Electronic Supplementary Material (ESI) for Integrative Biology.

Generation of 3D functional microvascular networks with mural cell-like human mesenchymal stem cells in microfluidic systems by vasculogenesis-like process

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This supplementary file provides images showing Live/Dead assays to assess cell viability immediately after seeding, SM22α and NG-2 immunofluorescent staining to characterize bone marrow-derived human mesenchymal stem cell (BM-hMSC) phenotypic transition toward a mural cell lineage, confocal reconstruction of patent microvessels wrapped by mural cells, 2D-3D skeleton reconstructions of a representative microvascular network and fluorescent dextran diffusion within a patent microvessel. Supplementary videos show microbead perfusion through endothelial cell EC+BM-hMSC microvascular networks (Movie S1), and the 3D confocal reconstruction of a vascular endothelial (VE)-cadherin stained microvessel (Movie S2).

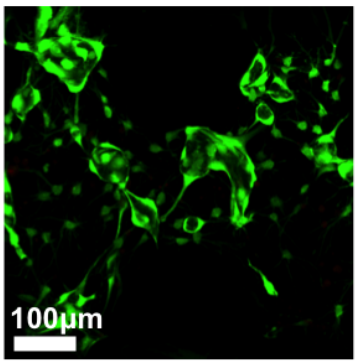


Fig. S1 Live/Dead assay showing cell viability at day 1. Cells were stained with 1 μl fluorescein diacetate (FDA, green; Sigma-Aldrich) and 3.5 μl propidium iodide (PI, red; Sigma-Aldrich) diluted in 1 ml phosphate buffered saline (PBS). Samples were incubated for 5 min, washed with PBS and observed through confocal microscopy.

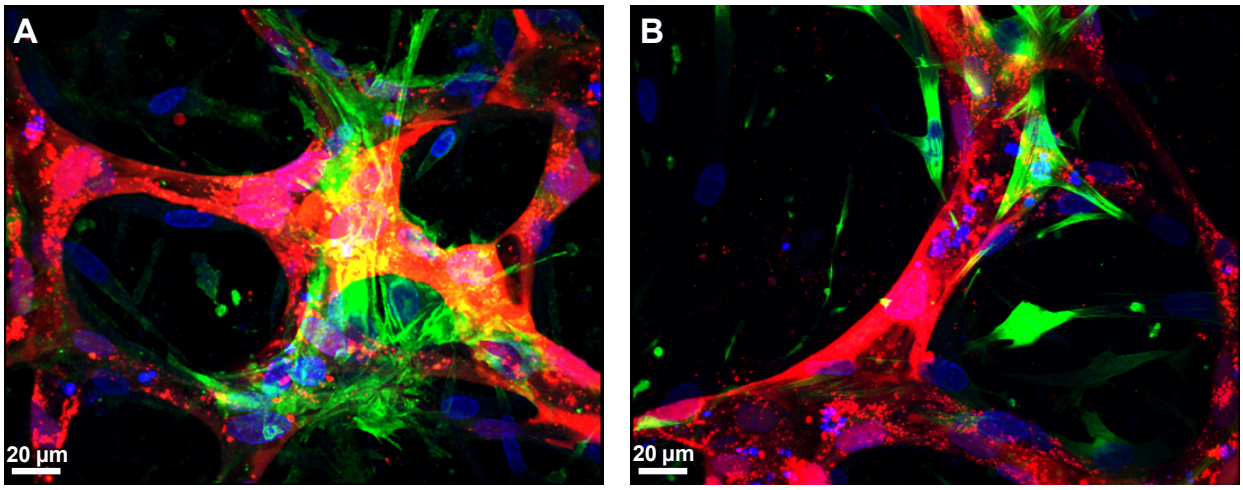


Fig. S2 SM22α and NG-2 immunofluorescent staining highlighting BM-hMSC phenotypic transition toward a mural cell lineage. Representative images showing red fluorescent protein (RFP)-transfected human umbilical vein endothelial cells (HUVECs) organized in a microvascular network co-localized with SM22α+ (green) (A) and NG-2+ BM-hMSCs (green) (B). Particularly, the presence of NG-2+ cells is a clear indication of a specific phenotypic transition toward pericytic cells. Cell nuclei were stained with 4'6-Diamidino-2-Phenylindole (DAPI, blue). These images refers to VEGF+Ang-1 supplemented microfluidic devices.

