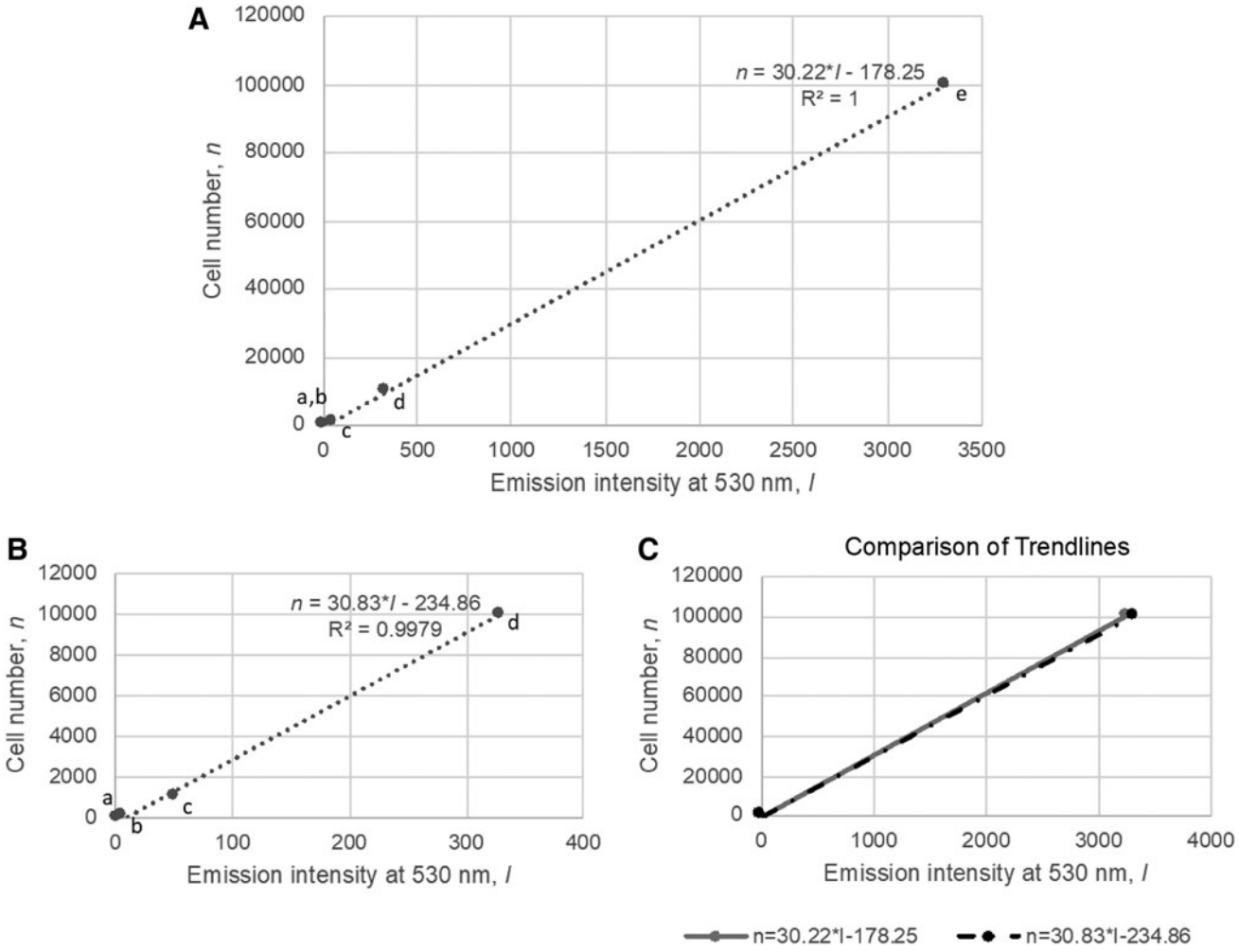


SUPPLEMENTARY FIG. S1. (*Continued*).

