

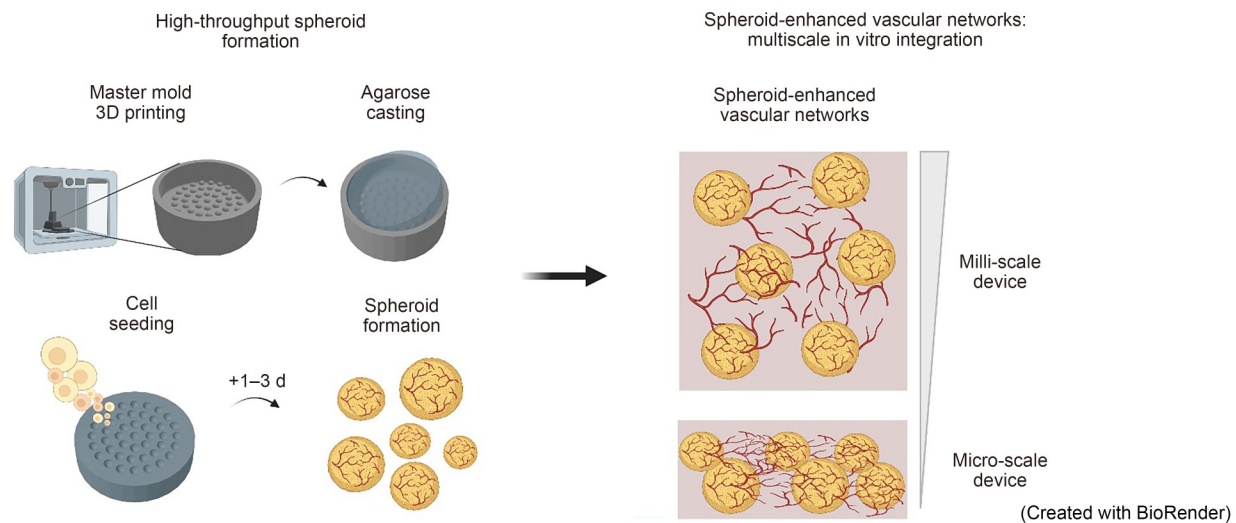


High-throughput generation of pre-vascularized 3D units to sustain vascularization of 3D constructs

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Graphical abstract



1 Introduction

The microvasculature is a crucial component of the tissue microenvironment, facilitating the delivery of nutrients and the removal of waste [1, 2]. Its central role in tissue function and regeneration has prompted extensive in vitro studies using both traditional and advanced modeling approaches.

Recent technological advances in microfabrication and bio-materials have enabled the development of organ-on-chip platforms that replicate physiological microenvironments in three dimensions, integrating relevant biochemical and mechanical cues [3–6]. In this context, microvasculature-on-chip systems have emerged as powerful tools for investigating endothelial behavior and vascular morphogenesis in controlled

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microenvironments, with applications across various fields, including cancer, bone, brain, placenta, and musculoskeletal research [7–12].

Microvascular network formation on a chip typically follows three main approaches: (i) patterned microchannels in hydrogels, (ii) vasculogenic-like self-assembly, and (iii) angiogenic-like sprouting [3, 13]. Patterned systems use pre-defined microchannels seeded with endothelial cells to guide vessel formation, while vasculogenic-like models simulate de novo vascular network formation by embedding endothelial cells in three-dimensional (3D) hydrogels, often supplemented with biochemical stimuli (e.g., vascular endothelial growth factor (VEGF)) or stromal cells. Conversely, angiogenic-like systems rely on sprouting from pre-existing endothelial monolayers toward a 3D matrix, driven by gradients and matrix remodeling. These approaches have successfully generated interconnected, perfusable microvascular networks within microfluidic devices.

However, scaling these processes to millimeter-scale or bio-printed constructs remains challenging [14]. The self-assembly and perfusion efficiency achieved in microfluidic channels often fail in larger, free-standing constructs due to diffusion limitations and reduced endothelial organization. To address this limitation, pre-vascularized modular units—microtissues or spheroids containing endothelial and stromal cells—have been proposed as building blocks to promote rapid vascularization upon assembly or implantation [15–17].

The presence of vascular-supporting cells is crucial for inducing vessel maturation, matrix deposition, and long-term network stability [18]. For instance, dermal spheroids combining fibroblasts and endothelial progenitors demonstrated basal lamina deposition (collagen IV and laminin) within 7 days, while tri-cellular spheroids of human umbilical vein endothelial cells (HUVECs), fibroblasts, and adipose-derived stem cells (ADSCs) exhibited capillary-like networks with basement membrane formation by Day 9 [19]. Similar outcomes were observed in mesenchymal stem cell (MSC)/HUVEC spheroids designed for bone-mimetic vascularization, confirming the relevance of multicellular interactions in vascular morphogenesis [20].

