Supporting Information

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SI Methods

Cell Culture and Reagents. ***Human mesenchymal stem cells.*** Human Bone marrow aspirates are obtained from donors after written informed consent, and human Mesenchymal Stem Cells (hMSCs) are isolated as follows. Aspirates are resuspended by using 20-gauge needles, plated at a density of 5×10^5 cells per square centimeter and are cultured in hMSC proliferation medium containing α-MEM (Life Technologies), 10% FBS (Cambrex), 0.2 mM ascorbic acid (Asap; Life Technologies), 2 mM l-glutamine (Life Technologies), 100 units/mL penicillin (Life Technologies), 10 μg/mL streptomycin (Life Technologies), and 1 ng/mL basic FGF (Instruchemie). Cells are grown at 37 °C in a humid atmosphere with 5% CO_2. Medium is refreshed twice a week, and cells are used for further subculturing or cryopreservation. For experiments, the hMSC medium (termed Exp. Medium) is serum-free and composed of Dulbecco's Modified Eagle's Medium supplemented with $10^{-}7$ M dexamethasone, 50 mg/mL ascorbate 2-phosphate, 40 mg/mL proline, 100 mg/mL pyruvate, and 50 mg/mL ITS1+Premix (Becton-Dickinson, MA: 6.25 mg/mL insulin, 6.25 mg/mL transferrin, 6.25 ng/mL selenious acid, 1.25 mg/ml bovine serum albumin, 5.35 mg/mL linoleic acid).

Human umbilical vein endothelial cells. Human umbilical vein endothelial cells (HUVECs) are purchased from Lonza (Lonza, group Ltd. Switzerland). Cells are grown at 37 °C in a humid atmosphere with 5% carbon dioxide (CO2) in endothelial growth medium-2 (Cambrex). Cells are routinely split at a 1:5 ratio and cultured in fewer than five passages. Only HUVECs from passage 3 or 4 are used to seed the coculture experiments.

MicroWell Array (MWA) and Shaped Well Array (SWA) Agarose chips. Micropatterned agarose chips for nonadherent cell culture are formed by replica molding. MWA: silicon wafers are etched to form cylindrical wells of 200 um diameter and 160 um deep. The interspace between wells is 100 um. Each chip contains 2,865 wells and a total diameter of 11.5 mm. SWA: SU-8 photoresist is patterned on top of a silicon wafer by depositing three layers to obtain a total height of 700 um. Wells are geometric (circle, square, triangle) and have a constant surface area of 1 mm^2. One SWA includes 15 geometric wells and has a total diameter of 11.5 mm. Structures from MWA and SWA are replicated in poly(dimethylsiloxane) (PDMS; Sylgard 184, Dow Corning) with a total thickness of 8 mm, individual chips are punched, cleaned, and sterilized in ethanol. Routinely, in the laminar flow cabinet, the PDMS stamps are deposited in the center of the well of a 6-well plate (microstructures on top) and a boiling solution of 3% agarose (Ultra pure agarose, Invitrogen) is poured on the PDMS stamp (8 mL), quickly centrifugated ($1,300 \times g$ for 1 min) to remove air bubbles and let to solidify. Upon solidification, agarose chips are demolded using a spatula and sterile gloves, punched to 11.5 mm diameter and placed in a 12 well plate. After wetting using corresponding Exp. medium (500 microliters), a concentrated suspension of cells (0.4 millions) is seeded, allowed to settle for 15 min on the chip and 1.5 milliliters of Exp. medium is then added. Cell clusters (approximately 500 cells/cell cluster) spontaneously fuse within 24 h are cultured for 150 h. Half of the medium is changed every day of culture. After 150 h, cell clusters are flushed out using Exp. medium, centrifuged ($340 \times g$, 1 min), resuspended in the Exp. medium and seeded on the SWAs. 20,000 cell clusters are seeded per SWA. Clusters are allowed to settle in the wells for 1 min then centrifuged ($340 \times g$, 1 min). Cell clusters which do not settle in the wells are harvested using a pipet and seeded again. For optimal seeding, 2–3 rounds of seeding are done. 1.5 mL of Exp. medium is added. Half of the medium is changed every day of culture. Cell clusters fuse within 24 h. Resulting tissues contain approximately 200.000 cells, have a surface area of 1 mm^2, and a thickness of 250 um.

