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Data Article

# "Data characterizing microfabricated human blood vessels created via hydrodynamic focusing"

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ABSTRACT

This data article provides further detailed information related to our research article titled "Microfabricated Blood Vessels Undergo Neovascularization" (DiVito et al., 2017) [1], in which we report fabrication of human blood vessels using hydrodynamic focusing (HDF). Hydrodynamic focusing with advection inducing chevrons were used in concert to encase one fluid stream within another, shaping the inner core fluid into 'bullseye-like" cross-sections that were preserved through click photochemistry producing streams of cellularized hollow 3-dimensional assemblies, such as human blood vessels (Daniele et al., 2015a, 2015b, 2014, 2016; Roberts et al., 2016) [2–6]. Applications for fabricated blood vessels span general tissue engineering to organ-on-chip technologies, with specific utility in *in vitro* drug delivery and pharmacodynamics studies. Here, we report data regarding the construction of blood vessels including cellular composition and cell positioning within the engineered vascular construct as well as functional aspects of the tissues.


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**Specifications Table**

| | |
|---|---|
| Subject area | *Bioengineering, microfluidics, materials science* |
| More specific subject area | *Synthetic human blood vessel fabrication* |
| Type of data | *Table, bar graph, immunofluorescence, videos* |
| How data was acquired | *Included data were acquired/generated using confocal microscopy (Nikon C1si) or computer-generated images, respectively.* |
| Data format | *Raw and analyzed* |
| Experimental factors | *None* |
| Experimental features | *We describe details of blood vessel construction, characterization and perfusion* |
| Data source location | *Washington, DC United States.* |
| Data accessibility | *All data described here is accessible within this article.* |

**Value of the data**

- The potential for organ-on-chip technology to modernize *in vitro* experimentation is within reach. Yet, due to the lack of an integrated functional vasculature the technology is hampered by the inability to maintain truly representative tissue constructs long-term
- Our methodology generates synthetic human blood vessels capable of angiogenesis, anastomosis, and perfusion
- This work reports critical aspects concerning the fabrication of the blood vessels such as requirements for synthesis; cellular composition and downstream applications

## 1. Data

Using hydrodynamic focusing (HDF) in conjunction with photocrosslinkable polymers, human blood vessel mimics were constructed. Data presented here describes parameters for creating human endothelial microvessels (HEMV) and multi-cell microvessels (MCMV) and the requirements for integration and perfusion. Fig. 1 shows HEMV and MCMV maturation. Engineered vessels have an average outer diameter (O.D.) of 250 μm, an inner luminal diameter of 100–125 μm, and a wall thickness of 50–75 μm (Table 1). HEMVs were embedded into an extracellular matrix, endothelial outgrowths were observed ( $< 10$ μm O.D.) (Table 1) [1]. Cellular growth was assessed in co-culture, variable media compositions were examined for three different cell types using their native media as diluents. The data showed endothelial cell proliferation was not affected until native growth media was diluted below 25% with vascular smooth muscle cell/pericyte growth medium (Fig. 2a and b). Smooth muscle cells were viable and displayed normal growth capacity (Fig. 2c and d). Pericytes displayed no changes in cell viability/proliferation (Fig. 2d and e). Distance limits growth-media diffusion [7,8] and engineered blood vessels were denuded from their walls. Fig. 3 shows collagenase-treated HEMV (liberated within 90 min); while the control HEMV remained intact. Immunofluorescence confirmed denuding did not affect structure. Similar results were obtained for mechanical disruption of the HEMV. Finally, HEMV were integrated into a perfusion device [1,5,6]. Videos 1 and 2 show microparticles entering and exiting the HEMV, respectively.

