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Review Article

Towards advanced regenerative therapeutics to tackle cardio-cerebrovascular diseases

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ABSTRACT

The development of vascularized organoids as novel modelling tools of the human cardio-cerebrovascular system for preclinical research has become an essential platform for studying human vascularized tissues/organs for development of personalized therapeutics during recent decades. Organ-on-chip technology is promising for investigating physiological in vitro responses in drug screening development and advanced disease models. Vascularized tissue/organ-on-a-chip benefits every step of drug discovery pipeline as a screening tool with close human genome relevance to investigate human systems biology. Simultaneously, cardio-cerebrovascular-on-chip-integrated microfluidic system serves as an alternative to preclinical animal research for studying (patho-)physiological processes of human blood vessels during embryonic development and cardio-cerebrovascular disease. Integrated with next-generation techniques, such as three-dimensional bioprinting of both cells and matrix, may enable vascularized organoid-on-chip-based novel drug development as personalized therapeutics.

1. Introduction

Human circulatory system that comprises hierarchical dense and intricate vasculatures, which is optimized to efficiently transport gas, nutrients, and metabolites to and from cells, spanning from the centimeter-scale aorta to micrometer-scale capillaries or lymphatic vasculature [1–5], is essential for physiologic functions of organs within human body [6,7]. Angiogenesis, the growth and morphogenesis of new vascular networks from existing ones that triggered by the disruption of the local oxygen supply, encoded at the signaling level by multiple combinations of diverse biochemical stimuli and juxtacrine cell interactions, is a key physiological or pathological process leading to the growth of new blood vessels from existing ones [8,9]. Tissue and organ viability depends on the proper systemic distribution of cells, nutrients, and oxygen through blood vessel networks. These networks arise in part via angiogenic sprouting [10]. Initiated with an endothelial sprout and ending with vascular tube maturation and vessel quiescence, the formation of new blood vessels through angiogenesis is a combination of processes. Proper blood vessel networks are necessary for constructing and re-constructing tissues and organs via delivering metabolic necessities throughout the body [11,12]. On the other hand, an understanding of vascular dysfunction has provided novel insight into mechanisms of pathogenesis and progression of ischemic diseases [13–15].

Cardio-cerebrovascular diseases remain as significant burden on global healthcare system, and a leading cause of death worldwide [6,16–21]. Cardio-cerebrovascular diseases together incur substantial morbidity and mortality, affecting millions of lives annually, and consuming heavy healthcare expenditure, impacts which are increasing due to an ageing population [22–26]. Importantly, the prevalence of comorbid cardio-cerebrovascular diseases is increasing globally. Coronary artery disease is one of the main causes of death in most patients who have transient ischemic attacks or stroke [27–29].

Generally, vascular pathology stems from a combination of genetic risk, environmental factors, and the biologic changes associated with ageing [30–32]. Genetic risk, likely compounded by epigenetic modifications, environmental factors, including diabetes and chronic kidney disease [33,34], and the plasticity of vascular smooth muscle cells to acquire an osteogenic phenotype are major determinants of age-associated vascular calcification [35,36]. Vascular endothelial cells form the inner layer of blood vessels where they have a key role in the

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development and maintenance of functional circulatory system [37].

Recent studies have discovered chemokine CXCL9-induced inflammatory clock of ageing, which was critically involved in cardiac ageing (mainly characterized by adverse cardiac remodelling and poor vascular function). Aged endothelial cells in human and mice exhibited loss of function, cellular senescence, and hallmark phenotypes of arterial stiffness. Interestingly, the aged cardiovascular phenotypes could be reversed by silencing CXCL9 [38]. Thus, to develop multilevel community-engaged interventions is required to improve cardio-cerebrovascular health [39,40].

