ACCSCIENCE PUBLISHING

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RESEARCH ARTICLE

Successful endothelial monolayer formation on melt electrowritten scaffolds under dynamic conditions to mimic tunica intima

Supplementary File

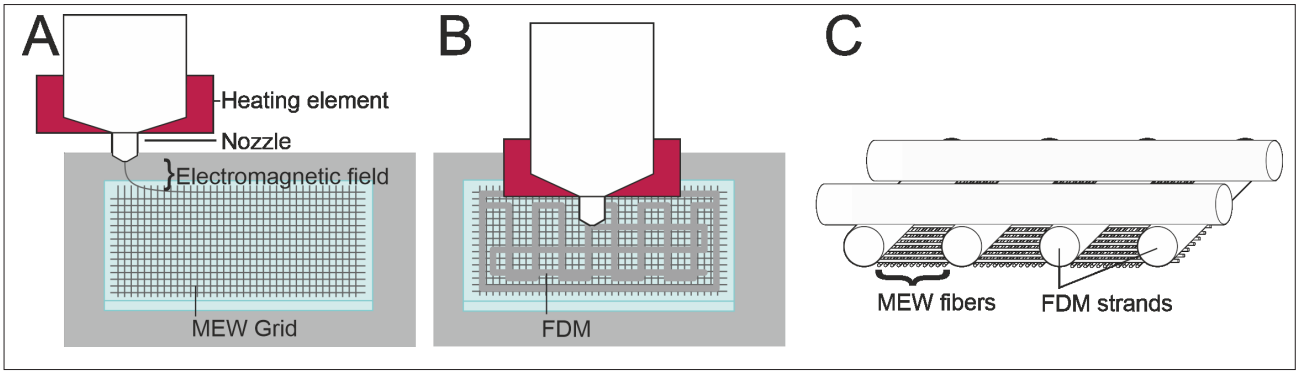

Figure S1. Printing process for vascular scaffolds. (A) The printing of microscale melt electrowriting (MEW) fibers using a strong electromagnetic field on a glass slide. (B) The printing of supporting structures on top of the MEW fibers using fused deposition modeling, after the printing of microscale fibers for cell adhesion. (C) Schematic illustration of the resulting scaffolds.