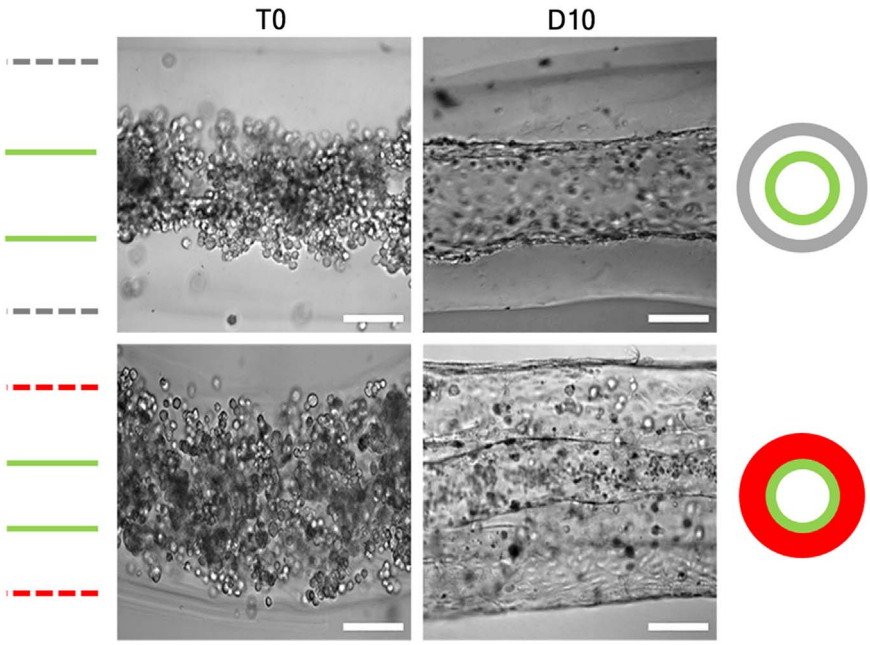



**Fig. 1.** Cellular Positioning within Microfabricated Blood Vessels. *Top panel*, depicts human endothelial microvessels (HEMV) grown over 10 days. *Top left*, the outer edges of the HEMV are marked with a grey dashed line, while the lumen of the HEMV is marked by a solid green line. *Far right*, depicts a cross-sectional view of the vessels in which the acellular walls of the HEMV are shown as white space bound by a grey circle; while the endothelial cells lining the lumen of the HEMV are depicted in green. *Lower panel*, shows a multi-cell microvessel (MCMV). Here, the outer edge of the MCMV is marked by a red dashed line indicating placement of the 1:1 mixture of smooth muscle cells (SMC) and human vascular pericytes (HVP). On the left, the solid green lines indicates the upper and lower boundaries of endothelial cells present in the lumen of the MCMV. Endothelial cells line the lumen of the MCMV creating a confluent monolayer of cells, while extensive proliferation of SMC/HVP fill the walls of the microvessel by day 10. Note: *Top right*, The extensive growth of SMC/HVP integrated into the wall of the MCMV when compared to the HEMV, *bottom right*. Scale bars, 50 μm.

**Table 1**
Parameters for Microvessel Fabrication.

| Type of Blood Vessel | Outer Diameter Range (μm) | Luminal Diameter Range (μm) | Wall Thickness Range (μm) | Bio-compatible (Y/N) | Endothelial Cell Density ($10^6$/mL) | Smooth Muscle Cell Density ($10^6$/mL) | Pericyte Cell Density ($10^6$/mL) |
|---|---|---|---|---|---|---|---|
| PEGDMA | 75–400 | 25–200 | 50–200 | N | – | – | – |
| Venule (HEMV) | 75–400 | 25–200 | 50–200 | Y | 30 | – | – |
| Arteriole (MCMV) | 75–400 | 25–200 | 50 - 200 | Y | 30 | 5 | 5 |
| (Observed) endothelial sprouts | 4–8 | 1 - 4 | N/A | Y | – | – | – |

## 2. Experimental design, materials and methods

### 2.1. Fabricating synthetic blood vessels

Human endothelial microvessels (HEMV; endothelial cell only) or multicell microvessels (MCMV; endothelial, smooth muscle cells and pericytes) are constructed as previously described [1,4]. Briefly,

