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Supplementary information

Perfusion and endothelialization of engineered tissues with patterned vascular networks

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Supplementary information for

Perfusion and endothelialization of engineered tissues with patterned vascular networks

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Supplementary Manual: Customization of perfusion chambers with multiple sets of inlets and outlets

In the main manuscript, we outline how our scriptable perfusion chambers can be easily modified to accommodate gels of various dimensions or incorporate additional features such as PDMS anchors and gaskets. Here we describe a more advanced set of options to specify the positions of an arbitrary number of ports for catheterizing the gel. These options are useful for changing the position of the default single inlet and single outlet, and also for adding new sets of ports which may facilitate experiments with multivascular networks or side-by-side configurations of perfusable channels.

The nomenclature for specifying port locations relies on an openSCAD variable called `portsVector`. `portsVector` is a vector of vectors and is declared between lines 88-98 of the R2 version of the perfusion chamber generator script. By default, the definition of `portsVector` is:

```
PortsVector = [
[0, 0, 0, TipBooleanDist, NeedleGauge, 1]
];
```

Generating a chamber with this default `portsVector` results in the placement of a single inlet and single outlet on the short sides of the chamber (Fig. 1a). Each port is exactly centered on its face in the default configuration. Note that `TipBooleanDist` and `NeedleGauge` are user-defined parameters in the code, which have default values of 11 and 18 (GA), respectively.

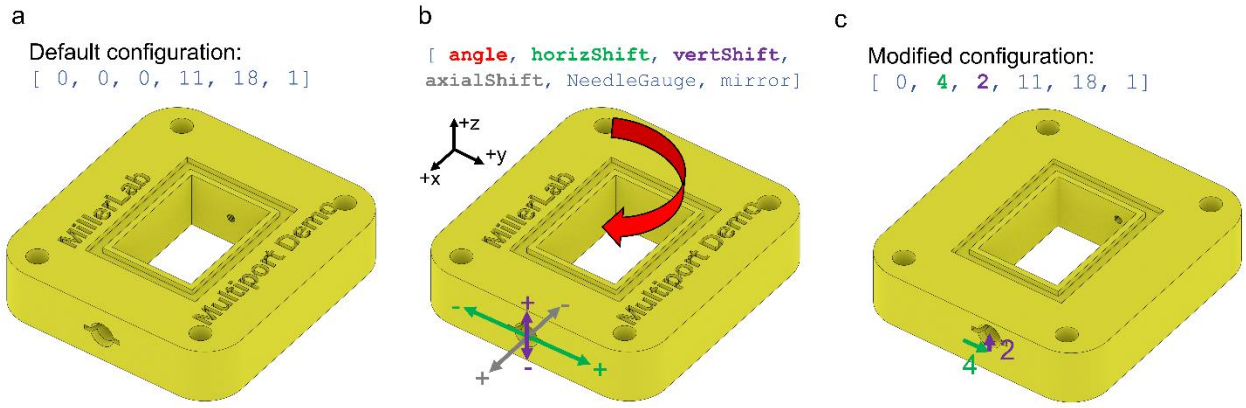

Figure 1 | Adjustable parameters for changing port placement. a, chamber generated from default `portsVector` settings. **b,** Annotation of rotation and translation functions available for port placement. **c,** Annotated example of translating an inlet/outlet port pair to an off-center position.

We can begin to understand the syntax for generating custom ports by adjusting the vector elements in the default port. Each vector within PortsVector has six elements which specify the geometry of the tip, as described below:

```
PortsVector = [
[ angle, horizShift, vertShift, axialShift, NeedleGauge, mirror]
];
```

Fig. 1b illustrates the effect of the first four elements in terms of the inlet position. Fig. 1c demonstrates the translation of the inlet port by altering elements 2 and 3, `horizShift` and `vertShift`.