molds were then coated in layers of crosslinking PVA solution. Two graft thicknesses were made: a thin-walled graft (1.5 mm, thin) and a thick-walled graft (1.5 mm, thick). Similarly, 4-mm grafts were made using plastic cylindrical molds coated with polydimethylsiloxane (PDMS, 4 mm diameter). The molds were plasma treated as before but for a total duration of 1 min 20 s. To fabricate PVA films, ~1.3 g of the crosslinking PVA solution was poured into polystyrene dishes. PDMS molds were used to replicate micropatterns on PVA as previously reported.[S1] The crosslinking PVA on the cylindrical molds and in the dishes was air-dried for 3 and 10 days, respectively, at 20°C, 70% humidity. PVA grafts were demolded using 10×phosphate-buffered solution (PBS) (11.9 mN phosphates, 137 mM sodium chloride, and 2.7 mM potassium chloride, pH 7.4; Thermo Fisher), 1×PBS, and deionized



SUPPLEMENTARY FIG. S2. X-ray powder diffraction spectra of **(A)** solvent cast PVA samples: untreated, EtO, and γ-treated PVA, and **(B)** comparison between solvent cast and dip cast PVA films.



SUPPLEMENTARY FIG. S3. **(A)** Standard curve specific to EA.hy926 cells for cell number quantification using CyQUANT® Cell Proliferation Assay Kit. **(B)** Expanded lower region of the standard curve between cell numbers 0 and 10,000 with the adjusted trend line. **(C)** Comparison of the two trend lines in **(A, B)**.

water, sequentially. Samples were randomly assigned to treatment groups [untreated, EtO, gamma(γ)] and were washed in DI for 24 h and then stored dry or hydrated depending on their treatment group. Untreated and γ-irradiated samples were stored hydrated and EtO samples were stored dry.

Sterilization processes

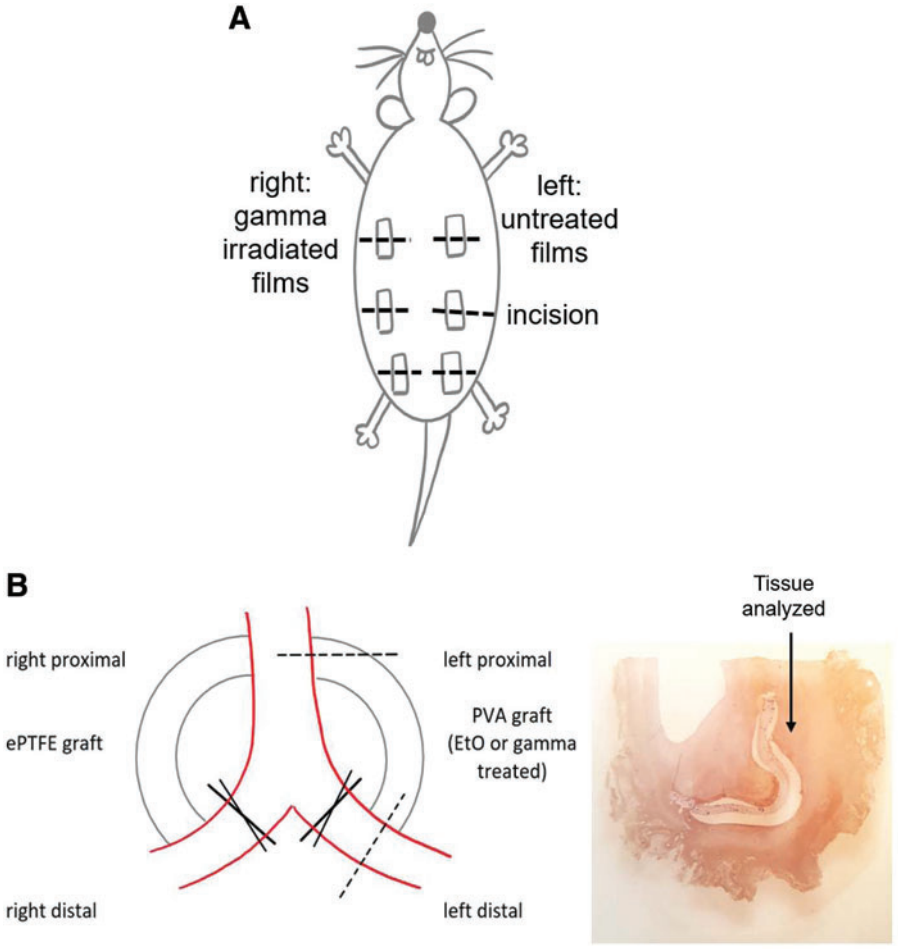
The untreated group samples were not treated with any sterilization process and were stored in DI water after fabrication. The EtO group samples were fully dried and sterilized by exposure to ethylene oxide gas (3M™ 8XL). The samples were preconditioned for 90 min at 55°C, 70% relative humidity. Samples were then sterilized for 60 min with an EtO concentration 759 mg L^{-1} at pressure 400–650 mBar (depending on load density). Pump down and purging were done for 60 min and the samples were aerated at 55°C for 12 h. The γ-irradiated group was treated while it was completely hydrated in DI water. Samples were sterilized with a Co-60 source (G.C. 220) at dose 25 kGy. The dose rate was calculated based on the half-life of the Co-60 source and the sterilization process was $\sim$3790 min.