Recent microfluidic and micropatterned platforms have enabled high-throughput formation and culture of such pre-vascularized spheroids, integrating perfusion and controlled aggregation. Examples include U-shaped microarray chips for spheroid formation under continuous flow and replica-molded agarose microwells, which enable rapid, uniform cell aggregation [16, 18, 21]. However, these units are typically integrated into in vivo models for regenerative applications or into standard in vitro setups (e.g., sprouting assays), making it difficult to systematically evaluate their ability to sustain vascularization in 3D in vitro constructs of defined size and geometry.

To address these limitations, we present a cost-effective, reproducible, and high-throughput method to generate hundreds

of 3D pre-vascularized units of HUVECs/human bone marrow mesenchymal stem cells (hBMSCs) per mold in a controlled manner. Furthermore, we demonstrate the efficient integration of these pre-vascularized units into in vitro 3D constructs, either as stand-alone units or in combination with pre-embedded cells, to enhance vascular network formation, providing a scalable strategy for vascular tissue engineering and organ-on-chip applications.

2 Workflow

The experimental workflow comprises three main stages: (i) mold fabrication, (ii) spheroid generation, and (iii) integration into microvascular models.

Step 1: Master mold fabrication

A 3D-printed master mold was designed and printed using a standard benchtop stereolithography (SLA) printer. The design includes arrays of negative hemispherical microwells optimized for uniform cell aggregation and spheroid retrieval. Positive agarose molds were cast from this master to generate a non-adherent surface suitable for controlled spheroid formation. The method is low-cost and easily reproducible in laboratories equipped with a 3D printer. The process enables high-throughput production, yielding up to several hundred spheroids per multiwell plate.

Step 2: Spheroid generation and handling

The agarose molds enable rapid, homogeneous cell seeding via simple pipetting, with a single seeding procedure for each mold (hundreds of microwells). The non-adherent surface facilitates spontaneous aggregation and self-assembly of the mixed cell suspension, resulting in compact 3D spheroids following a short incubation period. One significant advantage of this approach is that it offers a straightforward seeding and harvesting procedure compared with standard low-adhesion multiwell plates, allowing for efficient recovery without enzymatic treatment or mechanical disruption.

Step 3: Integration into in vitro microvascular models

Once formed, the pre-vascularized spheroids are used as biological building blocks to establish in vitro microvascular systems. The following two primary configurations were tested:

Stand-alone culture—spheroids were seeded in microwell plates or microfluidic chips to assess their intrinsic ability to form interconnected microvascular networks.

Supportive configuration—spheroids were co-cultured with single-cell suspensions within the same devices to evaluate their capacity to enhance vascularization and promote network maturation.

This modular workflow provides a scalable, cost-effective platform for producing and testing pre-vascularized units across various organ-on-chip applications.

3 Experimental methods

3.1 Design and production of the mold

The mold for spheroid production was designed using SolidWorks, drawing inspiration from the work of De Moor and colleagues [19]. Specifically, we created two types of master molds featuring a pattern of concave U-bottom microwells, with an interspace of 200 μm . We tested multiple configurations with different well diameters (d) and depths (h) to optimize spheroid formation: mold 1 ($d=400\text{ }\mu\text{m}$, $h=440\text{ }\mu\text{m}$, 673 microwells; Fig. 1b); mold 2 ($d=600\text{ }\mu\text{m}$, $h=660\text{ }\mu\text{m}$, 333 microwells; Fig. 1b). The mold diameter ($d=15.6\text{ mm}$) matches that of a 12-well culture plate. Once the master mold prototypes had been designed as a negative of the final device, they were converted into a .stl file and 3D printed using a 3D printer (Asiga MAX X, Asiga, Australia), which employs an ultraviolet (UV) laser light (40 mW/cm^2) to polymerize resin (Clear Resin, Formlabs, Massachusetts, USA) layer by layer (layer thickness 10 μm). After melting the agarose, micromolds were obtained by replica molding. Specifically, the TopVision Agarose powder was first UV-sterilized for 1 h and then dissolved in phosphate-buffered saline (PBS) to obtain a final 0.06 g/mL solution. The agarose solution was heated and poured over the negative master molds. After being solidified at room temperature, the agarose devices were removed from the templates and placed in a 12-well plate. They were then UV-sterilized for 1 h and equilibrated in EGM-2-MV culture medium (Lonza, Basel, Switzerland) for 24 h.

3.2 Cell culture

HUVECs expressing green fluorescent protein (HUVECs-GFP) and red fluorescent protein (HUVECs-RFP), sourced from Angio-Proteomie (MA, USA), were cultured with EGM-2-MV (Lonza) and utilized between passages 5 and 7. hBMSCs were isolated from 4 patients (2 males and 2 females, aged 55–60 years) undergoing total knee replacement who had provided informed consent. Cells from 4 patients (2 males and 2 females, aged 59–75 years) were pooled and utilized between passages 5 and 7. They were cultured in minimum essential medium Eagle-Alpha modification (Alpha-MEM, Gibco, USA) supplemented with 10% (volume fraction) fetal bovine serum (FBS; Hyclone, USA), 1% PenStrep (Gibco), 1% HEPES (Gibco), and 1% human basic fibroblast growth factor (FGF-2; PeproTech, USA).