Adjust element 1 to change which side of the chamber the port is added on. The default position is 0 degrees, which corresponds to short side in the *-x* direction. Use 90 degrees to get a port placed on the long side (in the *-y* direction). 180 gives a port on the short side in the *+x* direction, and so on. Values of element 1 not in 90 degree increments are allowed, but should only be used in the rare case that the vascular network itself does not form a right angle with respect to the gel. Fig. 2 provides a visual recap of the angle selection. **Note**: when combining an `angle` setting with a `horizShift`, note that the direction of the horizontal shift will always be in the *+x* or *+y* direction.

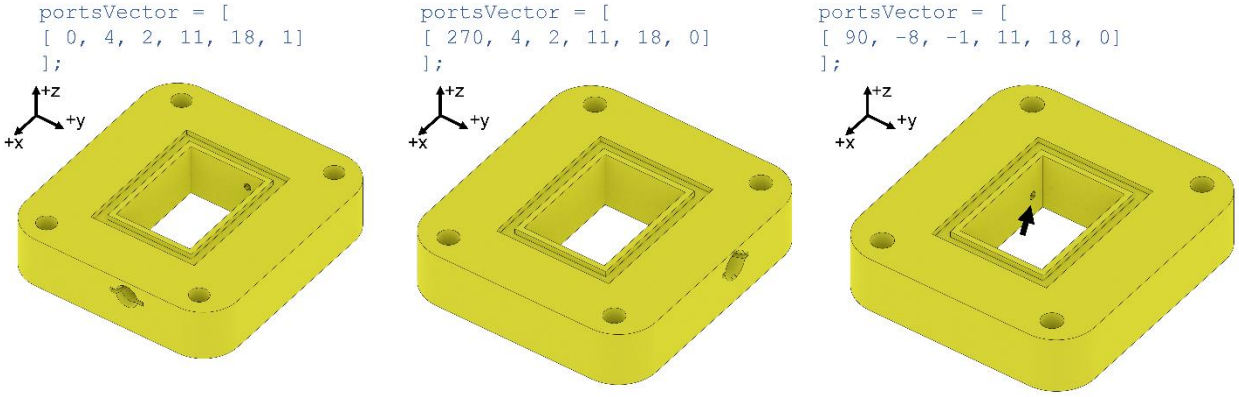

Figure 2 | Rotation angle parameter for port placement. Examples highlight the effects of adjusting element 1 in `portsVector` to describe the face where the port is placed.

Element 4, `axialShift`, controls where the cavity for the catheter tip is positioned relative to the chamber edge. This has the effect of controlling how far the tip will protrude into the chamber interior, and thus, into the gel. The correct value will depend on your catheter tip length and how far you want it to stick into the inlet. A larger value of axial shift will push the catheter tip out of the central cavity; a smaller value will make it stick further into the gel (see Fig. 3a). By default, `axialShift` is set to the openSCAD variable `TipBooleanDist`, which has default value is 11, an empirically determined value which has worked well in our studies.