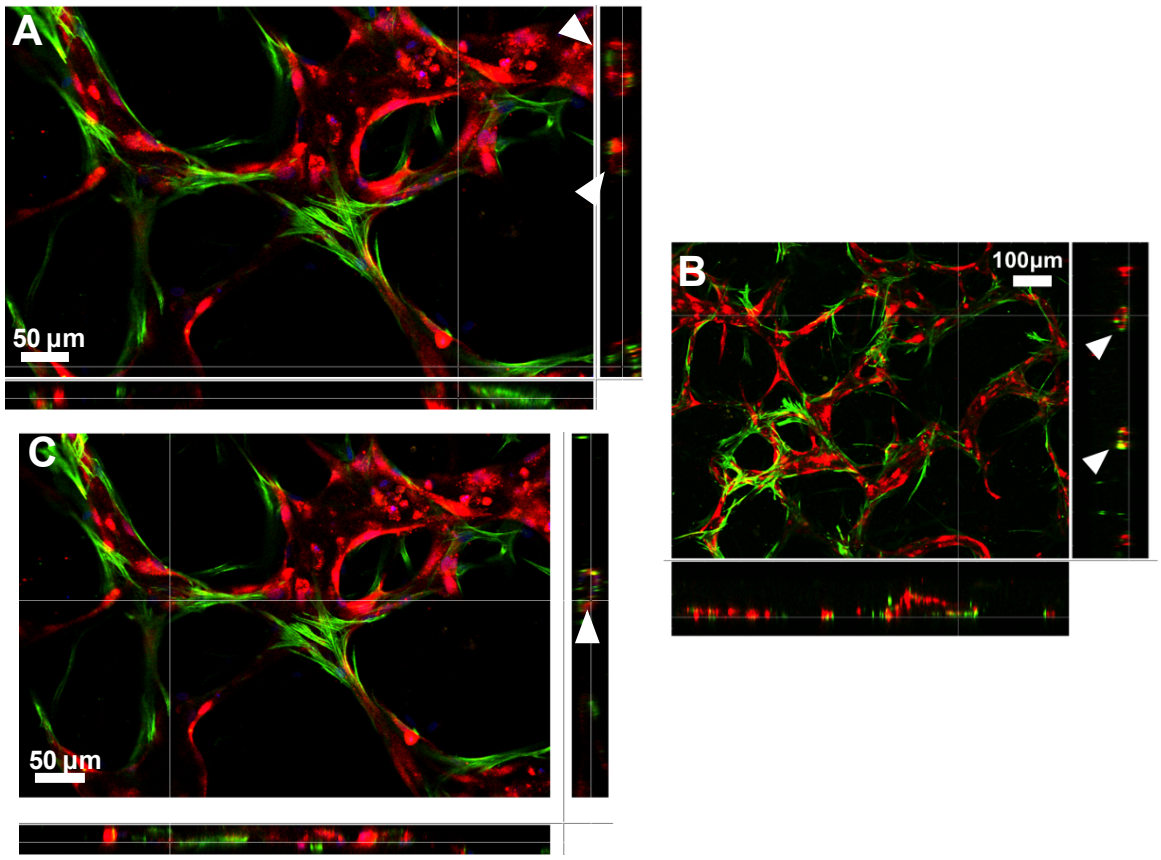


Fig. S3 Confocal microscopy images representing mural cell-like BM-hMSCs (α-smooth muscle actin, green) co-localization with ECs (red). Microvessel lumens are indicated by white arrowheads. These images refer to VEGF+Ang-1 supplemented microfluidic devices.