Swelling and water content testing

Fully hydrated grafts from all treatment groups were cut into 1 cm length. Excess water was removed before weighing the graft to get graft initial weight, w_0. Grafts were dried in a 60°C oven until there was no further weight reduction. Typically, a 24-h duration was sufficient for complete dehydration. The dry weight of the grafts, w_d, was then measured. Water content percentage was calculated according to the following formula: $(w_0 - w_d)/w_0 \times 100\%$. The fully dried grafts were then soaked in DI water for 24 h to examine swelling percentage. After the grafts were fully hydrated, each graft was dabbed dry and weighed to get hydrated graft weight, w_h. Swelling percentage was calculated using the following formula: $(w_h - w_d)/w_d \times 100\%$.

Compliance testing

The radial compliance test method was adopted from Refs.[S6,S7] Grafts were sealed at one end and at the other end an open-end tip connected to a bag of 0.9% (w/v) saline solution was inserted. Saline solution at 80 and 120 mmHg was supplied to the graft and the change in graft diameter at three



SUPPLEMENTARY FIG. S4. **(A)** Schematic of the subcutaneous implantation of PVA films in the ventral side of a rat model. Each film was implanted below the wound. **(B)** Schematic of PVA graft implant at baboon abdominal aorta iliac bifurcation.[S5] PVA, polyvinyl alcohol.

different locations along each graft was tracked and subsequently measured with ImageJ. Compliance was calculated with the following formula: $\%C_{rad} = (d_{120} - d_{80})/d_{80} \times 100\%$. Compliance is reported here with the units of %/40 mmHg.

Uniaxial tensile testing

Tensile testing was performed using a Universal Mechanical Tester (UMT) (UNMT-2MT, T1377; Center for Tribology, Inc.) outfitted with a 100-kg load cell and an extension rate of 10 mm/min. To ensure adequate gripping, the ends of samples were dried and wrapped in tape, while the middle of the grafts (2.0 cm gauge length) was kept hydrated.

Minimum bend radius testing

Concentric circles with outer diameters 1 to 16 cm (1 cm increments) were used to measure the minimum bend radius. Starting with the smallest circle, a 1.5-mm-diameter, 3-cm-long graft was wrapped around the circle. If a kink was present, the next largest circle size was tested. This continued until the graft could wrap around the chosen circle without kinking.