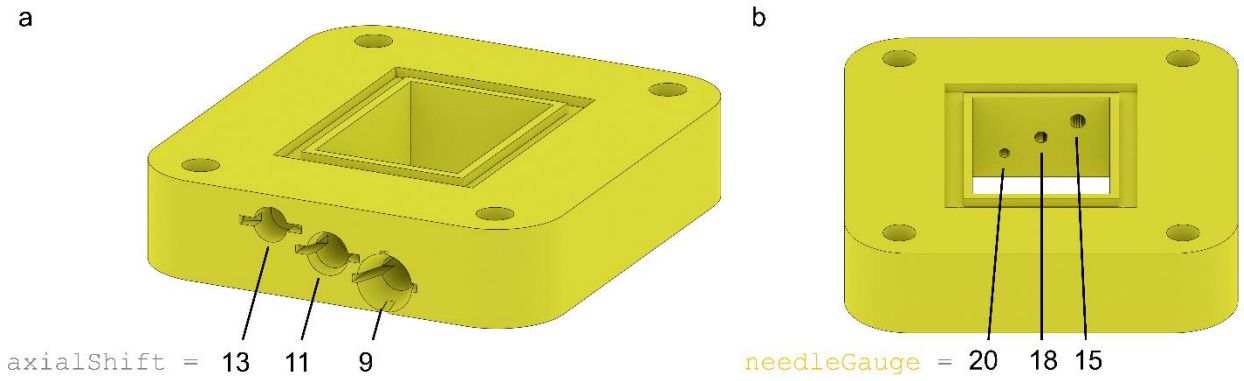

Figure 3 | Axial port positioning and tip diameter. **a,** Three example ports demonstrate the effect of the `axialShift` parameter on the geometry of the cavity where the Luer tip is wedged into the chamber. **b,** An array of three ports highlights the three Luer tip port sizes available by default within the perfusion generator script, selected with `needleGauge`.

Element 5, `needleGauge`, is simply the gauge of catheter tip you want to insert through the port. 15, 18, and 20 gauge needles have been optimized extensively within the code and are the pre-loaded size options (Fig. 3b). The default is 18 gauge.

Element 6, `mirror`, allows you to use a single entry in `portsVector` to define not a single port, but a pair of ports, e.g. an inlet and outlet. For a tip geometry described by `portsVector` elements 1-5, `mirror` specifies the number of axes across which the port should be reflected. `mirror` can be assigned the value 0, 1, 2, or 3, the effects of which are shown in Fig. 4. `mirror = 0` creates just one port, `mirror` > 0 creates a pair of ports by reflecting the specified port across 1, 2, or 3 axes. The first axis of reflection always places the reflected port on the opposite side of the chamber; the second and third axes reflect the port horizontally and vertically across the same side of the chamber.

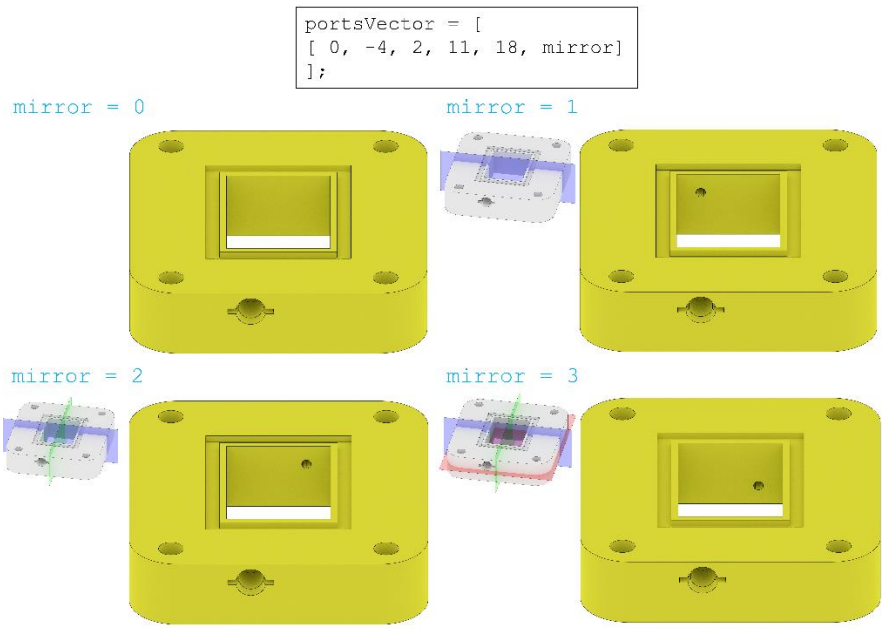

Figure 4 | Mirroring tips to create pairs of inlets/outlets. The resulting geometry is shown for each possible value of `mirror` applied to the same base port configuration. Chambers are viewed from the -*x* direction.