3.3 Building the 3D pre-vascularized units

HUVECs-GFP/hBMSCs were pre-mixed to obtain a 1:1 ratio. We seeded 500 μL of cell suspension in EGM-2-MV media into each mold. Cell density was calculated to ensure

the desired number of cells for each microwell (varying from 2×10^3 to 5×10^3 cells/microwell). The medium was refreshed 24 h post-seeding and then every 2 d thereafter. Spheroids were collected for analysis on Days 1, 3, and 7.

3.4 Spheroid characterization

Cell viability within the spheroids was evaluated using the LIVE/DEAD kit (L3224, Thermo Fisher Scientific, USA), and metabolic activity was assessed using the Alamar Blue indirect assay (DAL1100, Thermo Fisher Scientific). The geometrical characterization leverages the GFP signal from the HUVECs to analyze area, perimeter, major diameter, minor diameter, and circularity (ranging from 0 to 1, where 1 represents a perfect circle). Images were acquired using a fluorescence microscope (Eclipse Ti, Nikon, Japan) and analyzed in ImageJ using the “Analyze Particles” plugin.

3.5 Vascularizing 3D constructs

Spheroids were collected via pipetting after 1 and 3 d and subsequently seeded into 3D constructs (3D wells and microfluidic chips) within a fibrin gel (fibrinogen: 2.5 mg/mL; thrombin: 2 U/mL final concentration). To evaluate their potential to form microvascular networks as stand-alone units, three-day-old spheroids were seeded at three concentrations: 140, 70, and 35 spheroids/sample. Control cases with single cells were seeded with HUVECs-RFP and hBMSCs at final concentrations of 21×10^6 , 11×10^6 , and 5×10^6 cells/mL. To assess their capacity to function as supporting units within the constructs, one-day-old spheroids were pre-mixed with HUVECs-RFP and hBMSCs at final concentrations of 4×10^6 and 2×10^6 cells/mL.

3.6 Sprouting and network characterization

The spheroids were imaged on Days 1 and 3 using a fluorescence microscope (Eclipse Ti, Nikon). The sprouting analysis was conducted by a single investigator in a blinded manner. The number of sprouts per image was manually counted and normalized to the GFP area within the image. The microvascular network features were extracted using the μVES code [22, 23] following binarization in FIJI ImageJ. The heterogeneity index was calculated by analyzing a 4×4 matrix in the image, where the standard deviation of intensity across the 16 squares was divided by the image’s average intensity.

3.7 Immunofluorescence

After 7 d of culture, constructs were fixed in 4% paraformaldehyde (PFA; Sigma-Aldrich, Germany) for 20 min at room temperature, washed with PBS, and permeabilized with 0.2% Tween-20 for 15 min at 37 $^{\circ}\text{C}$ under agitation. Samples were incubated in 5% bovine serum albumin (BSA)

in PBS for 1 h at 37 °C to block nonspecific antibody binding. Primary antibodies for vascular endothelial cadherin (VE-cadherin) (441144G, 1: 400, Thermo Fisher Scientific) and collagen IV (14-9871-82, 1:150, Thermo Fisher Scientific) were diluted in 1% BSA in PBS and incubated overnight at 4 °C under gentle agitation.

4 Experimental results

The results are presented to show the entire workflow (Fig. 1a), from the production and characterization of spheroids to their use in sustaining vascularization in microfluidic chips and 3D mm-scale constructs.

