Gastruloids unlock pathways to perfusable heart and liver organoids

Dongliang Zhao[1] **and Hongxing Luo**[2,*]

[1]Institute of Biomedical Engineering, Shenzhen Bay Laboratory, Shenzhen 518132, China

[2]Institute for Surgical Research, Oslo University Hospital, Rikshospitalet, Postboks 4950 Nydalen, Oslo 0424, Norway

*Correspondence: hi.hongxing@gmail.com

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For fifteen years, organoid technology has aimed to make human biology experimentally accessible, yet every three-dimensional system has encountered the same physical barrier. Once a spheroid of brain, gut, lung or heart tissue grows beyond roughly three millimetres, cells at its centre are starved of oxygen/nutrients, a direct consequence of the absence of blood vessels. This size limit stalls maturation at embryonic-like stages, narrows the scope of disease modelling, and undermines graft survival. Although investigators have induced endothelial sprouting within organoids or grafted constructs into immunodeficient mice, a fully robust branching and lumen-forming vascular tree that co-develops *in vitro* with the organ parenchyma has remained elusive, even though flow culture can generate perfusable lumens and enhance maturation in kidney organoids.[1]

In a recent *Science* article, Abilez and colleagues propose a strategy that may help overcome this barrier.[2] The authors begin with human pluripotent stem cells, place them inside millimetre-scale micropatterned circular stencils, and let geometric confinement guide self-organisation (Figure 1). Within 48 hours the discs adopt concentric germ-layer rings reminiscent of early gastrulation. Onto this spatial template the team layers a carefully timed sequence of signalling cues. A short pulse of the Wnt agonist CHIR99021 initiates mesoderm formation. Fibroblast growth factor 2 supports proliferation, while subsequent Wnt inhibition with IWR-1 resets patterning. Vascular endothelial growth factor, together with angiopoietin and a transforming growth factor-β inhibitor, drives endothelial sprouting. Finally, platelet-derived growth factor combined with low-dose transforming growth factor-β1 promotes mural-cell wrapping and vessel stabilization. The complete sequence, referred to as Condition 32, emerges from a systematic screen of 34 permutations and is selected because it maximizes the synchronous appearance of cardiomyocytes, endothelial cells, and smooth-muscle cells in a fluorescent reporter line.

