ADVANCED HEALTHCARE MATERIALS

Supporting Information

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High-Scale 3D-Bioprinting Platform for the Automated Production of Vascularized Organs-on-a-Chip

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Supporting Information

1. Chip prototyping

Different chip geometries were tested by 3D-printing of various prototypes as shown for the pillar number, pillar size and channel width.

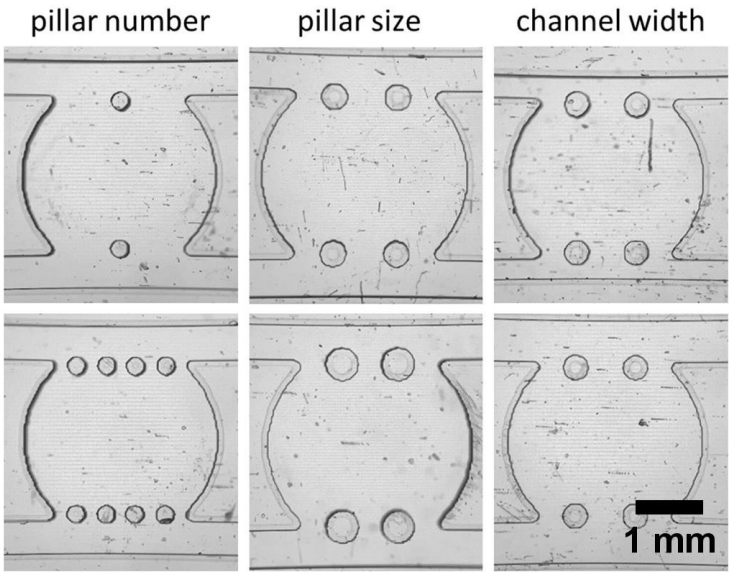

Figure S1: Different chip geometries were tested by DLP printed prototypes, including pillar numbers, pillar sizes and channel width.

2. Cell viability

Cell viability was tested with an FDA (live) and PI (dead) staining of cells 14 hours post-printing and compared to a non-printed control group with n = 5. Fibrinogen was crosslinked post-printing / post-pipetting by adding 25 µl of medium supplemented with 10 U/ml thrombin, following an incubation for 30 minutes before an additional 50 µl of medium were added.