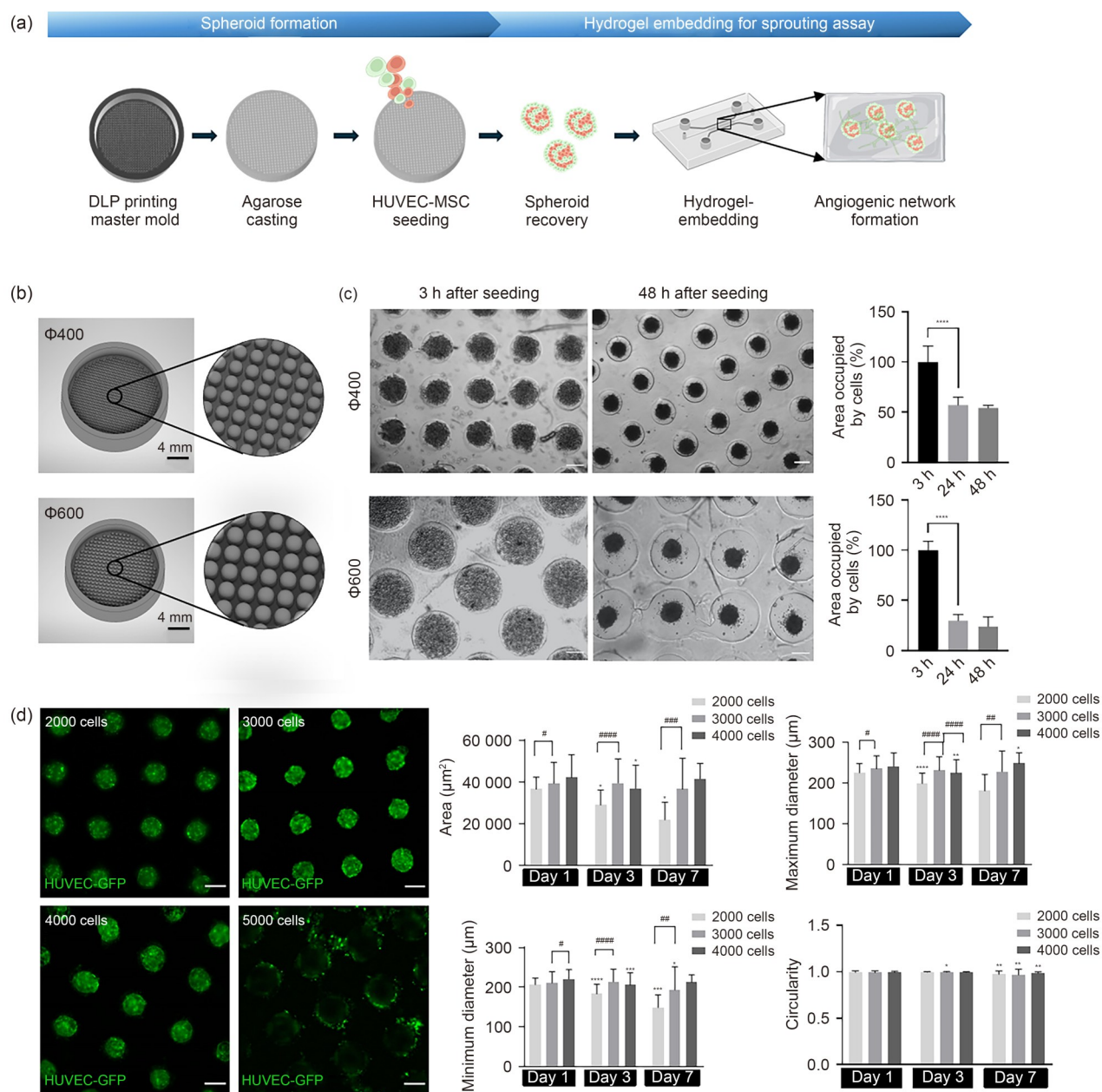


Fig. 1 Formation of pre-vascularized HUVEC/hBMSC spheroids. (a) Experimental timeline outlining spheroid formation and hydrogel embedding for sprouting assays (created with BioRender). (b) DLP-printed master molds with microwells 400 μm and 600 μm in diameter ($\Phi 400$, $\Phi 600$). (c) Left: cell aggregation over time in 400 μm and 600 μm diameter agarose molds. Right: area occupied by cells over time, normalized to microwell area, at 3, 24, and 48 h after seeding in 400 μm and 600 μm diameter agarose molds. Scale bars: 200 μm . One-way ANOVA, **** P < 0.0001. (d) Left: pre-vascularized spheroids after 3 d of culture formed at various cell concentrations (2000, 3000, 4000, and 5000 cells/microwell). Right: geometrical characterization of pre-vascularized spheroids formed at various cell concentrations (2000, 3000, and 4000 cells/microwell). Data are expressed as mean $\pm$ standard deviation ($n=10$). Two-way ANOVA, * P < 0.05, ** P < 0.01, *** P < 0.001, **** P < 0.0001, # P < 0.05, ## P < 0.01, ### P < 0.001, and #### P < 0.0001. * indicates significant differences within the same group between the current time point and the previous one; # indicates differences between groups at the same time point. DLP: digital light processing; ANOVA: analysis of variance

4.1 Spheroid production

No cytotoxicity of the agarose gel was observed (cell proliferation >70%) at both 1% and 6% concentrations. However, we observed a reduction in metabolic activity at the higher concentration (6% agarose vs. 1%, Fig. S1 in the supplementary information). We also analyzed cell viability over time. Cell viability in HUVEC-GFP/BMSC spheroids was 97.92% at Day 3, declining to 78.96% by Day 7 (Fig. S1 in the supplementary information). Although viability remained above 70%, the spheroids became less compact and lost their inner capillary-like structure at Day 7. Based on these data, the spheroid production time was limited to three days.

