ADVANCED FUNCTIONAL MATERIALS

Supporting Information

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High-Throughput Scaffold System for Studying the Effect of Local Geometry and Topology on the Development and Orientation of Sprouting Blood Vessels

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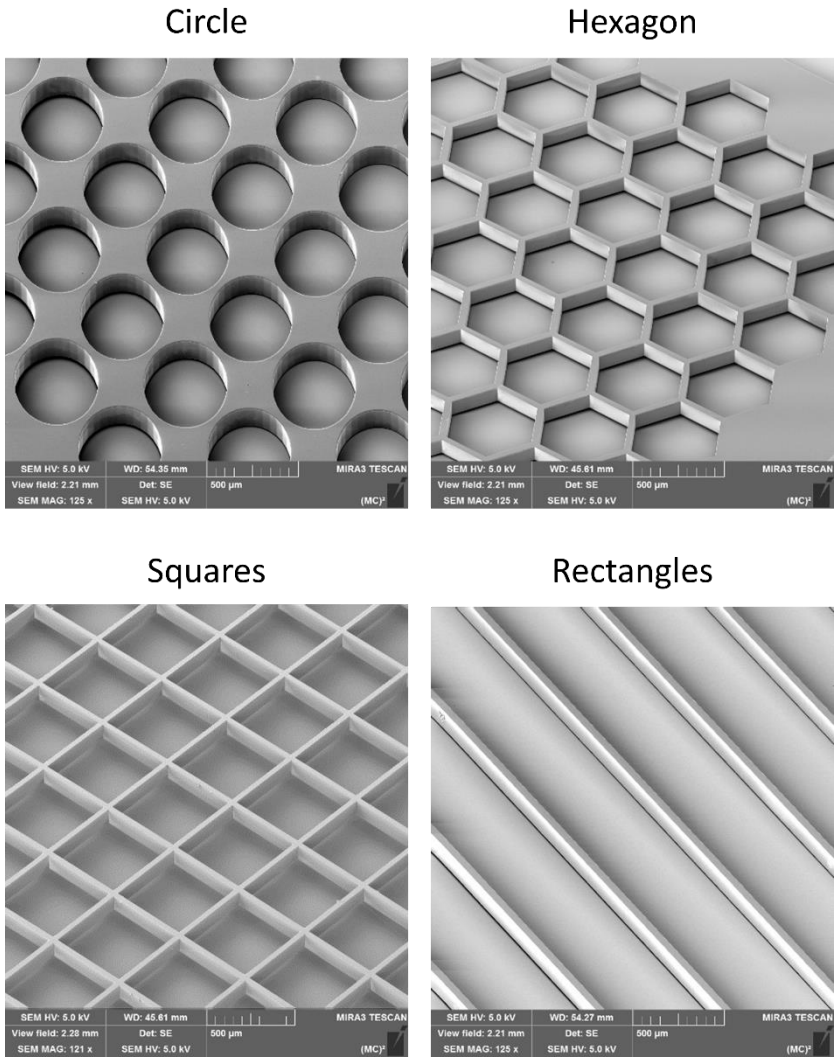

Figure S1. Scanning electron microscopy images of the fabricated scaffolds.