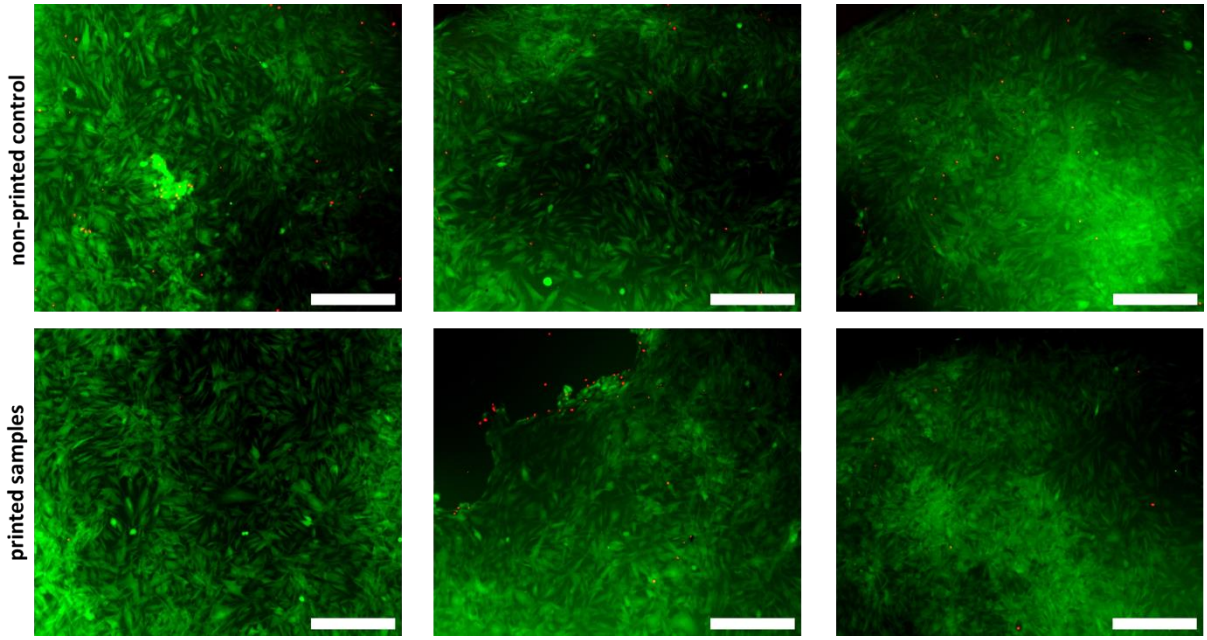

Figure S2: Viability staining and microscopy of HDF cells 14 hours post-printing as well as of a non-printed control group with live cells stained with FDA (green) and dead cells stained with PI (red). Scale bar showing 500 µm.

3. Confocal microscopy images of microvascular networks on day 14

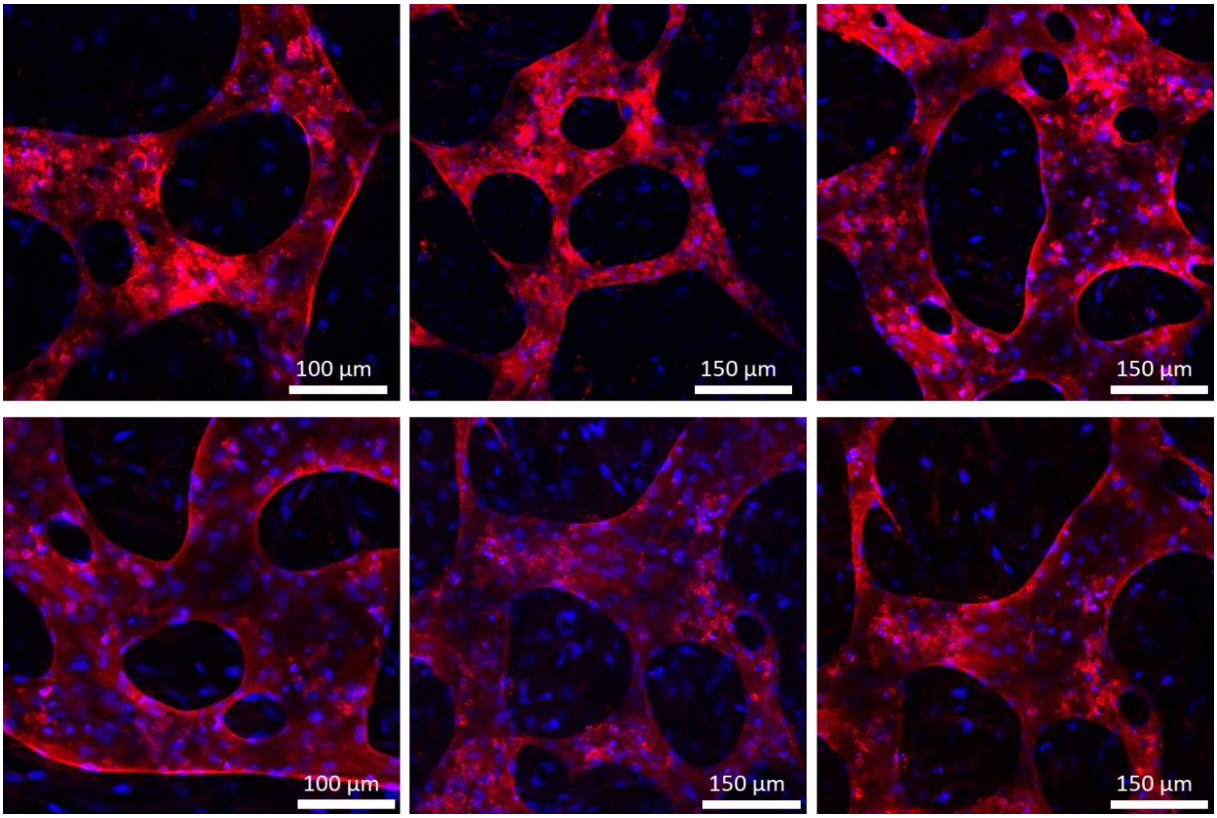

Figure S3: High magnification confocal fluorescence microscopy images of the microvascular network. Staining of CD-31 (red) and nuclei (blue).