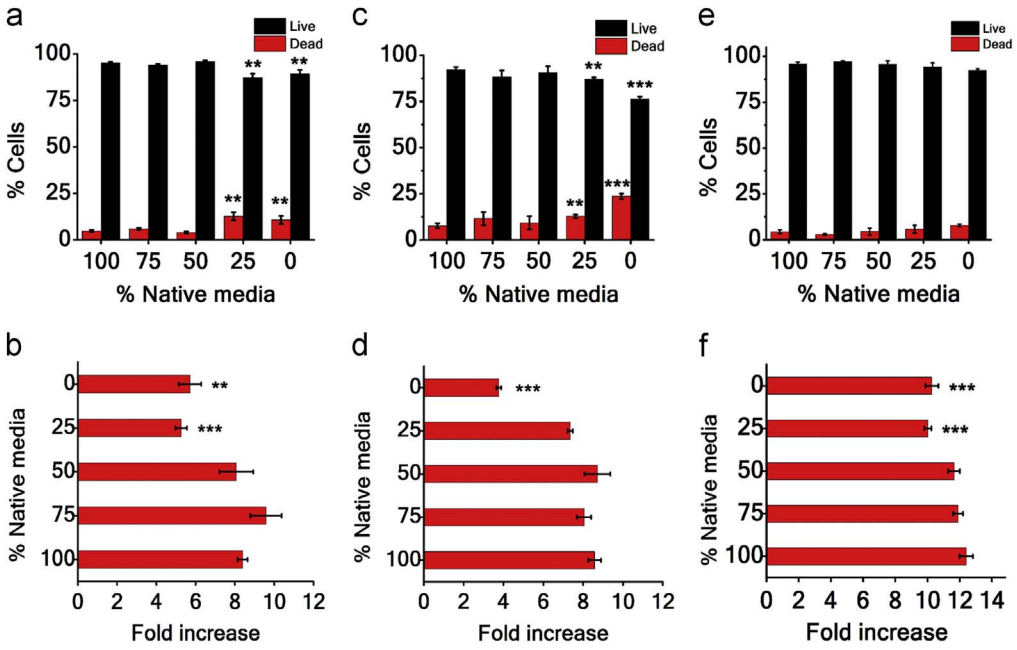



**Fig. 2.** Media compatibility assay. Primary endothelial, vascular smooth muscle and pericyte cell cultures were separately assessed for their growth capacity over a 5 day time-course using various composite media formulations. (a and b) Endothelial cells. (c and d) Smooth Muscle Cells. (e and f) Human Vascular Pericytes. Cell viability was performed via trypan blue exclusion; both live and dead cells are plotted. Cell proliferative capacity was addressed by plotting fold-increase in cell number after plating. Data shown in Fig. 2b,d,f are relative to data obtained in 100% native media.

tetrathiol- and acrylate-modified poly(ethylene) glycol based polymers are mixed at 1:1 ratio (Jen-Kem USA, Allen, TX). Lyophilized gelatin-methacrylamide (GelMA) is also included at a 1:1 ratio to provide cellular attachment. The final concentration for the microvessel is 6% w/w (3% poly(ethylene glycol), 3% GelMA). GelMA was synthetized as reported [9–11]. Resuspended polymers are introduced to a microfluidic device and photopolymerized into 3D synthetic blood vessels using ultraviolet (UV) light. Flow rates are previously described [1].

### 2.2. Cell lines and media compatibility assay

Endothelial growth medium-2 is used for endothelial cells (Lonza, Walkersville, MD). Human primary vascular smooth muscle cells (SMC) were obtained from ATCC (Manassas, VA) and grown in DMEM/F12 with 10% fetal bovine serum plus EGS supplement (Sigma, St. Louis, MO) as recommended. Human vascular pericytes (HVP) were obtained from ScienCell (Carlsbad, CA) and grown similarly. All cells were maintained at 37 °C/5% CO2 and not used beyond passage 5. Composite media formulations, beginning with 100% native media, were diluted using the native media of the other cell type down to 0% native media. Percent cell viability was determined *via* trypan blue; cell proliferative capacity was assayed by determining the fold increase in cell number by day 5.

### 2.3. Denuding the HEMV

For specific experimentation, removal of the outer polymeric wall of the HEMV is possible using collagenase digestion, however it is not required for advanced developmental processes [1]. HEMV were stained with anti-CD31 (Thermo Fisher Scientific) [1] to identify endothelial cells; digested for 90 min at RT using 1 mg/mL of collagenase (Sigma, St. Louis, MO) resuspended in TESCA buffer. Surgical forceps were used to separate the polymeric shell of the HEMV from the lumen.