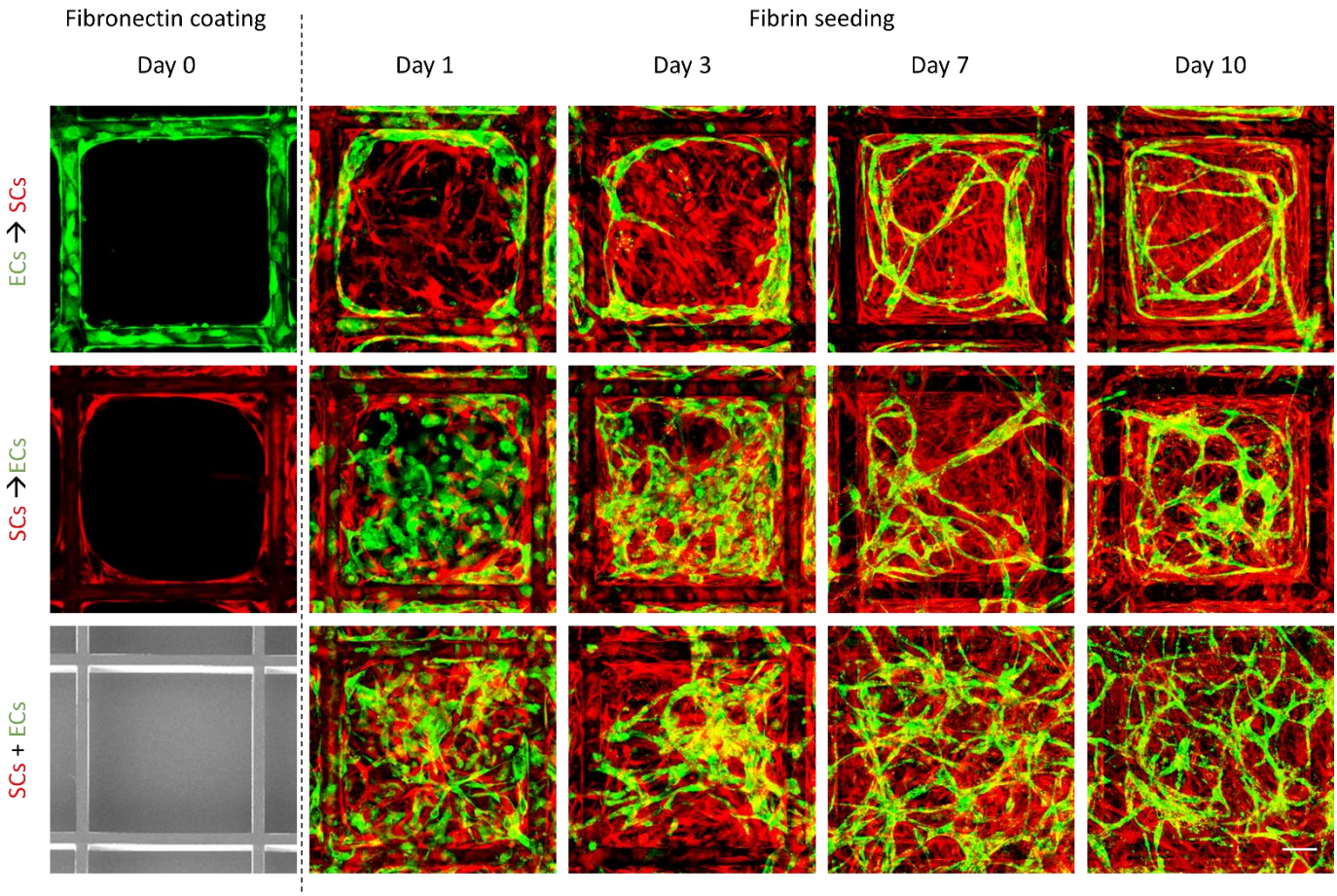

Figure S2. Different seeding orders for vascular network formation. Representative confocal images at different time points of vascular network formation for different seeding orders. Day 0 represents the day in which the second cell type is added on the already seeded scaffolds. Top: ECs (green) seeded on fibronectin-coated scaffold, and subsequent addition of SCs (red) suspended in fibrin gel. Center: SCs seeded on fibronectin-coated scaffold, and subsequent addition of ECs suspended in fibrin gel. Bottom: Both cell types suspended in fibrin gel and seeded on fibronectin-coated scaffold (Scale bar: 100 μm)