We observed an initial spheroid-packing phase, characterized by a reduction in spheroid diameter (Fig. 1c). Area significantly decreased over the first 24 h (approximately 40% in the 400- μm -diameter microwells and 70% in the 600- μm -diameter microwells), and this decrease was slightly greater at 48 h.

To test the homogeneity of the seeding, we measured the spheroid dimensions in five regions of the molds after 2 days of culture. We found no difference within the 400 μm mold, indicating that cells were uniformly distributed across the entire surface, resulting in 3D spheroids that were homogeneous in shape and size (Fig. S2 in the supplementary information). Conversely, the 600 μm mold resulted in a slightly more heterogeneous distribution with a slight preference for the central region (Fig. S2 in the supplementary information).

We further evaluated the spheroids' geometrical features (minimum and maximum diameters, area, and circularity) to assess possible differences in the geometry of the two molds (Fig. 1c; Fig. S3 in the supplementary information). During the first two days of culture, 3D spheroids grown in the 600 μm -microwell exhibited higher diameters and area values (first day, 600 μm mold: max $d=(258.47\pm3.44)$ μm , min $d=(231.37\pm3.50)$ μm ; 400 μm mold: max $d=(238.32\pm17.90)$ μm , min $d=(215.76\pm2.80)$ μm ; $P<0.0001$). Starting from the third day, the dimensions of the spheroids in the two molds became comparable (third day, 600 μm mold: max $d=(211.06\pm13.50)$ μm , min $d=(182.88\pm19.10)$ μm ; 400 μm mold: max $d=(213.12\pm47.24)$ μm , min $d=(190.93\pm44.20)$ μm). The area followed the same trends (Fig. S3c in the supplementary information). The circularity exhibited an average value of 0.98 across all conditions and time points. Based on these data, we selected the 400 μm microwell for further experiments.

We tested various cell densities (2000, 3000, 4000, and 5000 cells/microwell) to evaluate spheroid size control as a function of cell number (Fig. 1d). By Day 3, the 5000 cells/microwell group exhibited a reduced GFP signal, likely due to high mortality, which marked an upper limit for this setup and was excluded from further analysis. At each time point, the spheroid size was larger when seeding more cells, demonstrating that the initial cell number can be used to tune spheroid size.

Additionally, we tested the spheroids for key markers of endothelial and supporting cell organization, with immunofluorescence analysis demonstrating the presence of VE-cadherin and collagen IV (Fig. S4 in the supplementary information). VE-cadherin exhibited greater localization in the inner regions over time, from a wide distribution within the spheroid (Day 1) to early pre-vascular network formation (Day 3). Collagen IV was weakly detectable on Day 1 but increased by Day 3, particularly around the periphery, contributing to spheroid stabilization.

4.2 Vascularizing 3D constructs via spheroids

We evaluated the efficacy of spheroids as supporting units for creating vascularized models by testing microvascular network formation at varying seeding densities and using single-cell suspensions as controls (Fig. 2). Spheroids created a local inhomogeneity in cell concentrations at Day 1, and by Day 7, they achieved a homogeneous distribution throughout the entire gel (Figs. 2a and 2b). Conversely, single cells exhibited the opposite behavior. No difference was observed in vessel features such as thickness or average branch length (Figs. 2a and 2b). Conversely, single cells failed to produce an extensive connected network (Figs. 2a and 2b).

Additionally, the vascular networks originating from pre-vascularized units displayed protrusive structures reminiscent of endothelial sprouts as they matured. This supports the hypothesis that microvascular networks develop in an angiogenic-like manner under these conditions. The number of sprouts originating from each spheroid increased over time following hydrogel inclusion at all spheroid concentrations (Fig. 2c, Day 1: low concentration (0.62 ± 0.35) sprouts/ mm^2 , medium concentration (1.37 ± 0.64) sprouts/ mm^2 , high concentration (1.18 ± 0.65) sprouts/ mm^2 ; Day 3: low concentration (4.81 ± 1.00) sprouts/ mm^2 , medium concentration (4.86 ± 0.70) sprouts/ mm^2 , high concentration (2.73 ± 1.38) sprouts/ mm^2). However, the number of sprouts at Day 3 decreased in the condition with the highest number of spheroids, likely due to the formation of closed connections from previous sprouts.