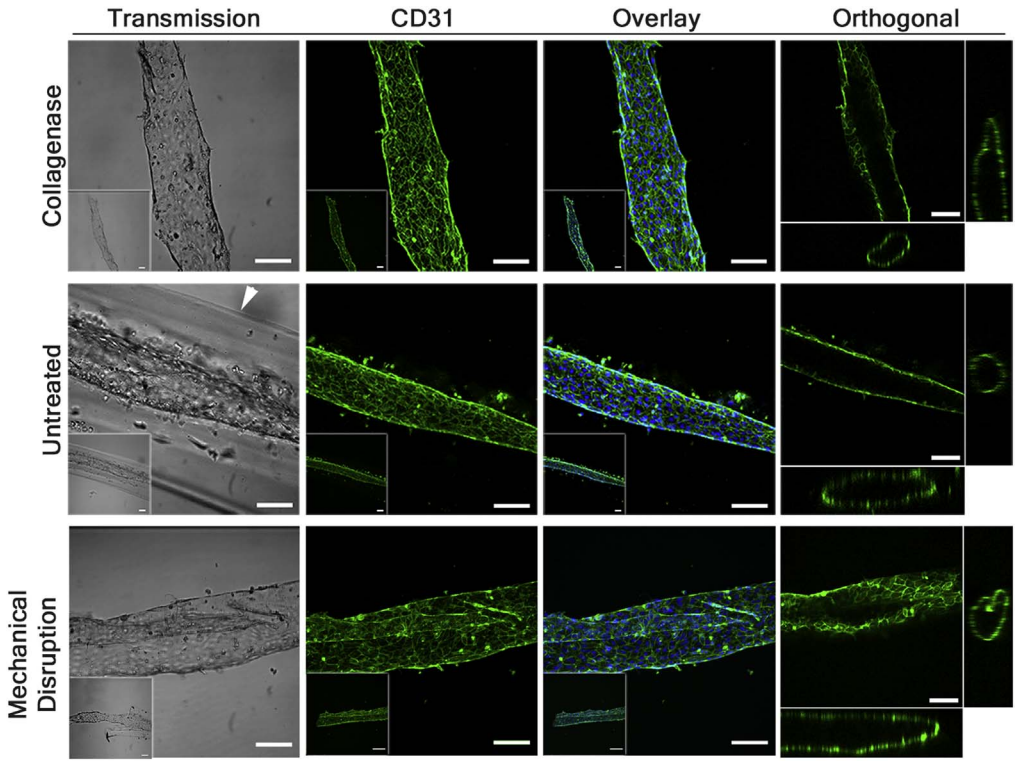
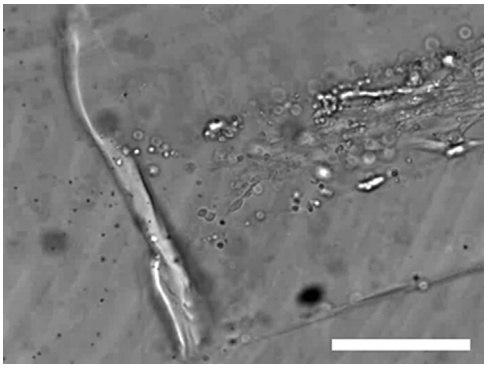
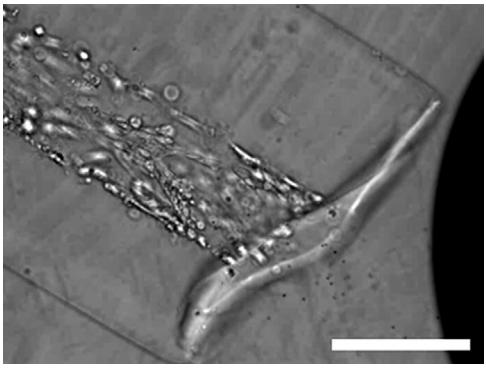



**Fig. 3.** Collagenase digestion or mechanical disruption of HEMV. *Top panel*, collagenase digestion illustrates the lumen of the microvessels were isolated from its polymer shell; while negative control microvessels retain their outer polymer shell (*middle panel*, arrowhead). *Lower panel*, shows the outer shell of the microvessel can also be mechanically separated using surgical forceps leaving an intact self-sufficient vessel. Anti-CD31 (green) immunofluorescence staining and DAPI (blue) are shown, highlighting the integrity of the denuded HEMV. Columns 1–3: Scale, 100 μm. Column 4: Scale bar, 50 μm.



**Video 1.** Human endothelial microvessels (HEMV) were integrated into a novel perfusion system. Microparticles (2 μm) were perfused through the HEMV, and real-time video was captured at the HEMV inlet (Video 1, left). Exit of the microparticles was captured at the outlet of the HEMV (Video 2, right). Scale bar, 100 μm.Supplementary material related to this article can be found online at http://dx.doi.org/10.1016/j.dib.2017.07.011.



**Video 2.** Human endothelial microvessels (HEMV) were integrated into a novel perfusion system. Microparticles (2 μm) were perfused through the HEMV, and real-time video was captured at the HEMV inlet (Video 1, left). Exit of the microparticles was captured at the outlet of the HEMV (Video 2, right). Scale bar, 100 μm.Supplementary material related to this article can be found online at http://dx.doi.org/10.1016/j.dib.2017.07.011.

### 2.4. HEMV perfusion

HEMV were integrated into a novel manifold device containing an inlet, outlet and center cavity filled with 5% GelMA [1,5,6]. A microperistaltic pump was used to recirculate endothelial growth medium or medium containing 2 μm O.D. microparticles (Shephrotech, Lake Forest, IL). Video were collected in real-time using a phase contrast microscopy and represent approximately 4.2 dyne/cm$^2$.

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## Transparency document. Supporting information

Transparency document associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.dib.2017.07.011.

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