Coculture. A coculture of 92% hMSC and 8% huvEC in Exp. Medium (as described above) are used for all experiments. Half the medium is replaced every day. Blebistatin (50 mM, Sigma-Aldricht) and Y-27632 (10 mM, Calbiochem) are added 24 h after seeding microscale clusters on the SWA. Half the medium is replaced every day with constant total concentrations of 50 mM of Blebistatin and 10 uM of Y-27632 until day 5. VEGF scavenging is supplemented to the culture medium at 2 μg/mL. The anti-rh-VEGF (R&D systems) antibody which binds both to VEGF165 and VEGF121. Pellet culture for the experiments of Fig. 4 *G* and *H* are done by pulling 0.5 million cells (92% hMSC and 8% huvEC) in a 10 mL tube, centrifuging to form a pellet ($340 \times g$, 2 min). 5 mL of medium is added. Pellets rounded up and form a sphere within 48 h and are cultured for 6 d.

Immunohistochemical Analysis. Tissue are cultured for 5 d after seeding on the SWA with or without Y27632+Blebistatin. After harvesting, tissues are frozen in Cryomatrix at −60 °C. Sections (7 μm) are cut with a cryotome. Sections are fixed in cold acetone for 5 min and air-dried. Sections are rehydrated for 10 min, after which they are incubated for 30 min with 10% FBS in PBS to block nonspecific background staining. Sections are incubated with monoclonal mouse antihuman PECAM-1 antibody (Dako), a monoclonal rabbit antihuman VEGF Carboxyterminal end antibody (Abcam), a monoclonal rabbit antihuman VEGFR2 antibody (Cell Signaling), a rabbit antihuman HIF1a antibody (Abcam), Phalloidin-Alexa fluor 488 conjugated antibody (Invitrogen), or a monoclonal mouse antihuman Phospho-Myosin Light Chain 2 (Ser19) (Cell signaling technology) for 1 to 4 h. Sections are washed in PBS and subsequently incubated with the secondary antibody (Alexa Fluor 488 or 594 antibody, Invitrogen). Samples are counterstained with Dapi (Sigma). Cellular proliferation is assessed by EdU (5-ethynyl-2'-deoxyuridine) incorporation over a period of 12 h ending at 24, 96, and 144 h of culture. The cell proliferation assay is performed using the Click-iT™ EdU kit (Invitrogen). Pictures are taken using a confocal microscope (Leica LSM500). To prevent biased measurements, quantifications of the area of PECAM-1^+ cells on Figs. 3 and 4 are done using a threshold applied on images based on size and circularity (Image J) to select for the PECAM-1^+ structures which elongated in the main plane of the tissue. Thresholded PECAM-1^+ structures are termed vascular structures (VS). Quantification on Fig. S5 is done without a threshold. For quantification of Figs. 3 and 4, each image is divided into 20 boxes from the center to the periphery. Each box is used for quantification of either the number of nuclei, the area of VS, the intensity of VEGF fluorescence, or the intensity of the VEGFR-2 fluorescence. Results are the average of five nonconsecutive cuts for each three different biological samples. Results are used to create the 20 boxes-graphics presented in Fig. 3*B* or pooled to create graphics (Fig. 3 *A*, *D*; Fig. 4 *A*–*F*). Color maps (Fig. 3) show values, in five consecutive regions ranging from the tissue center to the tissue corners, of VS density (scale from 1 to 4.8) and VS/nucleus density (scale from 1 to 2.5) normalized to the central region. The same scale is used for normal and impaired compaction. Contribution of tissue density to VS density is measured as

the expected VS density due to a strict effect of compaction as compared to experimental VS density. Color maps (Fig. 4) show values for VEGF density per nucleus (scale 1 to 3) and VEGFR2 intensity per PECAM-1$^+$ area (scale 1 to 17) in five consecutive regions ranging from the tissue center to the tissue corner and normalized to the region of weakest intensity (central region, impaired compaction).

ELISA Assay. ELISA assay (R&D systems) was performed as described by the manufacturer.