Now that we have described how the 6 elements of each vector entry in `portsVector` can be used to control port placement, we can begin adding multiple sets of ports by adding additional vectors within `portsVector`. Fig. 5 shows some examples which combine sets of ports. Note that vectors within `portsVector` must be comma-separated, and that lines of code within openSCAD can be commented out with a `//` to suppress them.

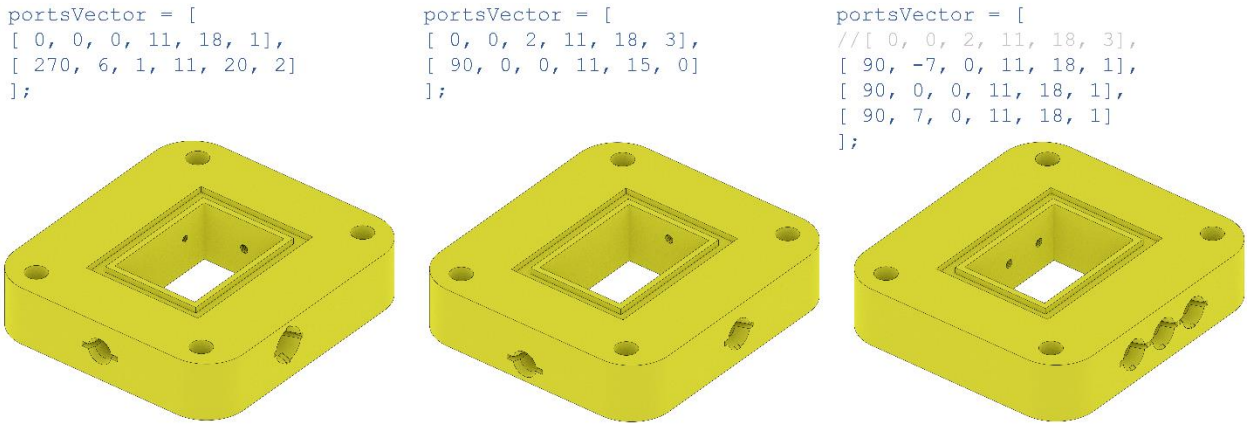

Figure 5 | Examples of configurations with multiple ports. We illustrate examples with 4, 3, and 6 total inlet/outlet ports to highlight the flexible configurations available.

Finally, we note that the other features of the perfusion chamber generator are fully compatible with the repositioning and addition of ports. In particular, if needle gaskets and PDMS anchor traps are enabled, these features will be automatically repositioned as needed when ports are relocated. We demonstrate these features in Fig. 6.

```
PortsVector = [
[0, 0, -2, 11, 18, 3],
[90, 7, -3, 11, 18, 2]
];
```

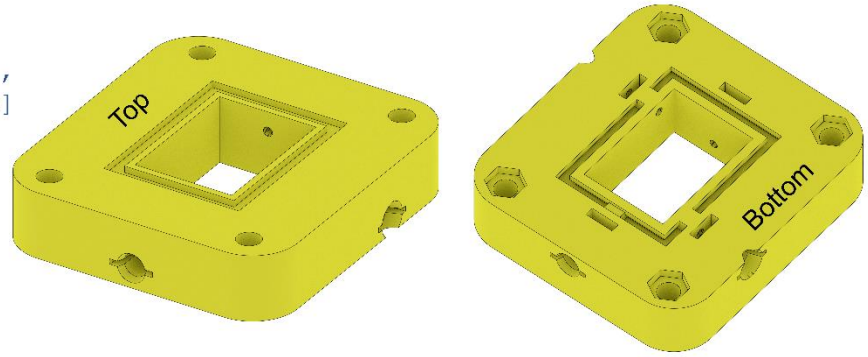

Figure 6 | PDMS trap features adapted to reconfigured ports. In this example, needle molds and PDMS anchor traps are enabled to create a stronger and longer-lasting airtight seal (see Fig. 2 in main Protocol). Note that when ports are added or rearranged, the needle molds follow the positions of the ports. As needed, a gap is created in the PDMS anchor trap perimeter for a Luer tip to pass through.