Burst pressure testing

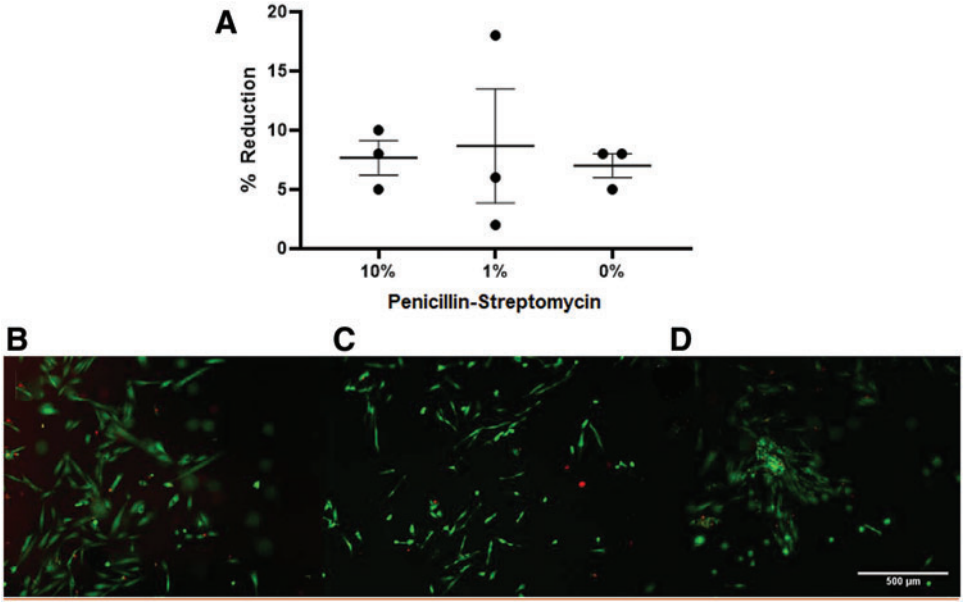
A close-ended graft was exposed to increasing intramural pressure via slow release of N_2 gas (NI-T, Praxair) until bursting. A blunted needle was fitted to the end of the gas delivery line and placed into one end of the graft. The other end of the graft was sealed at 2 cm (for 1.5 mm grafts) or 4 cm (for 4 mm grafts) from the needle attachment point on the other side. The pressure was measured in psi and converted into mmHg (1 psi = 51.71 mmHg).

Suture retention testing

Suture retention testing was performed using a UMT outfitted with a 10-kg load cell and an extension rate of 0.05 cm s^{-1}. One throw of a nylon suture (Ethicon, size 8–0 and 6–0 for 1.5-mm-thin and thick grafts, respectively) was used and was positioned 3 mm from the end of a fully hydrated graft. The suture was placed into the top clamp and the other graft end was placed in the bottom clamp. The weight of load at the rip was taken as the suture retention strength of the graft.

Scanning electron microscopy

Grafts were cut longitudinally and were dried flat on carbon tape in a controlled temperature and humidity



SUPPLEMENTARY FIG. S5. Effect of PS concentration used in the initial incubation of gelatin-conjugated PVA films on cell proliferation. **(A)** Percent reduction of Alamar blue by primary human umbilical vein endothelial cells (HUVECs) seeded on films pretreated with 10%, 1%, or 0% PS indicates that changing incubation concentrations of PS did not significantly affect subsequent cellular proliferation ($p > 0.9$). Data are presented as mean ± standard error, and each *dot* represents one data point. A live/dead assay of samples pretreated with **(B)** 10% PS, **(C)** 1% PS, and **(D)** 0% PS shows similar ratios of living and dead HUVECs. Across all groups, cells showed an elongated morphology and were randomly oriented. HUVECs, human umbilical vein endothelial cells; PS, penicillin/streptomycin.

chamber to ensure a slow drying process. Samples were sputter coated with gold for 30 s at 62.5 mTorr using a vacuum sputter coater (Desk II Sputter Coater; Denton Vacuum). Scanning electron microscopy was done using a high-resolution field emission scanning electron microscope (Zeiss 1550; Carl Zeiss AG) with an acceleration voltage of 7 keV.

Differential scanning calorimetry

Differential scanning calorimetry of dry PVA films was performed using a differential scanning calorimeter (DSC) Q2000 (TA Instruments). Samples were loaded in hermetic aluminum pans and then underwent a heating/cooling cycle of 30–260°C at a ramp rate of 10°C. An empty hermetic aluminum pan was used as reference. The fractional crystallinity was calculated from the second cooling cycle.