4.3 Spheroid-enhanced in vitro microvascular modeling

We then evaluated the spheroids' ability to function as supporting units in a 3D device, specifically by mixing them with standard single-cell seeding (Fig. 3). We therefore employed HUVECs-RFP for single-cell seeding and HUVECs-GFP for spheroid formation, using one-day-old spheroids to maximize their angiogenic potential (as shown by immunofluorescence and consistent with Carvalho and colleagues [24]). We observed the integration of microvascular networks, joining vessels from spheroids and single cells, in both the microfluidic

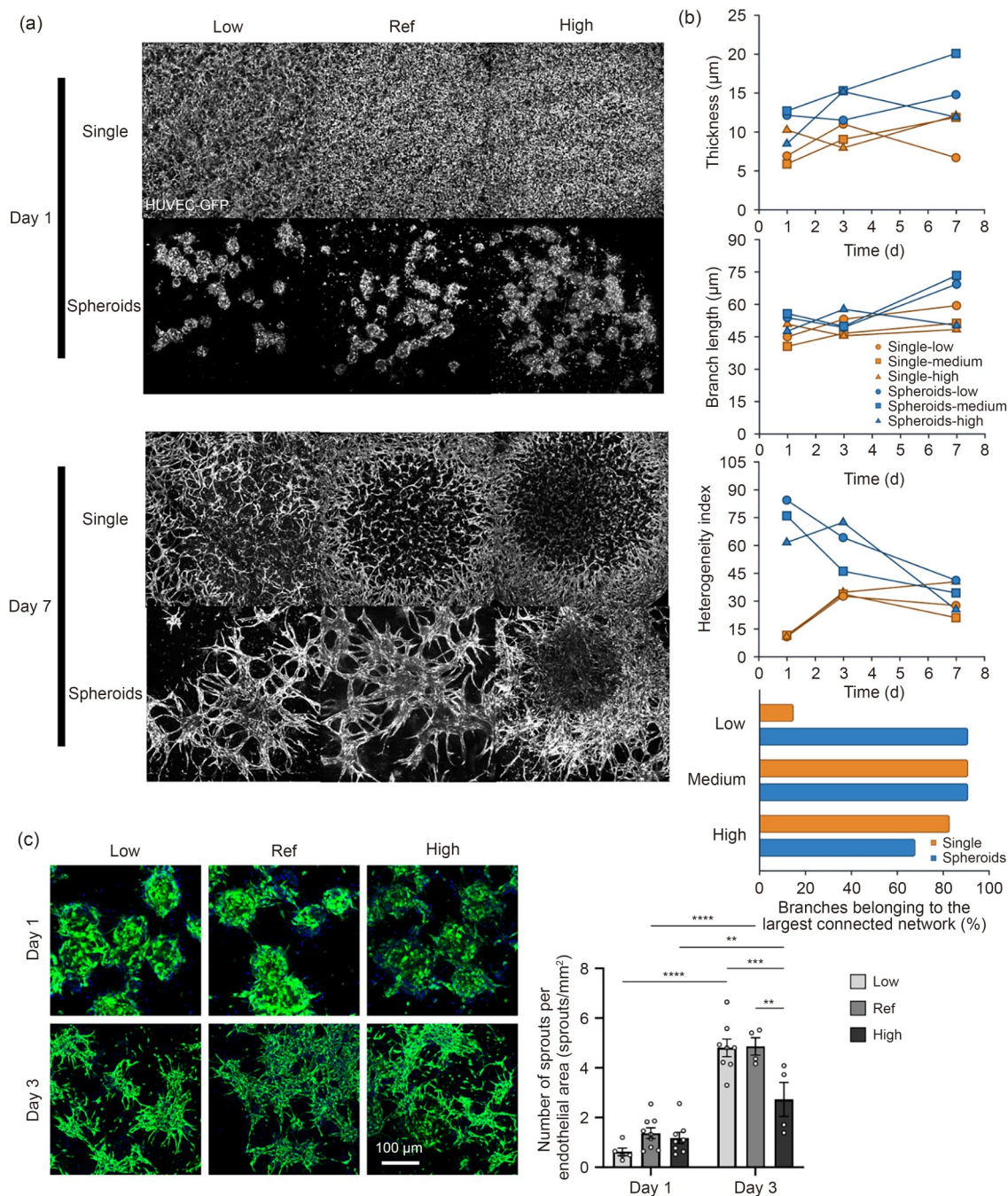


Fig. 2 Comparison of single cells and pre-vascularized spheroids at different concentrations in the formation of microvascular networks within a 3D device. (a) Formation of microvascular networks over time by various concentrations of single cells (single) and pre-vascularized spheroids, fibrin-embedded at low (single: 5×10^6 cells/mL; spheroids: 35 spheroids per device), reference (single: 11×10^6 cells/mL; spheroids: 70 spheroids per device), and high concentrations (single: 21×10^6 cells/mL; spheroids: 140 spheroids per device). (b) Quantifications of the images reporting the thickness of the vascular structures, the average branch lengths, and the spatial heterogeneity index. (c) Representative images of gel-embedded spheroids for sprouting analysis and the quantification of the number of sprouts per area of endothelial cells. Data are expressed as mean±standard deviation ($n=5$). ** $P<0.01$, *** $P<0.001$, and **** $P<0.0001$

and mm-scale devices (Fig. 3). Network development occurred primarily through the outgrowth of pre-existing sprouts, as the number of sprouts remained constant over time, while their length and area increased (Fig. 3, 3D device: Day 3, (11.63 ± 2.27) sprouts/spheroid, (79.89 ± 20.58) μm ; Day 7, $(13.00 \pm$