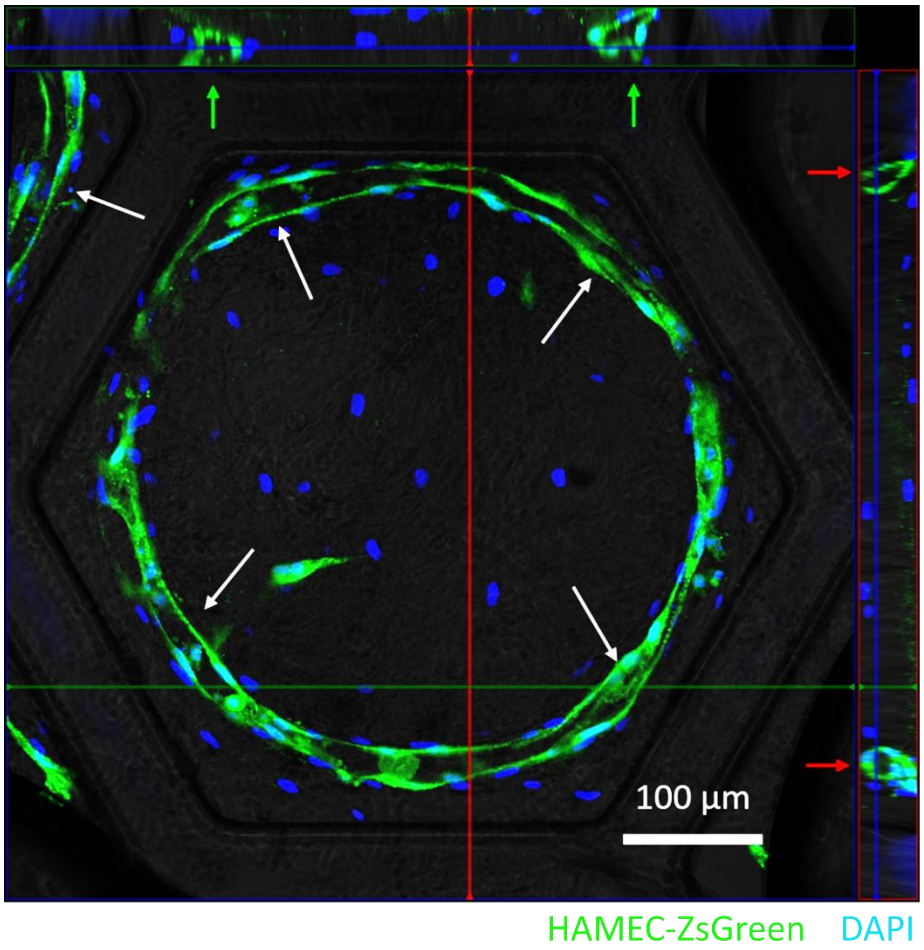

Figure S3. Vessels adjacent to the compartment walls are hollow. Representative image of the tubulogenesis phenomenon on vessels surrounding the compartment walls (green: HAMEC-ZsGreen, blue: DAPI). The blue, green and red boxes show cut views for the xy, xz and yz planes respectively. The arrows evidence the hollow structure adopted by the vasculature.

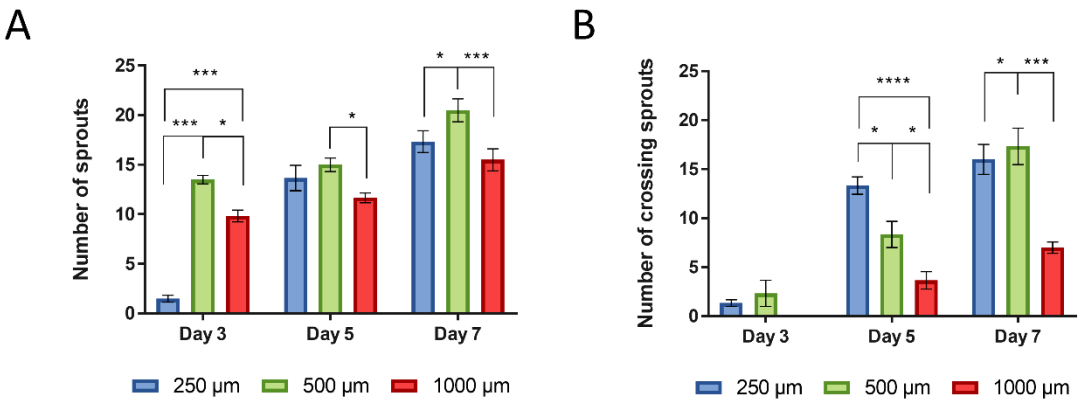

Figure S4. Effect of wall-to-wall distance on sprouting angiogenesis within rectangular compartments. Quantification of emerging sprouts from vessels adjacent to the compartment scaffold walls. Sprouts were quantified for scaffolds with wall-to-wall distances of 250 µm, 500 µm and 1000 µm, at days 3, 5 and 7. A) Quantification of emerging sprouts. B) Quantification of vessels emerging from a vessel and reaching to the opposing wall vessel (n>5; *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001).