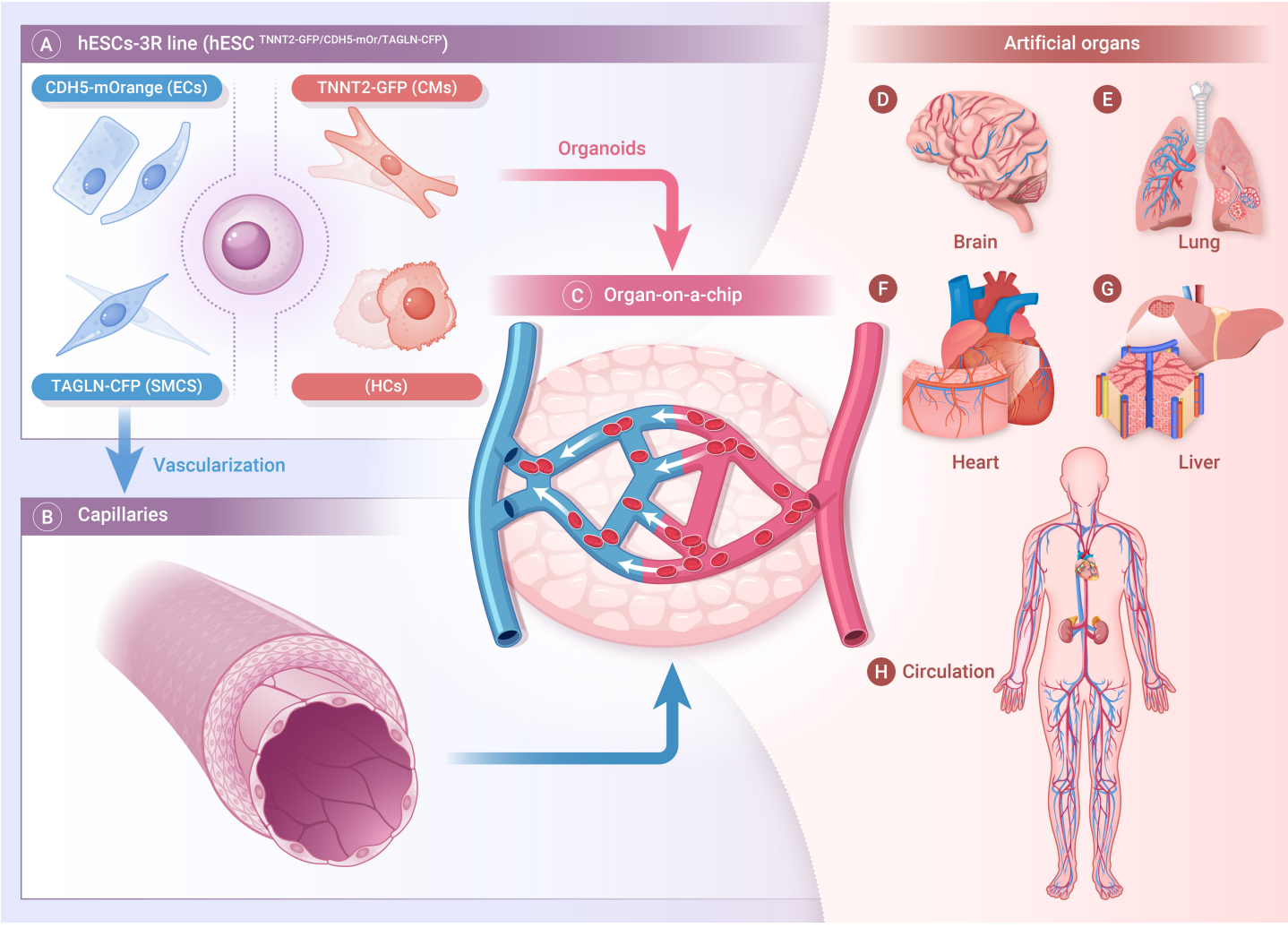

Figure 1. Gastruloid-guided generation of vascularised heart and liver organoids from a triple-reporter human embryonic stem cell (hESC) line Human embryonic stem cells carrying a triple fluorescent reporter (TNNT2-GFP for cardiomyocytes (CMs), CDH5-mOrange for endothelial cells (ECs), TAGLN-CFP for smooth muscle cells (SMCs)) are subjected to a gastruloid-style induction under geometric confinement (A), yielding vascularized heart organoids and liver organoids (B, C). The right panels show vascular maps of major human organs including brain, lung, heart, liver (D, E, F, G), and a full-body circulation silhouette (H), situating the *in-vitro* constructs within physiological context.

Sixteen days after induction, each disc has matured into what the authors

call a cardiac vascularised organoid (cVO). Confocal microscopy reveals a planar architecture with a striking doughnut-like appearance. Beating cardiomyocytes form a pulsatile inner ring, while an encircling layer of smooth muscle provides structural reinforcement. Surrounding these layers, a magenta vascular plexus envelops the construct, its tubules spanning approximately 4 to 40 micrometres in diameter. Perfusion assays indicate open lumens with effective fluid transport through the endothelial network, and functional readouts further point to an active endothelium. Electrophysiological recordings reveal spontaneous field potentials consistent with early foetal myocardium, and calcium-transient imaging confirms coherent contraction waves propagating across the tissue.

Single-cell RNA sequencing identifies 15 to 17 cardiac cell types, including atrial / ventricular cardiomyocytes, arterial / venous endothelial cells, smooth-muscle cells, epicardial / endocardial derivatives, neurons, and fibroblasts. These findings are consistent with current integrative single-cell analysis frameworks.[3] This diversity is comparable to the 16 populations catalogued in a six-week embryonic heart. Transcriptomic analyses implicate Notch and bone morphogenetic protein (BMP) signalling during vessel formation. Pharmacological inhibition disrupts the vascular plexus, and blockade of BMP signalling produces the most pronounced effect. The requirement for these pathways is consistent with decades of genetic data from animal models, supporting the view that the *in-vitro* system reflects authentic developmental logic.

The investigators then ask whether the same molecular choreography could be applied to an organ that arises from a different germ layer. They use an identical factor timeline and replace the initial mesoderm-priming cue with an endoderm specification step to generate hepatic vascularised organoids (hVOs) containing albumin-positive hepatocytes integrated with a sinusoidal-like endothelial network. Transcriptomic analysis demonstrates concurrent up-regulation of hepatic and angiogenic gene programmes, suggesting that early liver and heart share a conserved blueprint for vascular assembly despite their distinct embryological origins.