The vasculature throughout all major organs is essential for homeostasis and function throughout the body. Endothelial cells (ECs) comprise the innermost lining of blood and lymphatic vessels. ECs play a critical role in tissue homeostasis by regulating blood flow, delivering plasma-borne macromolecules, assisting vessel formation, and contributing to adhesion of circulating blood cells. ECs dysfunction would lead to various disease mechanisms, mainly including atherosclerosis [41], and peripheral artery disease [42,43], tumor vascularization [44], diabetic complications [45,46], small vessel disease [47,48], and neurodegenerative disease [49,50].

ECs from different tissues exhibited prominent transcriptomic heterogeneity [51]. Arterial, venous, and lymphatic ECs shared more markers in more tissues than did heterogeneous capillary ECs. ECs from different vascular beds (arteries, capillaries, veins, lymphatics) exhibited transcriptome similarity across tissues, but the tissue (rather than the vessel) type contributed to the ECs heterogeneity [52]. Tissue-specific differences in arterial, venous, and lymphatic specification of blood vessels and identification of transcriptomic and functional differences in larger vessels versus capillary ECs deserve further exploitation.

2. Blood vessel development and organogenesis

Blood vessel development is essential for normal embryogenesis. Vascularization of organs generally occurs by remodelling of the pre-existing vascular system during their differentiation and growth to enable them to perform their specific functions during development [53,54]. Organogenesis depends on orchestrated interactions between individual cells and morpho-genetically relevant cues at the tissue level. This is true for the heart, whose function critically relies on well-ordered communication between neighbouring cells, which is established and fine-tuned during embryonic development [55]. The development of stereotypical organs and body plans despite their incredible complexity, has captivated and confounded researchers. In 1942, Waddington proposed the concept of buffering to explain this remarkable characteristic of multicellular organisms, hypothesizing that there exists a buffering capacity based on an optimum and a threshold stimuli, beyond which the optimal response can no longer be achieved [56]. Cardiac development is achieved by the intricately coordinated actions of several key transcriptional factors and chromatin modifiers to guide timely activation of specific genes, thereby generating a complex four-chamber structure from the cardiac crescent [57]. Recent breakthroughs reported existence of a heretofore unknown capacity of embryo to repair and regenerate pharyngeal arch arteries, rescuing their morphogenesis into aortic arch artery, its branches, and the ductus arteriosus [4].

Cell-ECM (extracellular matrix) interactions play essential roles in the morphogenesis of pharyngeal arch arteries and their derivatives, the aortic arch artery and its major branches [58–60], illustrating the complexity of signaling events between different cell types that orchestrate arch artery development and the importance of the stabilization step in overcoming earlier defects and determining phenotypic outcome [61]. Maintenance of microvascular homeostasis is orchestrated through a dynamic interplay between endothelial cells and perivascular cells with the extracellular matrix (ECM) [14,62,63].

Employing a microphysiological model of capillaries on a chip mimicking the characteristics of healthy or fibrotic collagen, perivascular cells have been demonstrated as key mediators responding to collagen architectural and mechanical changes associated with ECM alterations around the vasculature via a *NOTCH3*-mediated mechanism of juxtacrine communication [63]. As a key regulator of neoangiogenesis and spatial control of endothelial sprouting, several computational models of Notch signaling have been developed to increase our understanding of Notch-regulated processes in various tissues [64,65]. Importantly, NOTCH signaling-mediated induction of AHE arterial-type hemogenic endothelium has been demonstrated as an important prerequisite for establishing the definitive hematopoietic program from human pluripotent stem cells [66].

3. Bioengineered microvasculature

Vascular trauma occurs when a blood vessel is injured such as a rupture of an artery in the extremities, which can lead to serious, life-threatening complications such as hemorrhage or blood clotting. When damage to an artery in the extremity occurs, urgent surgical repair is needed to restore normal blood flow. The current standard of care for patients with extremity vascular injuries can include procedures such as autologous vein grafting (surgical repair using the patient's own blood vessels) or implantation of a synthetic graft [67,68]. The vascular grafts presented herein are functional as arteriovenous conduits and as small-caliber arterial bypasses in the peripheral (carotid) and coronary circulations. However, conduits currently used in the clinic have suffered from substantial intimal hyperplasia, aneurysm, and calcification [69,70].

