# Supplementary Data

## Supplementary Materials and Methods

### *Cell culture*

HUVEC were cultured in VascuLife VEGF EC Culture medium (Lifeline Cell Technology #LL-0003). The VascuLife VEGF LifeFactors Kit (Lifeline Cell Technology #LS-1020) added to VascuLife Basal Medium (Lifeline Cell Technology #LM-0002) includes the following components (final concentrations are provided after dilution in Basal Medium): recombinant human (rh) vascular endothelial growth factor (VEGF, 5 ng/mL), rh epidermal growth factor (EGF, 5 ng/mL), rh basic fibroblast growth factor (bFGF, 5 ng/mL), rh insulin-like growth factor 1 (IGF-1, 15 ng/mL), ascorbic acid (50 μg/mL), hydrocortisone hemisuccinate (1.0 μg/mL), heparin sulfate (0.75 U/mL), L-glutamine (10 mM), and 2% fetal bovine serum.

### *Tissue processing*

Dissected (native) or decellularized human umbilical vein (HUV) samples were immersed in OCT medium and snap frozen. Frozen HUV scaffolds were cut into 8 μm sections using a cryostat and collected on glass slides for staining. Tissue sections were stored at −80°C until staining.

### *Hematoxylin and eosin stain*

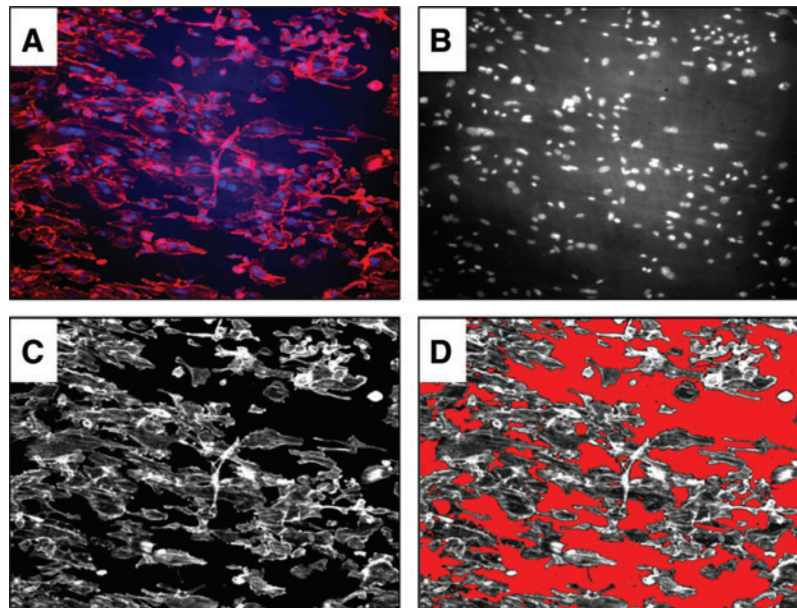
Tissue sections were fixed for 10 min using 10% neutral-buffered formalin (NBF). Sections were rinsed in deionized (DI) water for 30 s, stained with hemotoxylin for 1 min, dunked in tap water, and rinsed in running tap water for 1 min. Sections were immersed in bluing reagent for 30 s and then rinsed in DI water for 30 s. Sections were stained with eosin for 20 s, dunked in tap water, and rinsed in running tap water for 1 min. Sections were progressive dehydrated using 70%, 80%, 90%, and 100% EtOH for 30 s each before clearing and mounting.