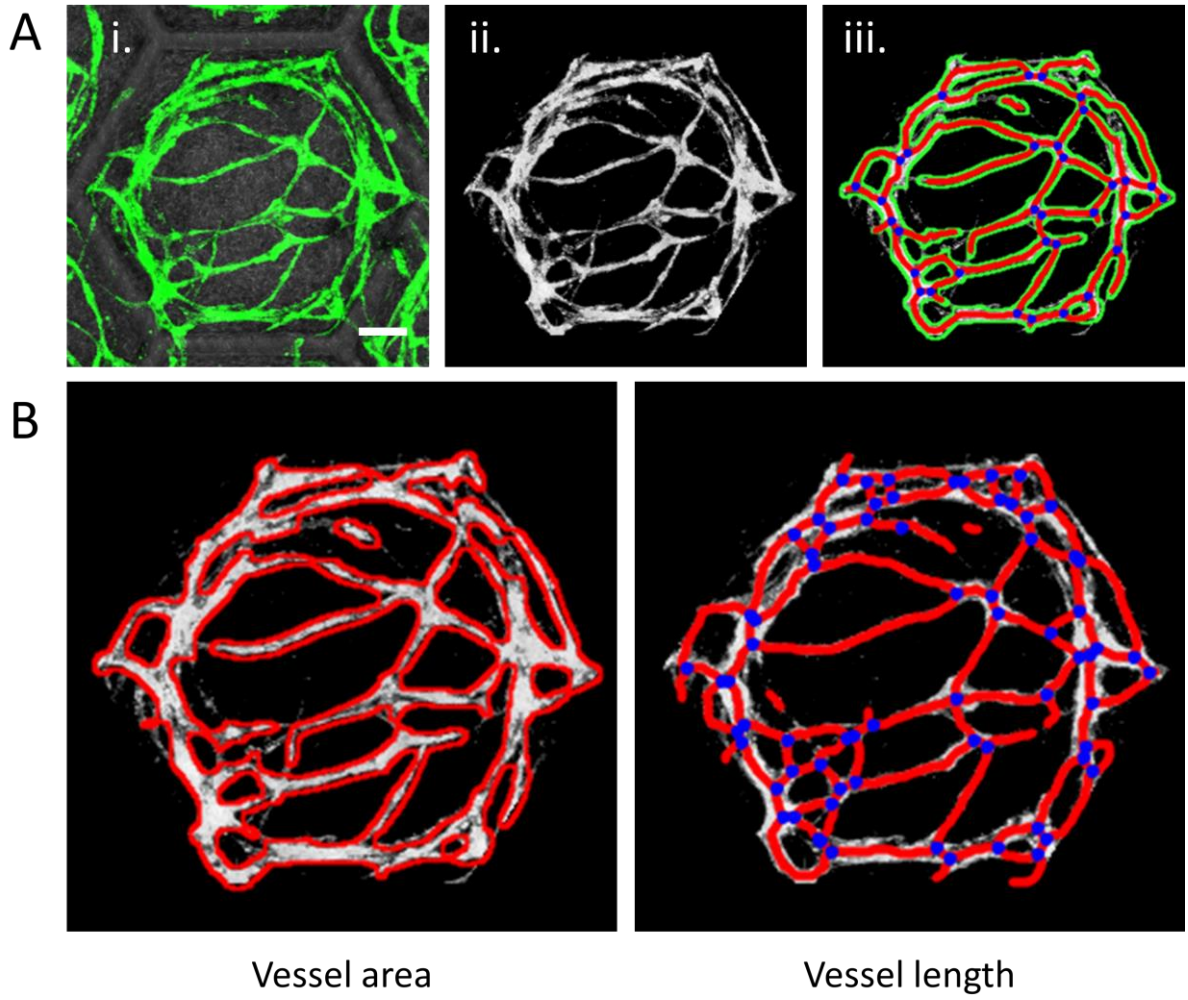

Figure S5. Quantification of vascular networks parameters. A) Representative images of the performed steps to quantify the vessel total length and vessel total area using the Angiotool software: i. Vessel networks (green) are imaged using confocal microscopy (scale bar, 200 µm). ii. To observe the vascular development within the compartments, vascular elements surrounding the compartments are eliminated. iii. Resulting images are analyzed using Angiotool software. The vessels are marked by red lines, following the network skeleton; the green perimeter shows the delimited vessel area; and the blue dots mark the branching points B) Visual results from the Angiotool analysis: left, the vessel area is calculated as the area of the vessels surrounded by the red perimeter; right, the total vessel length is calculated as the total length of the processed vessel skeleton, represented by the red lines.