X-ray photoelectron spectroscopy

X-ray photoelectron spectroscopy (XPS) survey spectra of dry PVA films were obtained using a monochromated Al Kα 1486.6 eV, ESCALab 250 microprobe (Thermo VG Scientific) at the Waterloo Advanced Technology Laboratory (University of Waterloo). The analytical chamber pressure was maintained at 2×10^{-9} mbar during measurement.

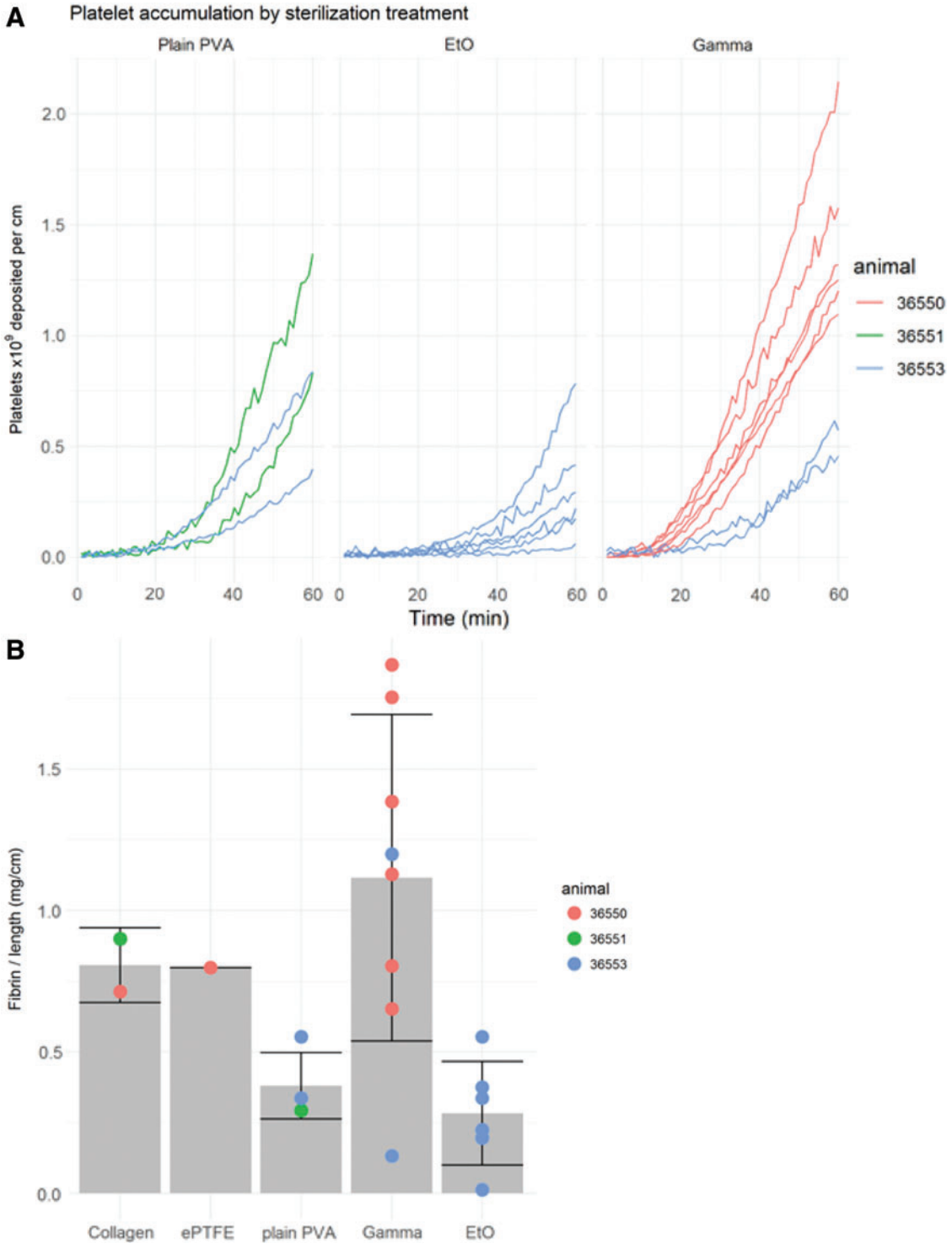
Water contact angle measurement

Before testing, PVA films were hydrated and then dabbed dry such that their surface had no water droplets. Using a custom-built contact angle measurement system, 3 µL of water was deposited on to the PVA surface and images were captured within 10 s of the droplet contacting the PVA film surface. The contact angle was measured using the Contact Angle plugin (written by Marco Brugnara) in ImageJ.

EA.hy926 cell culture on polyvinyl alcohol films

Untreated, EtO, and γ-irradiated PVA films that were unpatterned or patterned with 2 µm gratings (2 µG) were cut to fit in the wells of a 24-well plate. For consistency, the untreated, EtO, and γ-irradiated films were treated with UV for 20 min, and then incubated with 10% (v/v) penicillin/streptomycin (PS) (10,000 U/mL; Gibco) and 1% (v/v) amphotericin-B (VWR) for 1 h at 37°C. The films were then secured in a 24-well plate with autoclaved Silastic tubings (0.375″ ID, 0.625″ OD; Dow Corning) and were rinsed thoroughly with 1 × PBS. Next, to mimic the exposure of material to whole blood *in vivo*, the films were incubated with fetal bovine serum (FBS) (Gibco) followed by centrifugation at 1000 rpm for 30 min. The films were then stored in an incubator at 37°C for 30 min before cell seeding.