### *Masson's trichrome stain*

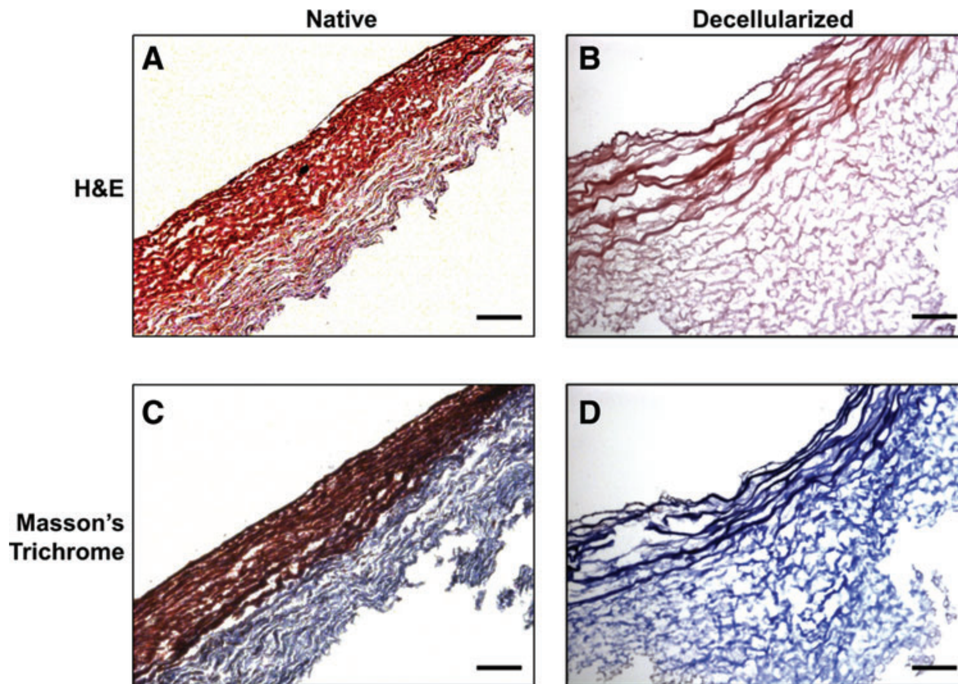
Masson's Trichrome stain was carried out according to kit instructions (Richard-Allen Scientific #87019). Tissue sections were fixed for 10 min using 10% NBF. Sections were rinsed in running DI water for 1 min and incubated in Bouin's Fluid at 56°C for 1 h. Sections were rinsed in running water for 5 min and then stained in Working Weigert's Iron Hematoxylin Stain for 10 min. Sections were rinsed in running DI water for 5 min and stained in Biebrich Scarlet-Acid Fuchsin Solution for 5 min. Sections were then rinsed in running DI water for 30 s, placed in Phosphotungstic-Phosphomolybdic Acid Solution for 5 min, and stained in Aniline Blue for 5 min. Sections were placed in 1% acetic acid, rinsed in running DI water for 30 s, and dehydrated in 100% EtOH twice for 1 min before clearing and mounting.

### *Color imaging*

Stained samples were imaged using a Zeiss Axio Imager.M2 microscope coupled with a Zeiss AxioCam MRc camera operated by AxioVision software version 4.8.




**SUPPLEMENTARY FIG. S1.** Image analysis for co-stained endothelial cell (EC) on the lumenal human umbilical vein (HUV) surface. EC-seeded HUV scaffolds were fixed and co-stained with rhodamine phalloidin/DAPI to visualize cytoskeletal F-actin (*red*) and nuclei (*blue*), respectively **(A)**. Images were separately obtained through DsRed and DAPI filters using a monochrome camera and analyzed using ImageJ software. Each DAPI image **(B)** was analyzed using the ITCN plug-in to quantify the number of cell nuclei in each image. Thresholding analysis was used to quantify the percentage of each DsRed image **(C)** devoid of F-actin **(D)**, which was subtracted from 100 to obtain percent coverage.




**SUPPLEMENTARY FIG. S2.** Morphological characterization of decellularized HUV scaffolds. HUV were decellularized using sodium dodecyl sulfate (SDS) and treated with deoxyribonuclease I. Shown are representative cross-sectional images of dissected, native **(A, C)**, or decellularized HUV **(B, D)** stained using H&E (*top*) or Masson's trichrome (*bottom*). The vessel lumen is shown in the *upper left corner*. SDS treatment resulted in removal of resident cells from the HUV scaffold, leaving behind an intact extracellular matrix composed of HUV smooth muscle and collagen-rich Wharton's jelly. Masson's trichrome: *black*, cell nuclei; *red*, smooth muscle/cytoplasm; *blue*, collagen. Scale bars: 100 μm.