Gene Expression Analysis by qPCR. The effect of tissue contraction and deformation on expression of angiogenic marker genes was analyzed from tissues cultured for 3 d on the SWA. RNA was isolated from a pool of five tissues by snap freezing the tissues in liquid nitrogen, crunching them using a pestle and by using an RNeasy mini kit (Qiagen). qPCR was performed by using SYBR green (Invitrogen) on a Light Cycler (Roche). Data were analyzed by using Light Cycler software version 3.5.3, using the fit point method by setting the noise band to the exponential phase of the reaction to exclude background fluorescence. Expression of angiogenic marker genes was calculated relative to DAPDH rRNA levels by the comparative ΔCT method. Primers were obtained from SAS Bioscience and used according to the manufacturer's protocol. Primers for VEGF isoforms had the following sequences:

human VEGF165 sense: ATCTTCAAGCCATCCTGTGTGC
human VEGF165 antisense: CAAGGCCCACAGGGATTTTC
human VEGF189 sense: ATCTTCAAGCCATCCTGTGTGC
human VEGF189 antisense: CACAGGGAACGCTCCAGGAC
human VEGF121 sense: GCGGATCAAACCTCACCAAG
human VEGF121 antisense: TCGGCTTGTCACATTTTTCTTG
human VEGF145 sense: GAATGCAGACCAAAGAAAGATAGAG
human VEGF145 antisense: TCGGCTTGTCACATACGCTCC

Statistics. Differences between groups are determined using a Student's T-test as described for each figure.

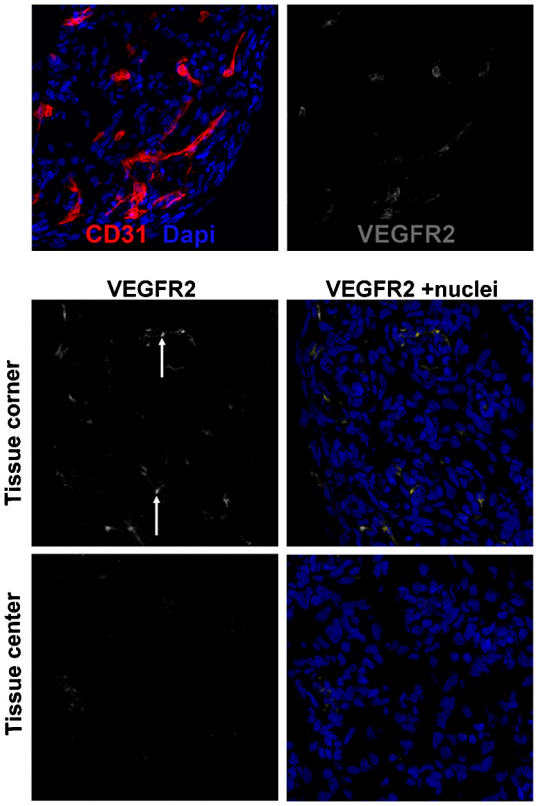


Fig. S1. VEGFR2+ cells strictly overlaps with PECAM-1$^+$ cells and form clusters in regions of high deformation. Top row: double staining for PECAM-1 and VEGFR2 shows that VEGFR2 expression is restricted to the PECAM-1$^+$ cells. Middle and bottom rows: VEGFR2 staining shows the formation of clusters of VEGFR2 at the cellular membrane, in regions of high deformation (middle row). Such clusters are not observed in the central region of the tissue (bottom row).

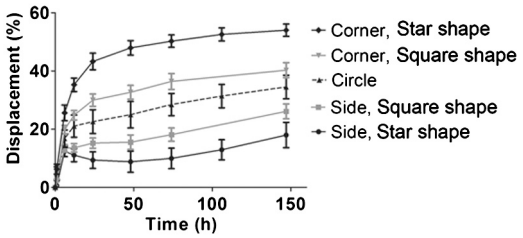


Fig. S2. Tissue displacement. The displacement of tissue borders is measured as relative to the initial distance between the center and the border using bright field pictures ($n = 5$). Displacement = 100% of the original distance. Standard deviations, $n = 10$.