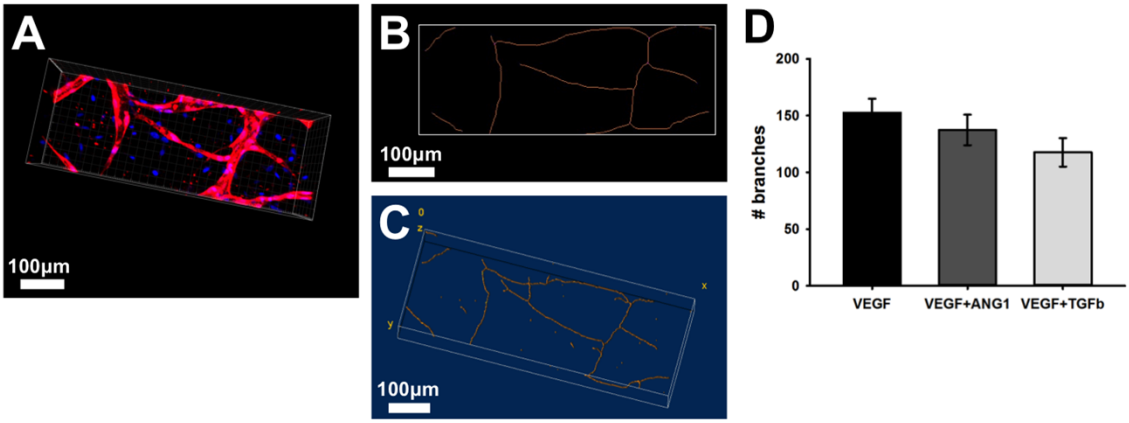


Fig. S4 Microvascular network analysis: number of branches. The 3D skeletonize plugin of the Fiji software was applied to compute the number of branches of the longest connected structure within each region of interest (ROI, 533x426 µm^2). A 25 µm threshold was applied to filter 3D skeleton data (main text). Representative images of a confocal 3D reconstruction (A), a 2D skeleton obtained with the 2D skeletonize plugin (B) and a 3D volumetric skeleton (C). 3D data for the three different experimental conditions (addition of VEGF, VEGF+Ang-1 and VEGF+TGF-β1). Average values were obtained for a minimum of n=8 regions within 2 or 3 independent devices per condition (D). VEGF: vascular endothelial growth factor; Ang-1: angiopoietin-1; TGF-β1: transforming growth factor-β1.

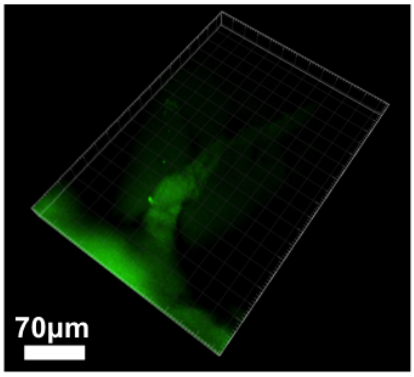
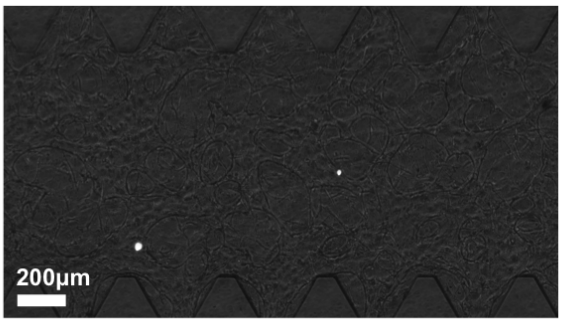
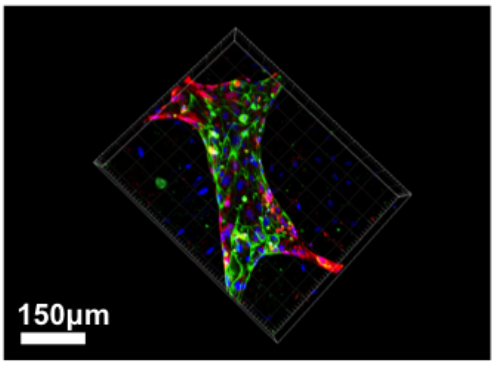


Fig. S5 Microvessel perfusion with 70 kDa fluorescent dextran revealing patent lumen and absence of focal leaks. Representative picture of a microvascular network composed by HUVECs and mural cell-like BM-hMSCs treated with VEGF and Ang-1.



Video S1 Perfusion of 10 µm fluorescent microbeads within a microvascular network composed of HUVECs and mural cell-like BM-hMSCs. A pressure drop was applied by aspirating the media from all the reservoirs of the microfluidic device and adding 40 µl microbead suspension within a single reservoir.



Video S2 3D confocal reconstruction of a representative microvessel stained with anti-VE-cadherin antibody (green). ECs (red) appear tightly connected through a network of vascular adherens junctions. Cell nuclei were stained with DAPI (blue).