### *Lumenal surface analysis*

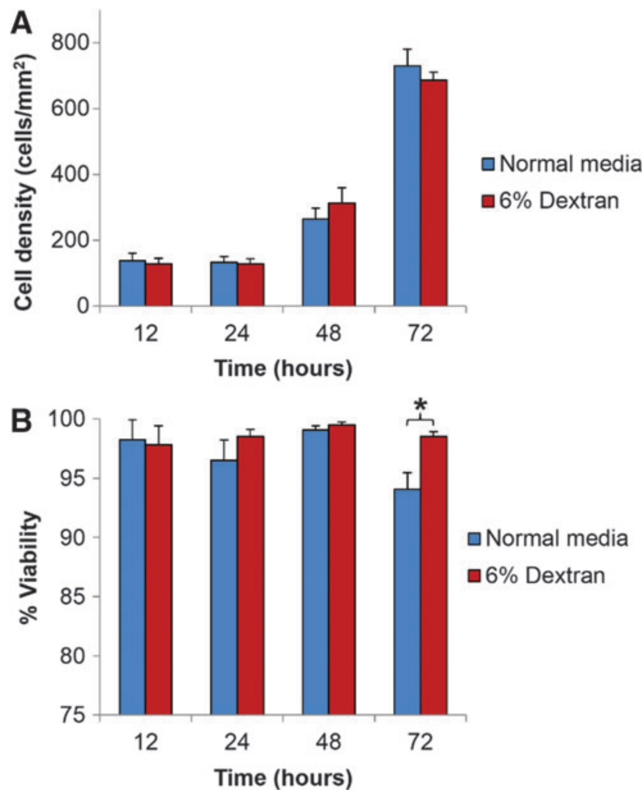
Scaffolds were fixed with 10% NBF for 10 min, cut open axially, and co-stained with rhodamine phalloidin and 4′,6-diamidino-2-phenylindole (DAPI; Invitrogen) as previously described (26). Each scaffold was imaged in 15 predetermined locations that were divided into 3 circumferential zones ($n=5$ images per zone) using a Zeiss AxioImager epifluorescence microscope. DAPI/DSRed channel images were exported as separate files for analysis using NIH ImageJ software (Supplementary Fig. 1). DAPI images were analyzed using the ITCN Automatic Nuclei Counter plugin ver. 1.6 to quantify the number of cell nuclei in each image, which was divided by the image surface area to calculate cell density. DSRed images were analyzed using threshold analysis to determine percent area coverage by endothelial cell (EC).

For rotational seeding, the mean cell density/percent coverage for each zone was ranked, so that zone 1 corresponded to the minimum, zone 2 to the median, and zone 3 to the maximum values for each scaffold. The mean cell density and percent coverage for each zone were compared among experimental seeding groups ($n=5$ scaffolds per group). For all other experimental groups, cell density/percent coverage values for each HUV scaffold were averaged across all 15 locations ($n=3$–6 scaffolds per group).