EA.hy926 (ATCC) cells (passage number 8) were seeded on the films at a density of 50,000 cells/cm^2. After seeding, the entire plate was spun down at 100 G (for 10 min) to help move the cells down closer to the PVA substrate. Cells were cultured for 13 days in DMEM (Gibco) supplemented with 10% (v/v) FBS, 1% (v/v) PS, and 0.1% (v/v) amphotericin B, and media changes were performed daily. The cell number was quantified using the CyQUANT® Cell Proliferation Assay Kit according to the manufacturer's protocol. The standard curve equation and



SUPPLEMENTARY FIG. S6. Individual animal data of the *ex vivo* shunt study. Charts were plotted for **(A)** platelet and **(B)** fibrin accumulation study. $n=4$ for plain/untreated PVA, $n=6$ for EtO-treated PVA, and $n=8$ for γ-irradiated PVA. Data are presented as mean ± standard deviation, and each *dot* represents one data point.

plot specific to EA.hy926 cells can be found in Supplementary Figure S3.

Cytotoxicity qualitative assessment

Qualitative assessment of the toxicity of the γ-irradiated and EtO-treated PVA films was performed by staining the EA.hy926 cells on the PVA samples after 13 days in culture with the LIVE/DEAD™ Cell Imaging Kit (Invitrogen) according to the manufacturer's protocol. Before adding the dyes, cell culture media were replaced with HEPES-BSS buffer (Lonza) and incubated for 15 min at 37°C. Samples were then washed with HEPES-buffered saline solution (HEPES-BSS) buffer twice for subsequent imaging.

Ex vivo *shunt testing*

Nonhuman primate studies were approved by the Oregon National Primate Research Center (ONPRC) Institutional Animal Care and Use Committee. Data were collected from three juvenile male baboons (*Papio anubis*) who were cared for at ONPRC following the ''Guide to the Care and Use of Laboratory Animals'' prepared by the Committee on Care & Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (International Standard Book, Number 0-309-05377-3, 1996).