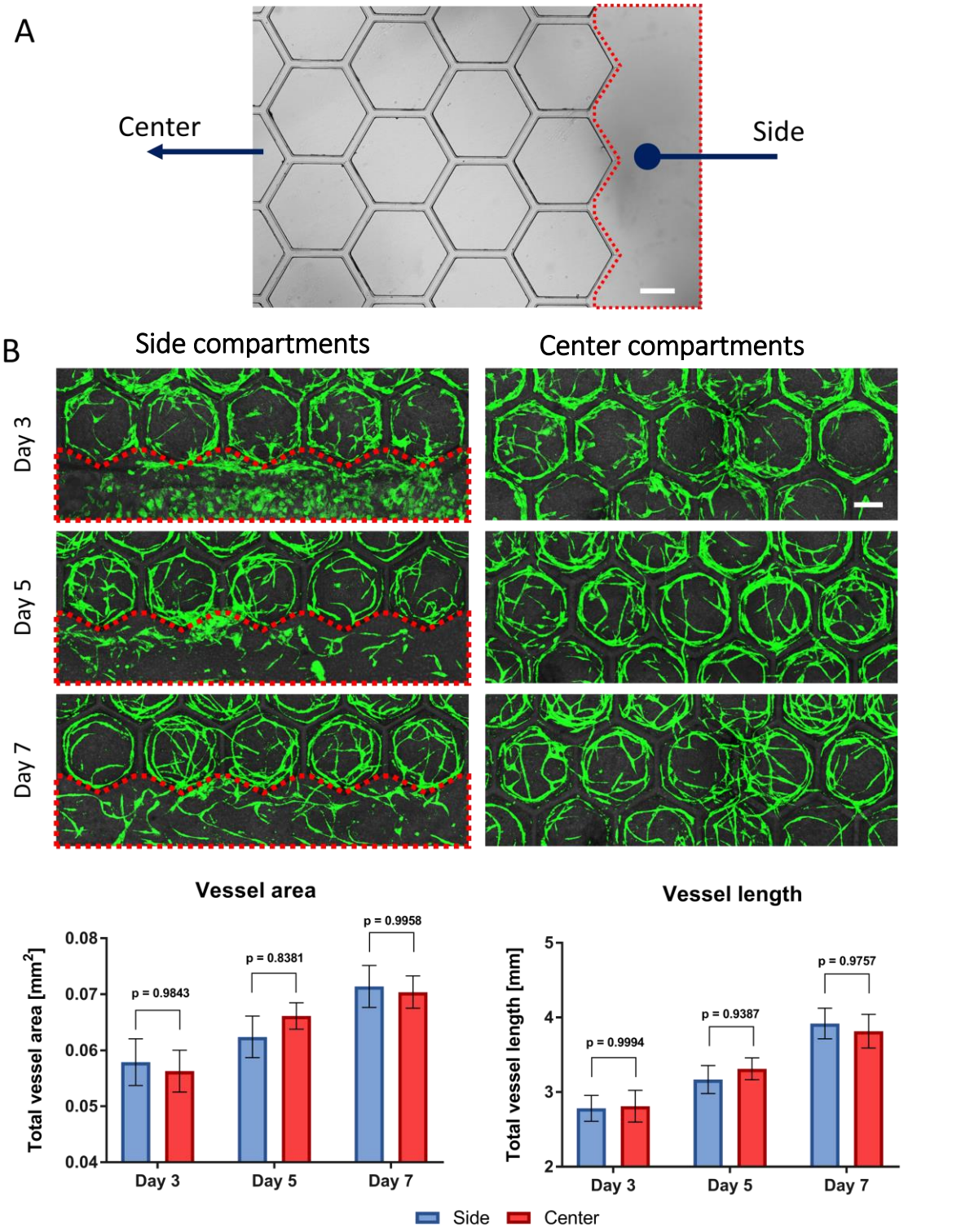

Figure S6. Cells located on the scaffold flat surfaces do not affect the network development within the adjacent compartments. A) Image of a hexagonal compartmented scaffold. The compartments can either be completely surrounded by other compartments (center compartments) or be adjacent to the scaffold side (side compartments), which has a wide flat surface (red dotted line; scale bar, 200 µm). B) Vascular network development of vessels

within compartments located at the center or at the side of the scaffold. Top: Representative images of vessel networks either within compartments located adjacent to the flat side surface (red dotted line) or compartments located center wise, for days 3, 5 and 7. The flat surface is populated by ECs that independently form vascular networks (scale bar, 200 μm). Bottom, comparison of total vessel area and total vessel length for networks located in compartments at the side versus