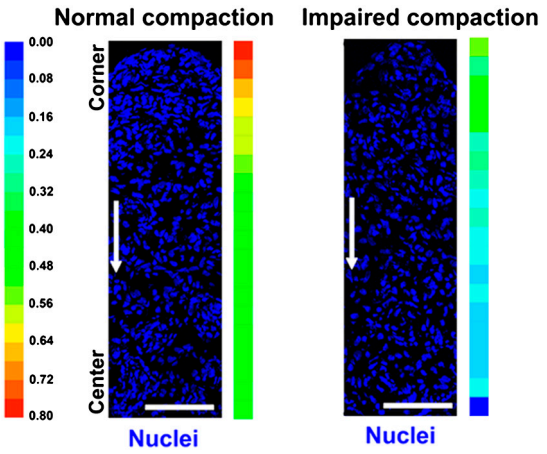


Fig. S3. Theoretical estimation of local strains. Local strains are estimated in star-shaped tissues for a strip from the center of the tissue to the corner (as displayed in Figs. 3, 4), based on nuclear density and macroscopic overall strain. As determined by light microscopy, normally contracting tissues display an overall strain of 0.54, whereas the overall strain is reduced to 0.27 in tissues with an impaired contraction. Based on these numbers, the average nuclear density in the unstrained state is estimated by dividing the average nuclear density in the strained state by 1 minus the overall strain. Finally, the local strains in twenty slices is determined by relating the local nuclear density in the strained state with the average nuclear density in the unstrained state.

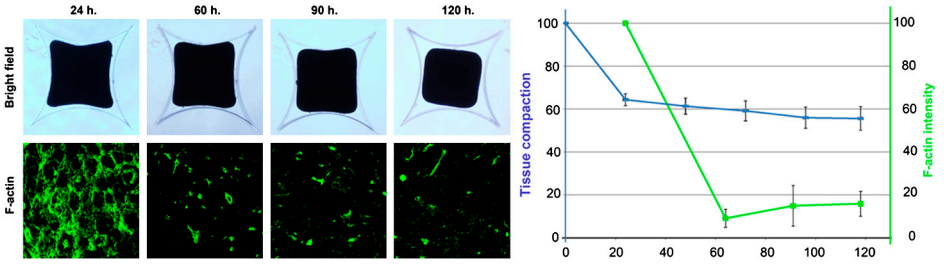


Fig. S4. The timeline of tissue compaction correlates with the F-actin intensity. Tissue contraction is assessed using bright field images and is quantified by measuring the projected area of tissues overtime ($n = 5$). F-actin fluorescent intensity is assessed using a phalloidin staining and is quantified using an histogram plot (Image J) (Standard deviations, $n = 5$).

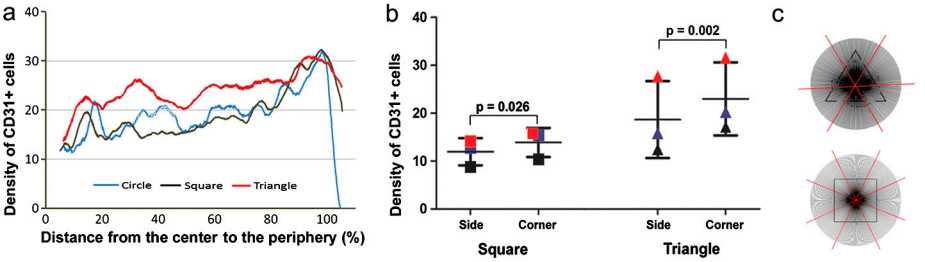


Fig. S5. Density of PECAM-1$^+$ cells. Radial-Profile Plot are done using five images of the total cross-section of a tissue for three different biological samples. Image J (http://rsbweb.nih.gov/ij/) is used to produce a profile plot of normalized integrated intensities around concentric circles as a function of distance from a point in the image. The intensity at any given distance from the point represents the sum of the pixel values around a circle. This circle has the point as its center and the distance from the point as radius. The integrated intensity is divided by the number of pixels in the circle that is also part of the image, yielding normalized comparable values (see details in http://rsb.info.nih.gov/ij/plugins/radial-profile.html). Shapes are divided into six or eight equal areas, as depicted by the red spokes (right schematics), values from each spoke and each area are averaged. The value is the average for one samples side and its respective corners. The statistical significance is calculated using a T-Test (one tail distribution, paired, standard deviations, $n = 3$).