Whole-blood, *ex vivo* shunt testing was run for 1 h at 100 mL/min in a nonhuman primate model as described previously.[S8] Briefly, platelet accumulation was quantified from the arteriovenous shunt every minute by testing the γ-radiation on a Brivo NM615 nuclear imaging camera (GE) from autologous platelets labeled with indium-111. Fibrin accumulation was quantified after the indium-111 decayed (10 half-lives) to yield an endpoint value. Untreated, EtO, and γ-irradiated PVA samples ($n = 4$–8 per group) were tested and were analyzed for statistical significance. Clinical expanded polytetrafluoroethylene (ePTFE, 4 mm ID, $n = 1$) and collagen I-coated ePTFE ($n = 2$) were tested to confirm reactivity of the model and comparison with historic data, but were not included in the statistical analysis due to the low replicates.

Implantation of PVA in rats, nonhuman primates, and histology

The small-animal study was approved by the Institutional Animal Care and Use Committee of the National University of Singapore. Four-week-old male Wistar Hannover Galas™ rats (InVivos) weighing 170–200 g were cared for according to the guidelines stated by the Institutional Animal Care and Use Committee of the National University of Singapore.

Both γ-irradiated and untreated, unpatterned PVA films were implanted subcutaneously in the rats. An incision was made in the abdominal skin along the ventral position. Three PVA samples were implanted on each side of the animal, which gave a total of six PVA samples (three untreated and three γ-irradiated PVA done before implantation) per animal (see schematic diagram in Supplementary Fig. S4). The rat was euthanized at day 21 postimplantation, and film samples were excised for histology analysis.

Nonhuman primate studies were approved (No. IP00000049) by the Institutional Animal Care and Use Committee according to the ''Guide for the Care and Use of Laboratory Animals'' prepared by the Committee on Care & Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (International Standard Book, No. 0-309-05377-3, 1996). Data were collected at the ONPRC at Oregon Health & Science University (OHSU) from juvenile male baboons (*P. anubis*, 13.4–17.1 kg). The ONPRC facility has an approved Assurance (No. A3304–01) for the care and use of animals on file with the Office for Protection from Research Risks at the National Institutes of Health (NIH), and has received full accreditation by the American Association for Accreditation of Laboratory Animal Care (AAALAC).

EtO and γ-irradiated PVA grafts were implanted end-to-side at the abdominal aorta to the left branch of the common iliac according to surgical procedures described before (see schematic diagram in Supplementary Fig. S4).[S9] Four baboons were involved for each of the treatment groups. The baboon was euthanized at day 28 postimplantation, and graft samples with the surrounding tissue were excised for histology analysis.

The immunohistochemistry staining was performed on formalin-fixed and paraffin-embedded tissue. Staining of the sectioned tissue was performed with hematoxylin and eosin to detect fibrosis, changes in PVA structure, and possible vascularization. Mac387 antibody staining detected M1 polarized macrophages by specifically detecting the MRP14.[S10,S11] The primary antibody dilution was 1:200 and incubation was overnight at 4°C. The total number of MAC 387+ macrophages per randomly selected 10 high-power fields of view (200×) in the surrounding tissue grafts was counted without prior knowledge of the sterilization treatment type. The results were analyzed as previously described.[S12]

Materials and method for Fourier-transform infrared spectroscopy

PVA grafts were cut longitudinally and then dried flat. Fourier transform infrared spectroscopy (FTIR) was performed at the Waterloo Advanced Technology Laboratory (University of Waterloo) using a Bruker Tensor 27 FTIR (Bruker) equipped with liquid nitrogen-cooled mercury cadmium telluride (MCT) detector. Measurements were obtained with attenuated total reflection mode with wave number ranges from 400 to 4000 cm^{-1}. Sixty-four scans were acquired at a spectral resolution of 4 cm^{-1}. Four 1.5-mm-thin grafts from each treatment group and a standard of uncrosslinked aqueous PVA film were tested. Analysis of FTIR spectra and production of FTIR figures were done using Spectragrphy.[S13]

Materials and method for X-ray diffractometry

X-ray diffractometry of thin PVA films was performed using the Bruker D8 FOCUS X-ray diffractometer. A 1.0 mm detector slit and scanning duration of 3 s/step were used to detect the reflected X-ray beam from 2θ equals to 5°–60°. Three PVA films were tested for each treatment group. Curve fittings of the obtained spectra were done using OriginPro software.