compartments located at the center (n>5). The high p-values demonstrate that the vessels located over the flat surface next to the compartments have no significant impact on the development of the vessels within the adjacent compartments.

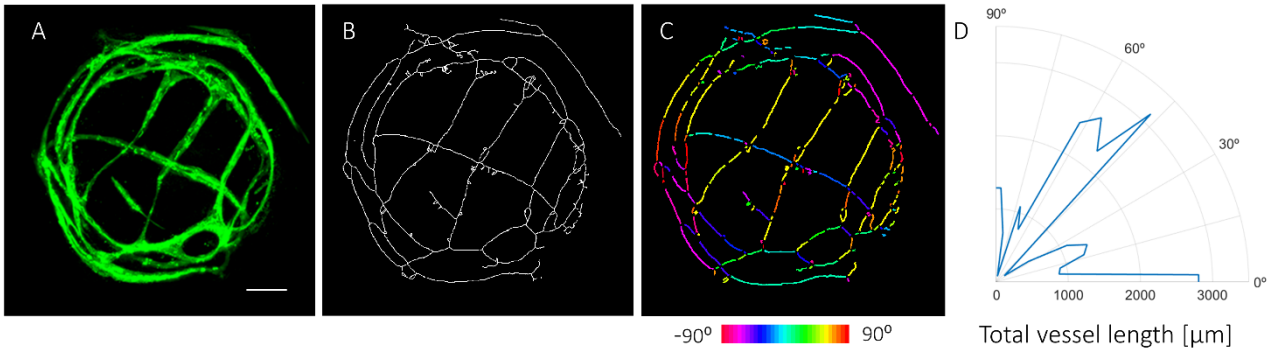

Figure S7. Quantification of total vessel length orientation. Representative images of the steps to quantify the vessel orientation: A) Vessel networks (green) are imaged using confocal microscopy (scale bar, 200 µm). B) Images are skeletonized and transformed into single vessel elements. C) Elements are classified according to the angle of their major length axis. D) The elements lengths are accumulated within bins of 5º and plotted on a radial axis. The distance from the origin represents the total length of all the binned elements, and the angle represents the orientation of the bin. Plots are represented from 0º to 90º due to the compartments symmetry along the x axis.