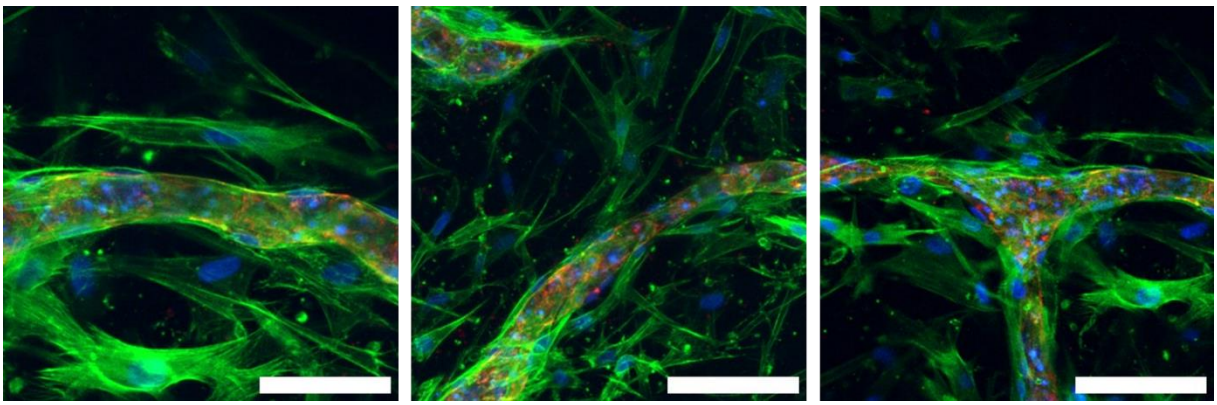

Figure S4: Confocal fluorescence microscopy images show HDF lining and stabilizing the walls of the microvascular network. Staining of CD-31 (red), actin filaments (green) and nuclei (blue). Scale bar showing 100 µm.

4. Perfusion of microvascular networks

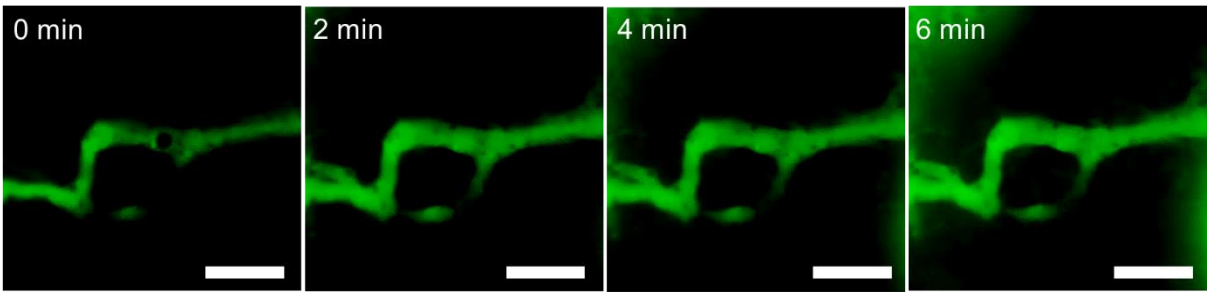

Figure S5: Perfusion with a 70 kDa FITC-dextran shows open lumen of vessel networks as well as tightness of vessel walls over the course of time. Scale bar showing 200 µm.