Supplementary figures

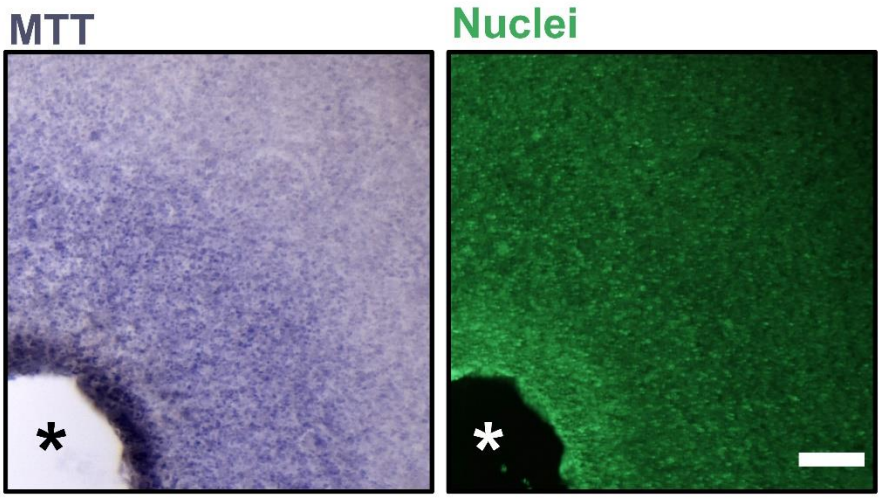

Figure S1 | Radial gradients of metabolic activity and cell density form around a perfused vessel. In an initially homogeneous population of HepG2 cells (ATCC Cat# HB-8065, RRID:CVCL_0027; 60e6 cell/mL), we observed the development of radially inhomogeneous patterns of metabolic activity (MTT stain) and proliferation (using nuclear intensity as a proxy for number of cells) after three days of perfusion (25 µL/min) using the protocols described in stages 1 and 3. The methods used and the quantification and analysis of these gradient patterns are fully described in our previous publication[1]. Scale bar = 200 µm; gels are 2 wt% low-melt agarose; asterisk marks the lumen. Images were obtained using a SteREO Discovery.V8 stereoscopic microscope (Zeiss) equipped with EOS 5DSR color camera (Canon).