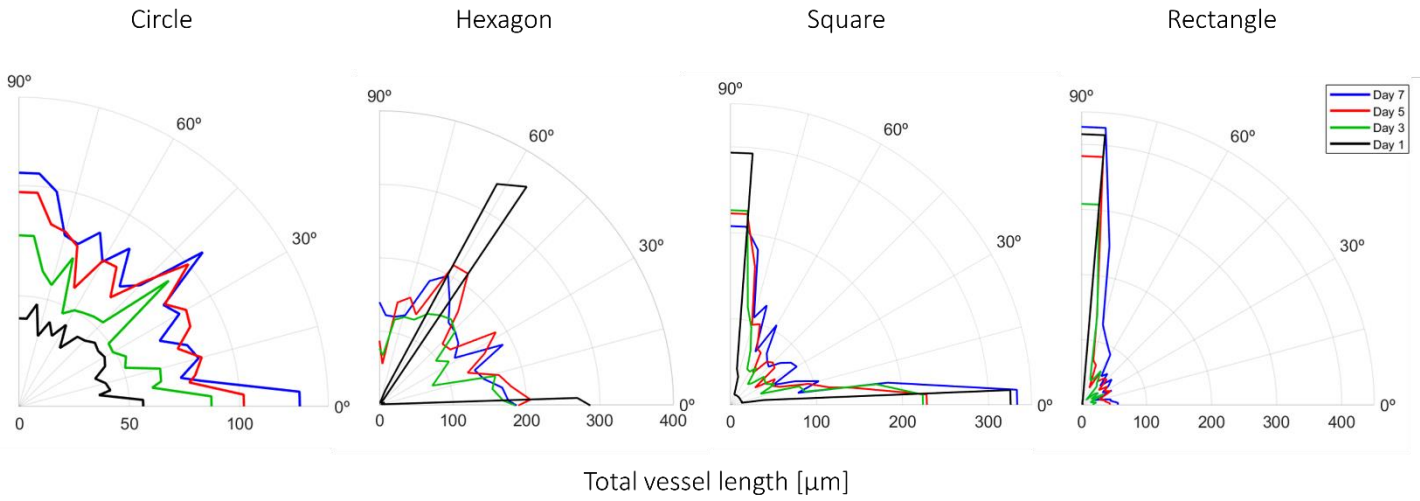

Figure S8. Total vessel length angle distribution without normalization to day 1. The polar plots show the length angular distribution at days 1, 3, 5, 7. It is evident the effect of the vessels located next to the scaffold walls. The necessity of reducing these peaks arise from their great magnitude in comparison to the signal of interest, i.e. the vessels in the interior of the compartments.

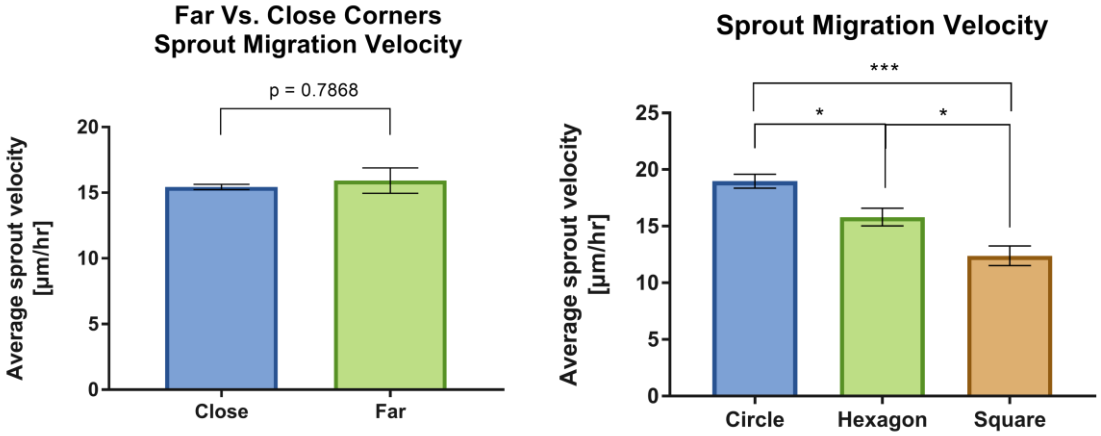

Figure S9. Vessel sprouting migration speeds depend on compartment topology in closed geometry compartments. Left: within hexagonal compartments, migrating vessels speeds were measured and compared for new sprouts reaching to the nearest corner or to the farthest corner. No significant differences where observed within the same geometry (n>5). Right, comparison between the migration speeds of sprouts emerging from a side and reaching for a corner (within the square and hexagonal compartments), or travelling to the opposite side of the geometry, for the circular compartments (n>5; *$p < 0.05$, ***$p < 0.001$).