5. Confocal microscopy images of microvascular networks on day 14 with z-slices

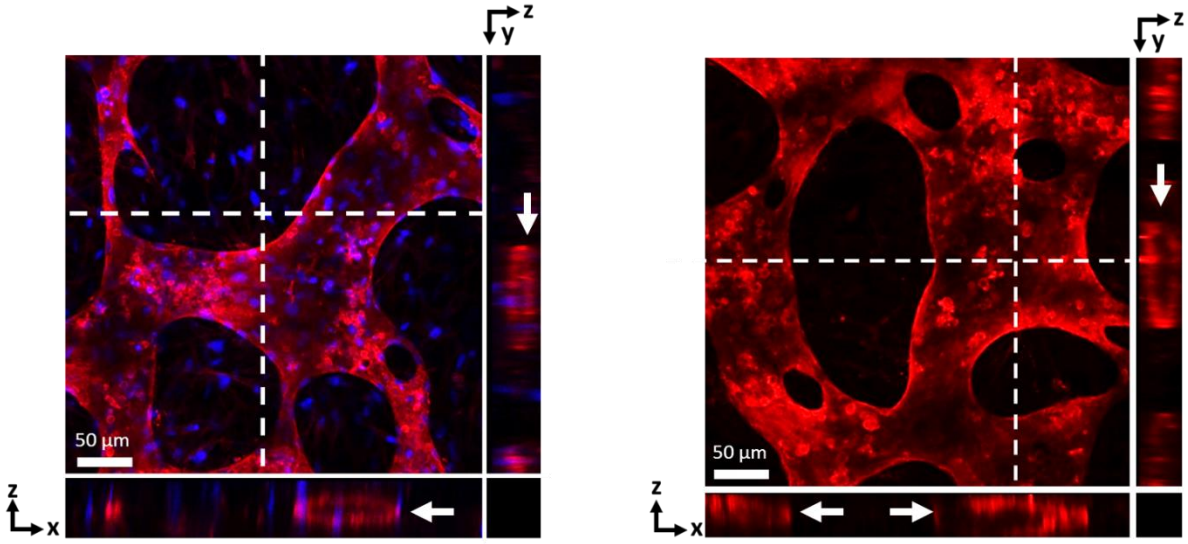

Figure S6: High magnification confocal fluorescence microscopy images of the microvascular network. Staining of CD-31 (red) and nuclei (blue). Z-slices in x and y direction are shown along the image edges. The slice line is indicated by a dashed white line. White arrows mark the open lumen visible in the z-direction images of microvascular networks on day 14. Stack height is 100 µm.

6. Vessel analysis

To obtain vessel parameters, confocal z-stack images were taken of the whole chamber with an image size of 800 x 800 µm^2 with 16 - 20 images per chamber at a pixel size per image of 1772 x 1772 pixels. In a first step, images were binarized using Fiji (Figure S7). In detail, images were binarized by thresholding them and adding further binarization operators such as dilation, erode, noise reduction and median. The binarized image was used to determine the number of white pixels n_{white} and to obtain the vessel area using a python script. The vessel area per chip A_v is a summary of the vessel areas per image $A_{v,i} = n_{white} \cdot \left(\frac{800}{1772}\right)^2$. The vessel percentage of the chip is then calculated as $C_v = \frac{A_v}{A_{chamber}}$. The next steps are based on the Matlab (The MathWorks, Natick, USA) image processing library. First, the image is binarized with the command *bwskel*. The branch and end points are determined and the branches cut free using *bwmorph*. The geodesic distance between end and branch points along the skeleton is obtained with *bwdistgeodesic* and gives the length of each individual branch. The mean thickness of each branch is determined using *bwdist* that measures the distance transformed along the skeleton to the edge of each vessel.

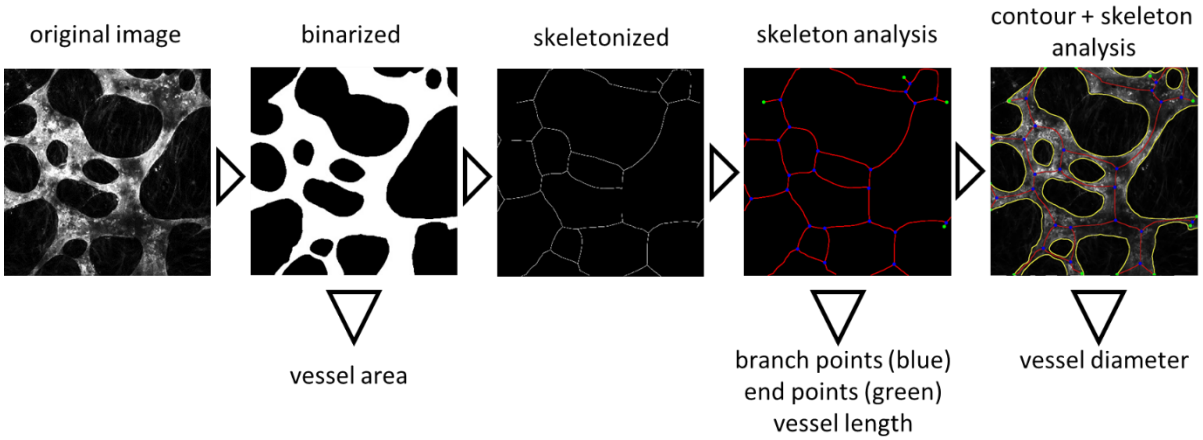

Figure S7: Image processing steps in the analysis of vessel parameters.