Proliferation assays to assess the effect of penicillin/streptomycin incubation on PVA gels

As a high concentration of PS was used to incubate the samples prepared in the EA.hy926 Cell Culture on PVA Films section, proliferation of cells grown on samples with incubations using lower concentrations of PS was tested to ensure that the subsequent washing of films was sufficient to prevent PS from interfering in cell proliferation.

PVA films were fabricated following the procedure outline in the Autoclaving of Polyvinyl Alcohol Solution and Crosslinking of Polyvinyl Alcohol Vascular Grafts and Films section. Gelatin was then grafted to these films using the methods previously described by Rizwan *et al.*[S14] Films were then cut to fit in the wells of a 24-well plate. Films were UV treated for 20 min and then incubated with 1% amphotericin-B (VWR) and 10% PS (10,000 U/mL; Gibco), 1% PS, or 0% PS for 1 h at 37°C. The films were then secured in a 24-well plate with autoclaved Silastic tubings

(0.375″ ID, 0.625″ OD; Dow Corning) and were rinsed thoroughly with 1×PBS. The films were then incubated with FBS (Gibco) followed by centrifugation at 1000 rpm for 30 min. The films were then stored in an incubator at 37°C for 30 min before cell seeding.

Primary human umbilical vein endothelial cells (Lonza) (passage number 3) were seeded on the films at a density of 50,000 cells/cm^2. After seeding, the entire plate was spun down at 100 G (for 10 min) to help move the cells down closer to the PVA substrate. Cells were then cultured in 500 μL of VascuLife VEGF Endothelial Medium (Lifeline Cell Technology). A complete media change was performed daily.

After 2 days, the Alamar blue assay was preformed to compare cell proliferation and viability among samples incubated with different amounts of PS. In each well, media were completely replaced with a solution of 0.1 μL Alamar blue/ml media. Samples were then further incubated for 4 h at 37°C, after which a 100 μL sample of media was taken from each well and put into a flat-bottomed 96-well plate. Proliferation was measured using absorbance at 570 nm using a Synergy 4 multidetection microplate reader (Bio-Tek). The positive control was made by autoclaving a solution of 0.1 μL Alamar blue/ml of media for 15 min, to ensure completely dye reduction. The negative control was a solution of 0.1 μL Alamar blue/ml of media placed in a well with no cells.

After the Alamar blue assay, samples were washed with fresh media three times and then allowed to continue to incubate at 37°C. After 2 days, the LIVE/DEAD Cell Imaging Kit (Invitrogen) was used to test proliferation according to the manufacturer's protocol. Before adding the dyes, cell culture media were replaced with HEPES-BSS buffer (Lonza) and incubated for 15 min at 37°C. Samples were then washed with HEPES-BSS buffer twice for subsequent imaging.

Statistical analyses

Statistical analyses were done using one-way ANOVA and Tukey's *post hoc*, except for XPS survey, swelling, water content, and *ex vivo* shunt data analysis (both platelet and fibrin data). XPS survey, swelling, and water content data were analyzed using unpaired two-tailed *t*-test, whereas platelet data in *ex vivo* shunt studies were analyzed with a multiway, repeated-measures, linear mixed-effects model against device and time. To increase model precision, a fractional polynomial term [time × ln(time)] and all relevant interactions were added as model fixed effects. After determining significance, Bonferroni-corrected *post hoc* contrasts were run. Fibrin data were analyzed with a one-way ANOVA and Tukey's *post hoc*. Data were considered statistically significant when $p < 0.05$. To better fit the normality and homogeneity of variance assumptions of ANOVA, platelet and fibrin data both were natural log transformed, with 0.01 added to each value to avoid exclusion of any zero values. All data are presented as mean ± standard error, except for *ex vivo* shunt studies where data are presented as mean ± standard deviation. In all figures, *denotes statistical significance with $p \le 0.05$, **$p \le 0.01$, ***$p \le 0.001$, and ****$p \le 0.0001$, with 95% confidence level. Sample size is reported in the legends of individual figures.

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