$2.53)$ sprouts/spheroid, (228.87 ± 50.57) μm). Regarding network development over time, the microfluidic chip produced a slightly faster development, possibly due to the more restricted environment relative to that of the 3D device (z dimensions: 300 μm in the microfluidic chip vs. 1.6 mm in the

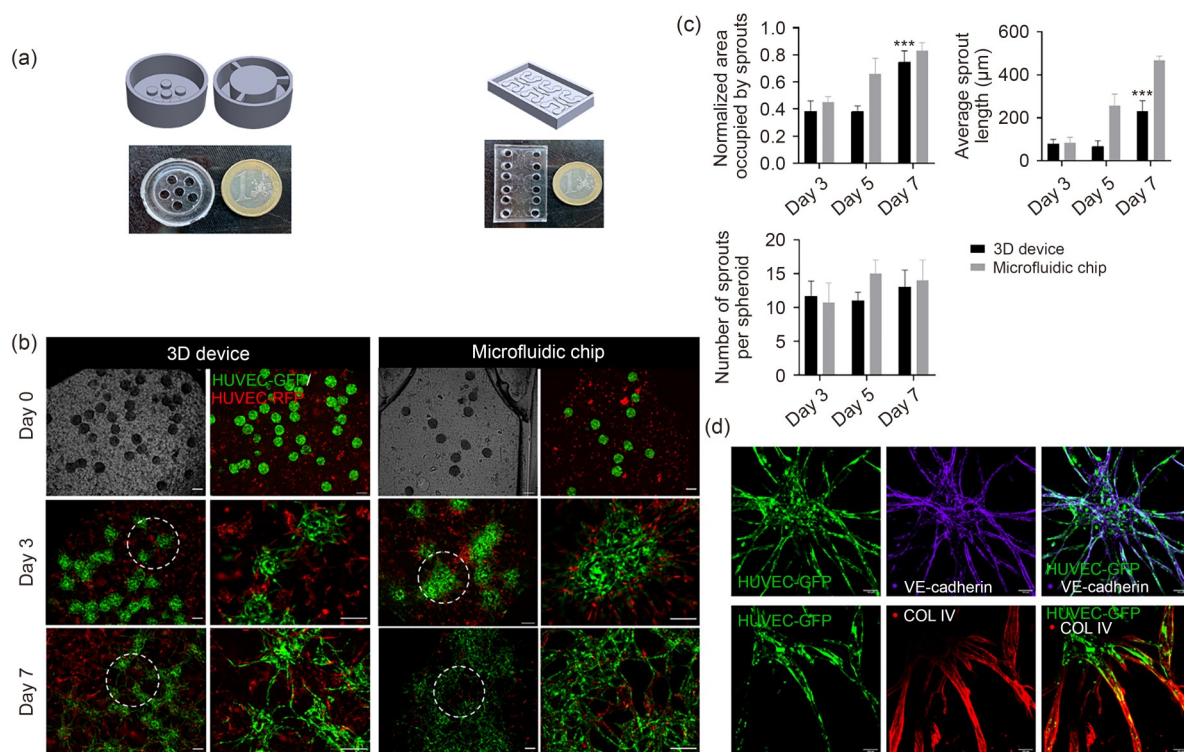


Fig. 3 Formation of interconnected microvascular networks in devices of different scales. (a) Left: master mold of the 3D device, comprising 6-mm-sized wells. Right: master mold of the microfluidic chip, in triplicate. (b) Sprouting activity over time of fibrin-embedded spheroids cultured in the 3D device and microfluidic chip. Scale bars: 200 μm. (c) Measurements of area occupied by sprouts (normalized on the entire spheroid area), average sprout length, and number of sprouts per spheroid over time for spheroids embedded in the 3D device and microfluidic chip. Data are expressed as mean±standard deviation ($n=5$). Two-way ANOVA, *** $P<0.001$. * indicates significant differences within the same group between the current time point and the previous one. (d) Detection of key endothelial markers by immunofluorescence for tight junctions (VE-cadherin) and basal membrane (collagen IV) in microvascular networks formed by pre-vascularized spheroids. Scale bars: 200 μm. ANOVA: analysis of variance

3D device; gel volume: 7 μL in the microfluidic chip vs. 20 μL in the 3D device).

In addition to morphological results, we assessed the presence and localization of tight junction proteins and the basal membrane to demonstrate the biological significance of the microvascular networks generated from the pre-vascularized units. Notably, we detected VE-cadherin and collagen IV signals in these networks as markers of the tight junctions between endothelial cells and basal membrane deposition, respectively (Fig. 3d).