The study features a translationally-oriented design with significant technological value. The investigators established a scalable micropatterning platform integrated with automated high-content imaging. Vascularization efficiency is quantified through measures such as vessel density, branching frequency, and lumen formation. This systematic approach improves comparability across experiments and helps to reduce variability, a long-standing obstacle in organoid research. When applied to multiple human induced pluripotent stem cell (hiPSC) lines from diverse genetic and demographic backgrounds, the platform reveals inter-individual differences in vascularisation efficiency, suggesting its ability to capture clinically relevant diversity. Because the workflow is modular, it can be adapted to include additional functional assays, including calcium-transient mapping of electromechanical coupling, metabolic profiling of mitochondrial activity, and permeability measurements in endothelial networks. The combination of geometric precision, time-controlled factor delivery, and automated analysis increases reproducibility and allows results to be validated across laboratories. Collectively, these features establish a foundation for personalised disease modelling of vascular disorders and for systematic screening of candidate therapeutic interventions.

This advance may carry several immediate implications for developmental modelling, safety assessment, and regenerative applications. First, it offers developmental biologists a human model of the earliest stages of embryogenesis, a period that is normally inaccessible for ethical reasons. Researchers can now manipulate signalling inputs, matrix mechanics or metabolic states to explore how these parameters influence the emergence of cardiovascular circuits. Systematic variation of Wnt signalling or BMP cues, substrate stiffness or oxygen availability could help define developmental thresholds that remain difficult to study in animal models. Second, the system enables structured evaluation of teratogenic risk. As a proof of principle, the authors exposed cardiac organoids to fentanyl and observed an increase in vessel density, a result that resonates with concerns about opioid exposure in utero and that may provide a framework for testing other clinically relevant compounds. Third, functional vasculature is a prerequisite for tissue grafts designed to repair organ damage. Current myocardial patches often lack intrinsic blood vessels and therefore survive poorly after transplantation. A pre-vascularised construct capable of rapid anastomosis with host circulation may persist and integrate more effectively. This effect could be enhanced if vessel calibre and mural-cell coverage are tuned in advance. Because the induction strategy also applies to hepatic vascularised organoids (hVOs), it has the potential to extend to other organs, broadening its impact on disease modelling and regenerative applications.

Nevertheless, the organoids contain very few haematopoietic or immune cells, preventing any exploration of inflammatory crosstalk or immune rejection. Integrating yolk-sac-derived progenitors or circulating leukocytes is an obvious next step. Although both cVOs and hVOs develop branched vascular networks, significant disparities persist in vascular density, diameter distribution, perfusion efficiency, and vascular permeability compared to native organs. Furthermore, the dynamic processes of vascular development, including pruning and remodelling, have not been monitored in real time, thus limiting the ability to recapitulate the spatiotemporal complexity of *in-vivo* vascular morphogenesis. Electrophysiological and metabolic profiles still resemble those of a foetal heart. Additional maturation strategies such as cyclic stretch or electrical pacing will probably be required to approximate adult-like performance.[4] These approaches have already been shown to advance ultrastructural, metabolic and functional maturation of engineered human cardiac tissues. Future research should focus on developmental remodeling of microcirculatory networks across organs and on their synergistic interactions with parenchymal cells. Key examples are barrier function in the brain, alveolar-microcirculatory coupling in the lungs, and hemodynamic regulation in the heart. Incorporating controlled fluid shear, which has been shown to set thresholds for angiogenic sprouting, may further refine network density and architecture.[5] Finally, the micropatterning method excels in reproducibility but fabricates only two-millimetre discs. Scaling to clinically relevant dimensions will demand integration with light-based bioprinting or continuous-flow microfluidic networks capable of delivering nutrients through the same vascular tree that the organoid now possesses. Patient-derived hiPSCs enable the generation of disease-specific organoids for evaluating personalized drug efficacy and safety. Beyond organoid platforms, progress in large-scale solid-organ bioprinting will also be critical. Integrating cultured microvessels with 3D-bioprinted constructs will resolve the key challenge of microvascular anastomosis, thereby achieving macro-micro vascular integration. This strategy addresses persistent perfusion deficits in bioprinted tissues, ultimately enabling long-term organ culture and clinical translation.

Despite these challenges, the Stanford protocol ends a long search for a simple and portable strategy that gives organoids their own blood supply. By grounding the design in the earliest events of human development and demonstrating its applicability from the heart to the liver, the authors broaden what may be achievable in organoid science. Their work promises deeper insight into congenital disease, more predictive drug-safety assays, and a more realistic path toward regenerative grafts. Once immune components, adult-level maturation cues, and scalable perfusion are in place, tissue models that begin life in a Petri dish may finally move into the realm of therapeutic reality.

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AUTHOR CONTRIBUTIONS

All authors contributed to and approved the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.