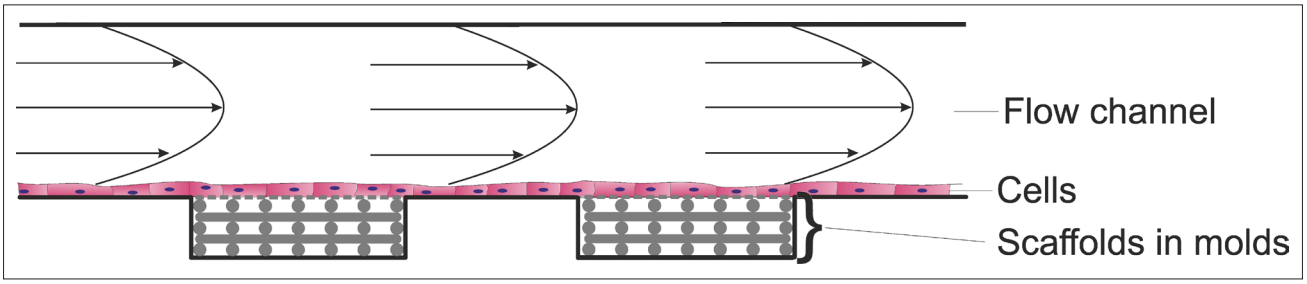

Figure S2. Experimental setup for cells under flow. Scaffolds were placed into flow chambers (μ-slides I Luer 3D, ibidi GmbH, Gräfelfing, Germany), and the whole chambers were seeded with endothelial cells. After 7 days of static culture conditions to promote cell adhesion to the scaffold, flow was applied to simulate *in vivo* blood flow for 3 days. Flow resulted in shear stress reaching up to 10 dyn cm^{-2}. After dynamic cultivation, the scaffolds were removed from the chambers and fixated, stained, and evaluated.

```
//Fiji setup
run("Options...", "iterations=1 count=1 black do=Nothing");
run("Set Measurements...", "area mean standard modal min centroid center perimeter bounding fit shape feret's integrated median
skewness kurtosis area_fraction stack display redirect=None decimal=3");
run("Clear Results");

//image preparation
input_dir = getDirectory("image");
originalImage = getTitle();
run("Split Channels");
selectWindow("C1-"+originalImage);
rename(originalImage+" _analyzed");
run("Duplicate...", " ");
selectWindow(originalImage+" _analyzed")

//image processing:
run("Pseudo flat field correction", "blurring=50 hide");
run("Subtract Background...", "rolling=100");
run("Enhance Contrast...", "saturated=0.1");
run("Find Edges");
run("Unsharp Mask...", "radius=20 mask=0.80");
setAutoThreshold("Default dark no-reset");
setOption("BlackBackground", true);
run("Convert to Mask");
run("EDM Binary Operations", "iterations=2 operation=close");
run("Invert");
run("Fill Holes");
run("Invert");
run("Skeletonize");
run("EDM Binary Operations", "iterations=1 operation=dilate");
run("Invert");

//object analysis
run("Analyze Particles...", "size=200-3500 display clear exclude clear overlay add composite summarize");
selectWindow("Results");
saveAs("Measurements", input_dir + originalImage + "_Auswertung.tsv");
selectWindow(originalImage+" _analyzed-1");
roiManager("Show None");
roiManager("Show All");
saveAs("Tiff", input_dir + originalImage+ "_ausgewertet.tif");
```

Figure S3. Fiji macro used for cell detection. The macro accomplished the image processing for evaluation and conversion to a binary image for measurement. Therefore, flat field corrections, background subtraction, contrast enhancements, and image filters, such as "find edges" and "unsharp mask," were used for image enhancements. After image processing, the respective image was converted to a binary image and measured using boundaries to exclude image artifacts. The result of this macro can be seen in Figure S2.

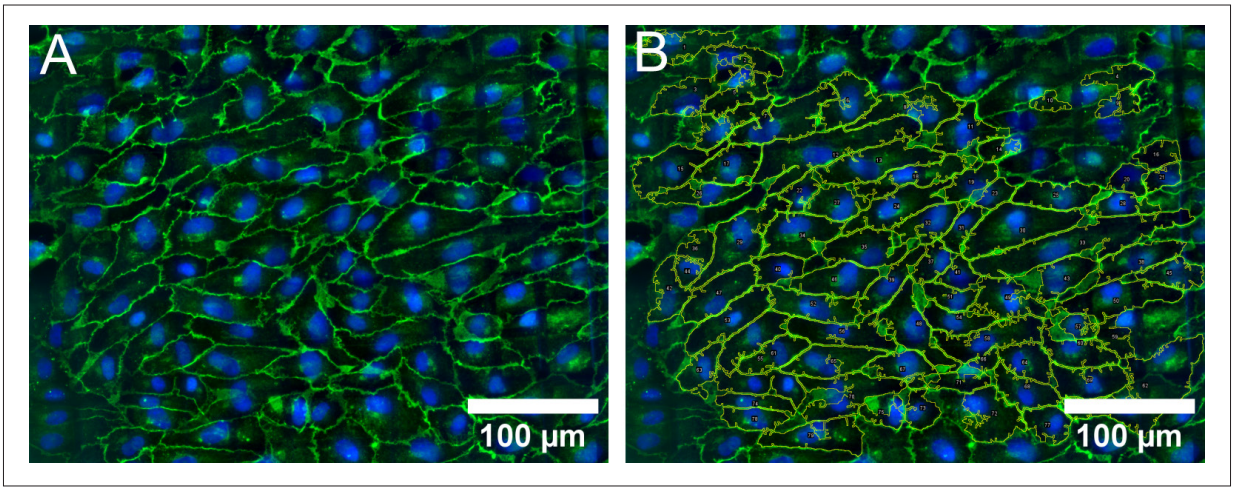

Figure S4. *Cell detection before and after accomplishment of the Fiji macro.* (A) Cell staining before Fiji macro image enhancement. Cell nuclei were stained in blue, and VE-cadherin was stained in green (B). After the Fiji macro procedure, non-peripheral cells in the cell staining images were detected with a high probability.