5 Discussion

We presented a high-throughput, cost-effective method for generating HUVEC/hBMSC spheroids as modular units for vascularized in vitro models via self-assembly. The workflow includes (i) master mold fabrication, (ii) the production of 3D pre-vascularized spheroids, and (iii) the integration of these units within fibrin gels.

In the first two steps, we developed a custom 3D-printed micromold to produce hundreds of spheroids simultaneously.

Although conventional techniques, such as the liquid overlay technique (LOT), are considered most suitable for generating homogeneous spheroids [17], our seeding approach ensured uniform distribution (Fig. S2 in the supplementary information). When using the mold with 400 μm wells, the system enables more than 600 spheroids per well (over 7200 across a full 12-well plate), with size tunable via seeding density (up to 4000 cells in 400 μm wells). We provided data demonstrating the homogeneity of the geometrical features of the obtained spheroids, along with biological validation using collagen IV and VE-cadherin. The evolution of these markers over time also aligned with the literature, suggesting the possibility of maximizing stability or angiogenic potential [24]. Furthermore, the system's throughput can be appreciated by considering the effort required to change the media for the spheroids, quantified by the number of pipetting steps. Considering 7200 spheroids (a full 12-well plate with micromolds), a complete media change can be achieved by pipetting 12 times. Conversely, when using the standard LOT (employing 75 low-attachment 96-well plates) with a multichannel pipette, the number of times to achieve this change increases to 900. This scale enables the application

of these methods to larger constructs, for instance, bioink enrichment.

For the final step, spheroids were embedded in fibrin gels to evaluate their vascularization potential. From a methodological standpoint, the physical principles underlying spheroid formation in both the proposed method and the standard LOT are comparable, as both rely on low-adhesion, round-bottom (U-shaped) wells. Therefore, we do not expect intrinsic differences in early spheroid-driven vascular potential. However, the two methods differ substantially in terms of throughput and experimental feasibility, which are critical for sustaining microvascular network formation. While a direct comparison of vascular network quality would be scientifically informative, it falls outside the practical applicability of standard LOT. For this reason, we compared the proposed method with standard vasculogenic-like models and demonstrated that spheroid-based networks exhibited similar maturation kinetics, despite differing developmental processes (increase vs. decrease in heterogeneity index; Fig. 2b). This difference may be related to the proposed method's distinct key mechanisms, which appear closer to angiogenic sprouting than to vasculogenesis. It also helps the spheroid-based network form a connected microvasculature, especially at lower vessel density (consistent with literature data from a different setup [25]). Indeed, spheroids formed angiogenic hubs that promoted network connectivity even at low seeding densities, whereas single cells created a microvascular network with numerous unconnected structures (Fig. 2a). Although additional molecular or functional analyses would be required to conclusively distinguish angiogenesis from vasculogenesis, the morphological and temporal features observed in our system are consistent with angiogenic sprouting (Fig. S5 and Videos S1–S4 in the supplementary information). Furthermore, spheroid-based sprouting assays are commonly used in vitro to model angiogenic processes, as they recapitulate the outgrowth of capillary-like structures from a preorganized endothelial unit [26–28].

We note that while the proposed method was not developed with the primary goal of generating fully perfusable microvascular networks, it could be used to support vasculogenic-based microvascular network generation aimed at microvascular perfusion (Fig. 3). The perfusability of in vitro microvascular networks is strongly influenced by the geometry and boundary conditions of the model (e.g., channel width and layout in microfluidic devices) and has been most consistently demonstrated in chip-based systems [3]. Given the broader applicability of our approach to generic 3D constructs, perfusion is not considered a primary outcome of the method; rather, the focus is on promoting the formation of connected, branched microvascular-like structures. We presented here a cost-effective, reproducible, and high-throughput method for generating hundreds of 3D spheroids in a co-culture of HUVECs and hBMSCs. This technology can

therefore be used to produce large numbers of spheroids and, by design, its application is not limited to pre-vascularized units. Thanks to these features, particularly high throughput, this method can support applications beyond microfluidic and microwell systems, such as bioprinting, where pre-vascularized spheroids serve as bioink building blocks [14]. Furthermore, the workflow is open to hosting different cell types, potentially enabling the combination of various spheroids within the same matrix, leading to a heterogeneous distribution within the same hydrogel.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1631/bdm.2500611>.

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Author contributions LP, MVC, and MM conceived the study. AS, DP, and LP conducted the experiments and wrote the original draft. AU, CA, SB, and MM contributed to data analysis and manuscript editing. All authors reviewed and commented on the manuscript.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval Human-derived specimen sampling was conducted in accordance with the guidelines, regulations, and procedures of the Ente Ospedaliero Cantonale (Bellinzona, Switzerland) and was approved by the local Institutional Review Board (Approval number: 2020-00029, from Comitato Etico Cantonale).

Data availability The data that support the findings of this study are available from the corresponding author upon reasonable request.

Use of generative AI tools No generative AI tools were used in the preparation of this work.

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