Recent years have seen the fusion of stem cell biology, physiology, and engineering to now allow for the creation of human tissues that can truly function in the setting of vascular repair and replacement for curing a broad spectrum of vascular diseases, mainly including cerebral dysfunction due to stroke, peripheral artery disease, diabetic foot, and aortic aneurysms [71–74].

To recapitulate and dissect major signaling pathways of vessel sprouting during physiological and pathological processes, functional engineered vasculature (such as 3D bioprinting, and angiogenesis-on-a-chip) for restoration of cardio-cerebrovascular system has been successfully established, elucidating three different spatial sprouting patterns [74–77] (Fig. 3). Such tissue-engineered vascular grafts may provide a readily available option for patients without suitable autologous tissue or for those who are not candidates for synthetic grafts.

Notably, autologous human induced pluripotent stem cells-derived blood vessels in vivo have been demonstrated as a potential translation in long-term vascularization for tissue engineering and treatment of vascular-associated diseases. Moreover, scientists have successfully generated endothelial cells and mesenchymal precursor cells from type 1 diabetic patient-derived human induced pluripotent stem cells lines, and generated blood vessels in vivo, which is an important milestone for clinical translation [78]. Metabolic transcriptome reprogramming during pathological vessel sprouting has been uncovered [45]. Furthermore, studies have proved that terminally differentiated vascular endothelial and smooth muscle cells closely resembled the in vivo counterparts as a faithfully modelling for studying human diseases of vasculopathies and microvascular integrity [79]. Thus, microvascular and tissue engineering are also making important strides, pointing towards a future that could enable revascularization of solid organs.

To date, tissue-engineered vascular grafts formed by seeding autologous bone marrow cells onto a copolymer of L-lactide and ε-caprolactone [80], or culturing autologous fibroblasts, and endothelial cells (ECs) without a scaffold [81], have shown promising functional results in early clinical trials. Encouragingly, SYMVESS, a universally implantable, bioengineered human vascular conduit for use in arterial replacement and repair at commercial scale, has been successfully approved as a new therapeutic product based on novel medical technology representing important progress in addressing a significant unmet medical need.

4. Defining spheroids and organoids

Compound attrition rates remain challenging for current biopharmaceutical industry [82]. Part of the attrition challenge is attributed to low animal-to-human translational success rates, so-called "translational failure" [83]. To overcome these shortcomings, there is a huge demand for more human predictive translational platforms and technologies, defined as New Approach Methodologies (NAMs). One promising example of NAMs are Complex in vitro models (CIVMs), i.e. organoids and microphysiological systems (MPS), which are complex three-dimensional (3D) "mini-organ" like structures [82,84].

Three-dimensional (3D) models are changing the paradigm of preclinical animal research as they more closely resemble the complex tissue environment and architecture in vivo. Among 3D models, spheroids and organoids represent the most versatile and promising models in that they can recapitulate the heterogeneity and pathophysiology of human organs de novo, and of filling the gap between conventional 2D in vitro testing and animal models as a revolutionized novel drug development pipeline (Fig. 1). Such 3D spheroids provide a more physiological model than traditional culture systems, which could be applied to various applications, including stem cell research and drug screening [85,86]. The use of single-cell and multi-cell spheroids has proven to be an efficient system to optimize and overcome limitations associated with ex vivo conventional systems [87].

The term "organoid" refers to mini-clusters of growing cells that are capable of self-assembled in vitro and differentiating into functional cell types, resembling an organ 3D structure, function, and cellular heterogeneity [88]. Generally, the term "organoid" is mainly used to describe such structure derived from stem cells [87]. Organoids are derived from pluripotent stem cells or isolated organ progenitors that differentiate to form an organlike tissue exhibiting multiple cell types that self-organize to form a structure not unlike the organ in vivo, which exhibit properties that recapitulate human organ development and that cannot be observed in animal models [89].