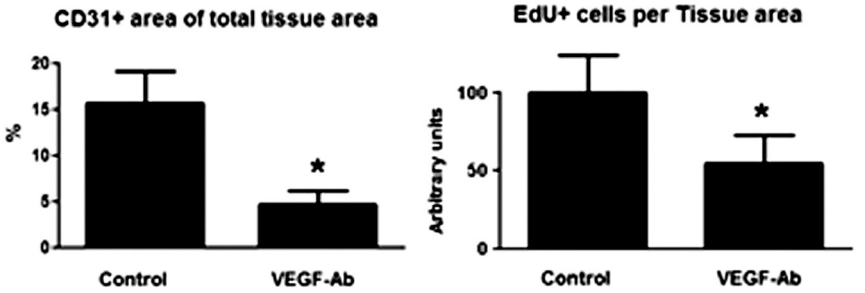


Fig. S6. VEGF scavenging with an antibody decreases PECAM1+ area and proliferation. In cell pellets, we observed that the scavenging of endogeneous VEGF using an antibody induces a decrease in the area covered by PECAM1+ cells and a decrease in cellular proliferation (EdU incorporation). Student T-Test, $n = 2$.

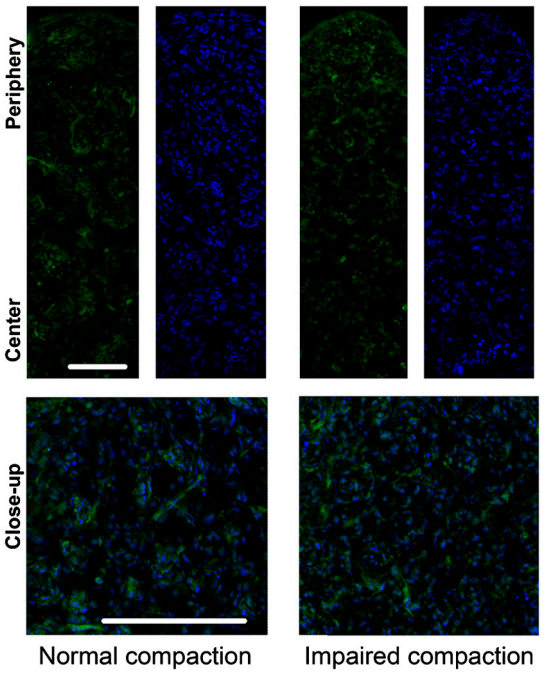


Fig. S7. Tissue hypoxia is homogeneous. Vascular morphogenesis is driven by local hypoxia which attract blood vessels through the activation of HIF1a which regulates VEGF production. HIF1a is constantly produced and degraded in the cytoplasma of cells and translocate to the nucleus upon hypoxia. Here, using antibody staining, we found that HIF1a is partly cytoplasmic and homogeneously expressed across the tissues. The homogenous expression of HIF1a suggests that the gradient of VEGF is not controlled by an underlying gradient of hypoxia. Scale bars are 100 micrometers.

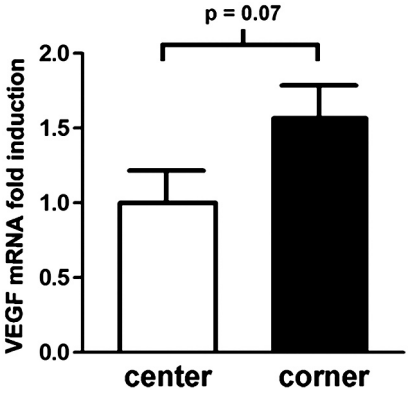


Fig. S8. VEGF expression. The corners of microfabricated tissues are dissected under a microscope and are separated from the center of the tissues (day 5 on SWA, $n = 15$ microfabricated tissues). qRT-PCR for VEGF-A mRNA is performed ($n = 3$ groups) as described in *Methods* (main text). VEGF-A mRNA is 1.5 times higher in tissue corners as compared to tissue centers (n.s. $p = 0.07$).