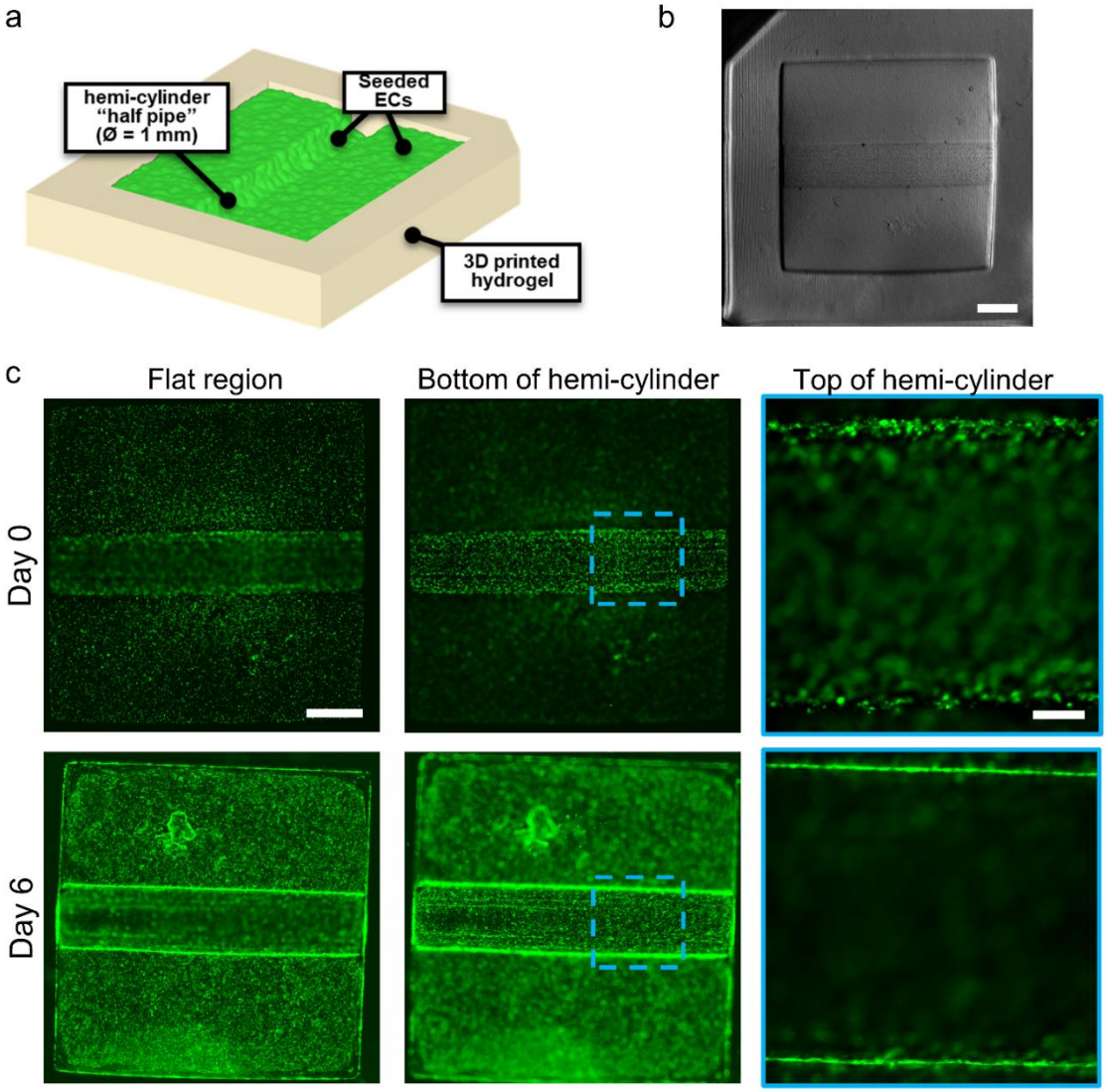

Figure S2 | Endothelial cell seeding on hemi-cylinders. a, We employ a "half-pipe" hydrogel as a 2.5D model for endothelial cell attachment to patterned hydrogels. The half-pipe architecture includes curved and flat regions to evaluate the adhesion and morphology of ECs in each topology. **b,** Here, half-pipe models were printed via hydrogel stereolithography from 10 wt% GelMA mixed with 3.25 wt% PEGDA (3.4 kDa). GFP-HUVEC cells were seeded over 4 h at a density of 400 × 10^3 cell cm^{-2}, which was calculated as equivalent to our suggested cell density of 30 × 10^6 cell ml^{-1} for injection into hydrogel vascular networks. Scale bar = 1 mm. **c**, Longitudinal epifluorescence images show effective adhesion of HUVECs to the flat and curved regions of the half-pipe. Magnified view of the top of the hemi-cylinder shows that initially rounded cells (Day 0) adopt a spread morphology over 6 days in culture. Gels were gently washed with PBS on Day 0 to remove unadhered cells prior to imaging. Images were acquired on a Ti-E epifluorescence microscope (Nikon) equipped with Zyla 4.2 sCMOS camera (Andor). Scale bars = 1 mm (low magnification); 200 µm (high magnification).

References in supplementary information

1. Kinstlinger, I. S. *et al.* Generation of model tissues with dendritic vascular networks via sacrificial laser-sintered carbohydrate templates. *Nat. Biomed. Eng.* (2020) doi:10.1038/s41551-020-0566-1