5. Vascularized organoids

As the ultimate goal of tissue engineering and regenerative medicine is to restore specific functions to damaged cells or tissues, cell therapy and tissue engineering open up new opportunities for tissue revascularization of diseased and damaged tissue and for surgical vessel replacement and tissue revitalization [90–93]. Tissue engineering aims to fabricate functional and viable 3D tissues outside of the body that can be tailored in size, shape, and function according to the respective needs before implanting them into the body. First clinical experiences have been published using bioengineered vascular grafts [94,95], and the ongoing preliminary research towards 'bioartificial beating heart' with increasing interest [96–100].

Vascularized organoids are emerging as powerful research models for studying endothelial differentiation and development [101], which has emerged as a novel modelling tool for preclinical research and personalized therapy for studying human vascularized tissues/organs derived from every certain healthy donor or patient for development of personalized medicine with promising therapeutic potential [102,103]. Recent breakthroughs have discovered that transplantation of vascular organoids embedded with dynamic hydrogel is capable of re-assembly of arterioles and restoration of cardiac function in infarcted hearts, offering effective therapeutic efficacy and translational applications in regenerative medicine [104].

5.1. Vascularized cardiac organoids

Cardiac organoids are comparable to age-matched human fetal cardiac tissues at the transcriptomic, structural, and cellular level. The creation of human induced pluripotent stem cells (hiPSCs) technology has provided an unprecedented opportunity to study tissue morphogenesis and organ development through 'organogenesis-in-a-dish' via human/patient-derived personalized hiPSCs [105]. Studies have successfully established protocols for generation of specific hiPSC lines derived from patients with inherited diseases for forming 3D cardiac microchambers, for studying the disease mechanisms of cardiac malformations at an early stage of embryogenesis [106]. By micropatterning and differentiating hiPSCs, further studies have engineered spatially organized cardiac organoids with contracting cardiomyocytes that surrounded by stromal cells distributed along the pattern perimeter as an in vitro embryotoxicity assessment tool [107]. Further studies have developed sophisticated internal chambers with well-organized multilineage cardiac cell types, recapitulate heart field formation and atrioventricular specification, develop a complex vasculature, and exhibit robust functional activity [108] (Figs. 2 & 3).

5.2. Vascularized cerebral organoids

Human brain organoids are three-dimensional tissues that are generated in vitro from pluripotent stem cells and recapitulate the early development of the human brain. Brain organoids consist mainly of neural lineage cells, such as neural stem/precursor cells, neurons, astrocytes, and oligodendrocytes [109,110]. Vascularization of human brain organoids is of great importance for potential clinical optimization following vascularization, circulation restoration and functional