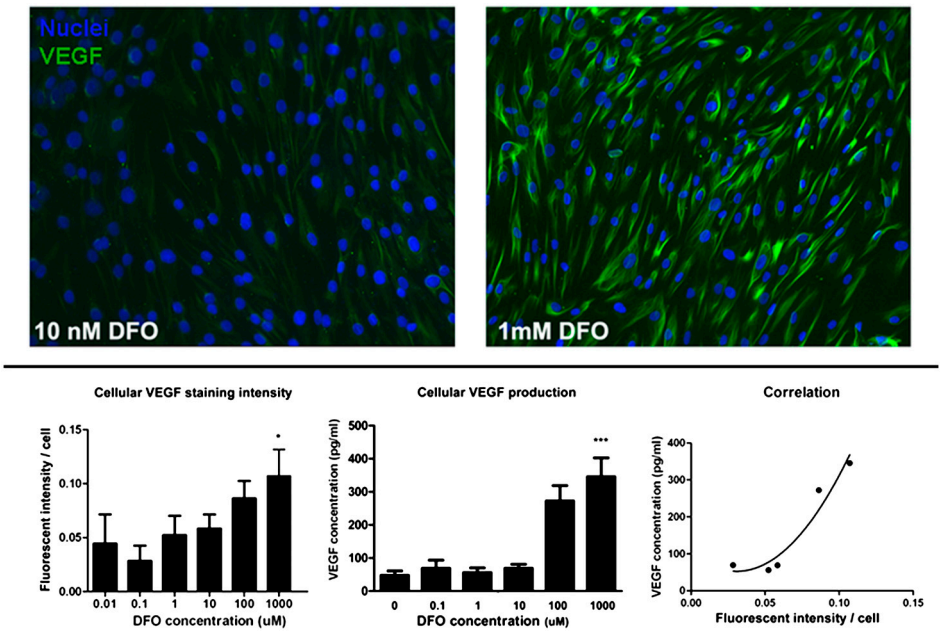
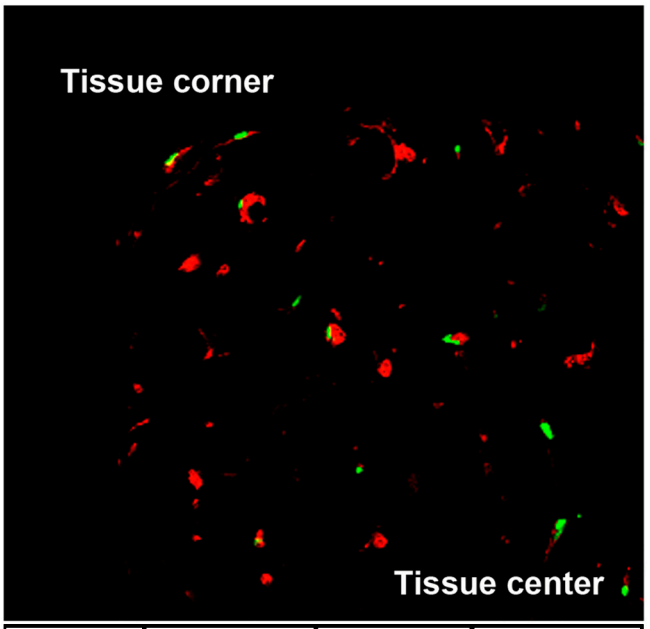


Fig. S9. A twofold increase in VEGF immunofluorescent intensity correlates with a fivefold increase in VEGF protein concentration. We tested the correlation between VEGF immunofluorescent intensity and VEGF protein concentration. Deferoxamine (DFO, a HIF-1a inducer) lead to a twofold increase in VEGF immunofluorescent staining intensity (top row and bottom left). In these samples, we assessed the production of VEGF protein by ELISA assay on cell lysates (ELISA assay for isoforms 165 and 121). This twofold increase in fluorescent intensity correlated with a fivefold increase in the intracellular production of VEGF (bottom middle). The correlation between fluorescent intensity and VEGF protein concentration was linear in the ranged depicted in this study (bottom right).



	24 hours	96 hours	144 hours
Circle	0.34 +/- 0.22	1.03 +/- 0.42	0.42 +/- 0.26
Triangle	0.49 +/- 0.14	0.38 +/- 0.24	0.63 +/- 0.15

Fig. S10. Cellular proliferation. Cellular proliferation was assessed by EdU incorporation over a period of 12 h ending at 24, 96, and 144 h of culture and measured as a percentage of the total number of cells. Proliferating cells (green) are restricted to the PECAM-1$^+$ cells and are not limited to the tissue periphery (Standard deviations, $n = 3$).