Table S1. Compartment shapes characteristic parameters

Shape	Characteristic length d= 500 µm [a)]	Area [µm^2]	Perimeter [µm]	Area-to-perimeter ratio [µm^2/µm]	Circularity factor (Isoperimetric quotient) [c)]
Circle	Diameter	196,349	1570.79	125	1
Hexagon	Minimal diameter	216,506	1732.05	125	0.9069
Square	Side	250,000	2000	125	0.7854
Rectangles	Wall-to-wall/Gap distance	247,500 [b)]	1000	225	N/A

[a)] See Figure 1Aiii;

[b)] Area of ROI – Area of single band = 500·500 [µm^2] – 50·500 [µm^2];

[c)] $C = f_{circularity} = \frac{4\pi \cdot Area}{Perimeter^2}$

Table S2. Statistical analysis of Figure 2

Day	Compared geometries	Vessel area		Vessel length		Normalized vessel thickness	
Day 1	Circle vs. Hexagon	0.0295	*	0.5241	ns	0.0007	***
	Circle vs. Square	0.9548	ns	0.9639	ns	0.9849	ns
	Circle vs. Rectangle	<0.0001	****	0.0033	**	0.4699	ns
	Hexagon vs. Square	0.0021	**	0.193	ns	0.0005	***
	Hexagon vs. Rectangle	0.1145	ns	0.1506	ns	0.1167	ns
	Square vs. Rectangle	<0.0001	****	0.0001	***	0.5863	ns
Day 3	Circle vs. Hexagon	0.5233	ns	0.8024	ns	0.457	ns
	Circle vs. Square	0.0025	**	<0.0001	****	<0.0001	****
	Circle vs. Rectangle	<0.0001	****	<0.0001	****	<0.0001	****
	Hexagon vs. Square	0.2173	ns	0.0003	***	0.0002	***
	Hexagon vs. Rectangle	<0.0001	****	<0.0001	****	<0.0001	****
	Square vs. Rectangle	<0.0001	****	<0.0001	****	0.0035	**
Day 5	Circle vs. Hexagon	0.9924	ns	0.9957	ns	0.8682	ns
	Circle vs. Square	0.1055	ns	0.0004	***	0.0016	**
	Circle vs. Rectangle	<0.0001	****	<0.0001	****	0.5143	ns
	Hexagon vs. Square	0.0518	ns	0.0006	***	0.0099	**
	Hexagon vs. Rectangle	<0.0001	****	<0.0001	****	0.9005	ns
	Square vs. Rectangle	<0.0001	****	<0.0001	****	0.0963	ns
Day 7	Circle vs. Hexagon	0.5987	ns	0.3376	ns	0.9206	ns
	Circle vs. Square	0.0005	***	<0.0001	****	0.0629	ns
	Circle vs. Rectangle	0.014	*	0.4741	ns	0.1879	ns
	Hexagon vs. Square	0.048	*	0.0004	***	0.2258	ns
	Hexagon vs. Rectangle	0.0006	***	0.0228	*	0.5002	ns
	Square vs. Rectangle	<0.0001	****	<0.0001	****	0.9488	ns

Movie S1. Time-lapse of multicolored endothelial cells forming *de novo* vessels (tubulogenesis) and new sprouts emerging and migrating within the scaffold compartment.

Movie S2. Fluorescent and contrast imaging of vessel development in square tessellated scaffold.

Movie S3. Red fluorescent beads show the displacement within the gel for the initial tubulogenesis stages.