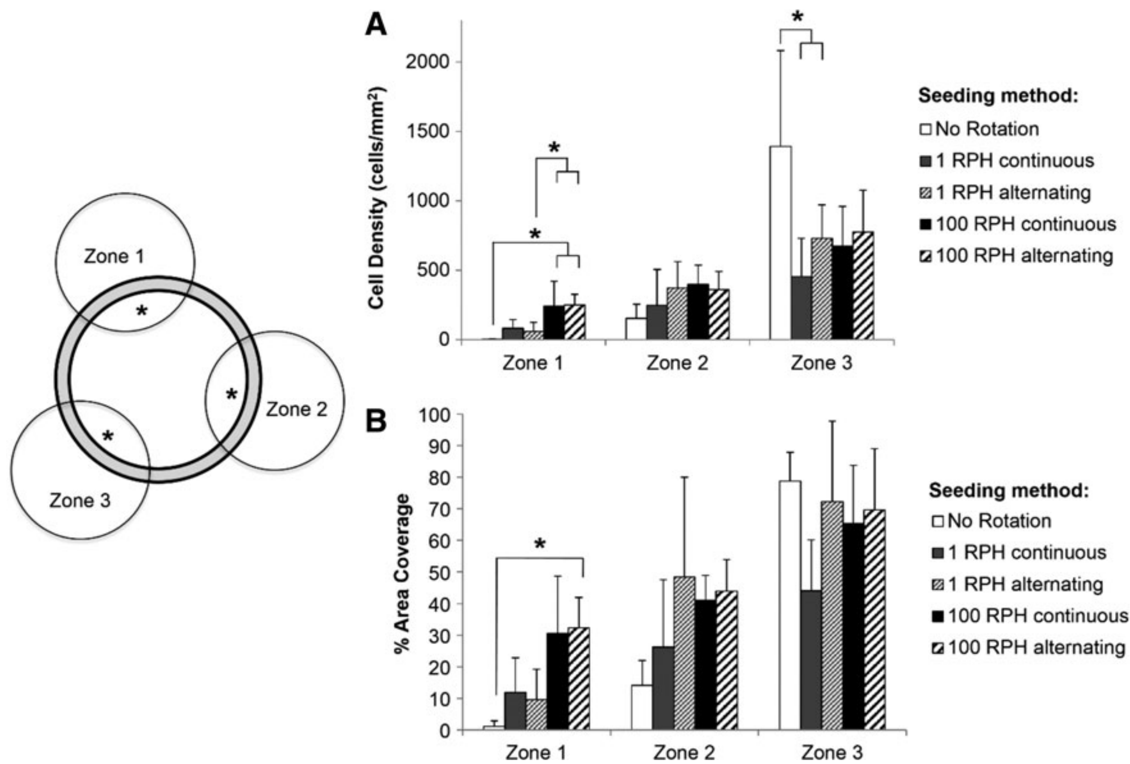
## Supplementary Results

### *Rotational seeding improves the circumferential distribution of EC on the lumenal HUV surface*

Decellularized HUV scaffolds were seeded with EC under static or rotational seeding conditions. Under static conditions, the majority of EC adhered to the bottom zone of the HUV surface, where the cells settled gravitationally after an injection (Supplementary Fig. 4A). However, the increase in cell density in this zone did not correlate with a significant increase in the total percentage of surface area coverage relative to other seeding groups (Supplementary Fig. 4B). No rotation resulted in a significantly lower mean cell density in zone 1 (the circumferential portion with the lowest coverage for each scaffold) than continuous rotation ($4\pm2$ vs. $241\pm162$ cells/mm$^2$, $p=0.004$) or alternating rotation ($4\pm2$ vs. $251\pm76$ cells/mm$^2$, $p=0.003$) at 100 revolutions per hour (RPH; Supplementary Fig. 4A). The 100 RPH alternating rotation also facilitated significantly greater surface coverage by EC than no rotation ($32\pm10$ vs. $1\%\pm2\%$, $p=0.016$) in zone 1.




**SUPPLEMENTARY FIG. S3.** EC culture in dextran-supplemented culture media. High-molecular-weight dextran was added to culture media to increase viscosity. EC growth was monitored over time to determine whether addition of dextran had any effect on proliferation and viability. **(A)** Cell density increased over 72 h and at no time point was any significant difference observed between EC cultured in normal or dextran-supplemented media. The doubling times for EC grown in normal and dextran-supplemented media during exponential growth were calculated to be 19.9 and 19.5 h, respectively, indicating similar growth characteristics. **(B)** The viability of EC cultured in 6% dextran was significantly higher than in normal media only at 72 h, but this was attributed to increased detachment of cells due to over-confluence at this time point. Results are presented as mean + SD. *Asterisk* indicates a significant difference in means ($p < 0.05$).




**SUPPLEMENTARY FIG. S4.** EC adhesion to the HUV as a function of seeding technique. HUV scaffolds were seeded overnight with EC using various rotation methods, then rinsed, fixed, and co-stained with DAPI/rhodamine phallodin to visualize cell nuclei and cytoskeletal F-actin, respectively. Scaffolds were then cut open axially, imaged in three pre-determined zones on the lumenal surface (see schematic at *left*), and analyzed in ImageJ. The mean cell density **(A)** and percent area coverage **(B)** are reported in three zones ($n = 5$ images per zone) along the graft circumference for each seeding method, ordered by number. *Zone 1*: minimum; *Zone 2*: median; *Zone 3*: maximum. Results are presented as mean + SD. *Asterisks* indicate a significant difference in means between seeding groups within each zone ($p < 0.05$). RPH, revolutions per hour.