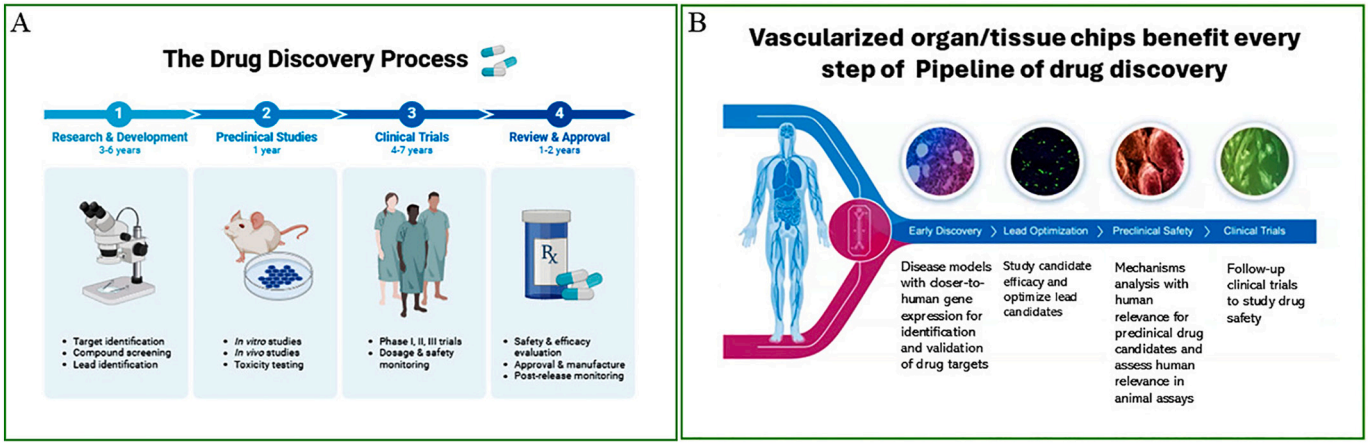

Fig. 1. Vascularized tissue/organ-on-a-chip benefits every step of drug discovery pipeline as a screening tool with close human genome relevance to reduce costs and time duration of animal models as a revolutionized drug discovery pipeline.

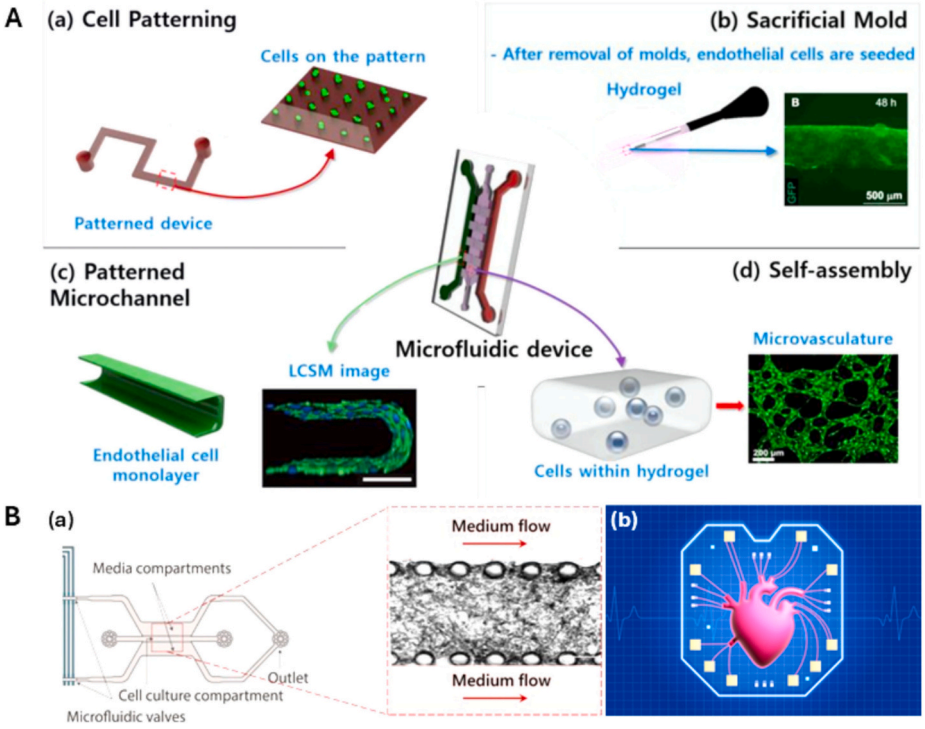

Fig. 2. Microvasculature-on-chip (A) [179–182] and heart-on-chip (B) as novel research models of cardio-cerebrovascular system equipped with microfluidic device and artificial intelligence. LCSM: laser scanning confocal microscope

integration of human brain organoids in vivo after transplantation [111–115].

In 2018, human cerebral organoids were transplanted into the retrosplenial cortex of NOD-SCID mice for the first time. Organoid grafts exhibited progressive neuronal differentiation and maturation, gliogenesis, integration of microglia, and growth of axons to multiple regions of the host brain, with potent therapeutic potential for restoration of the delivered oxygen and nutrients though functional circulation [112]. Moreover, the fusion of heterogeneous neuronal organoids has been well-documented [116–118]. Transplantation of fused brain-vascular organoids into immunodeficient mice, followed by the engraftment of human hematopoietic stem cells, will enable researchers to establish vascularized human brain organoids with a human vascular system. Prevascularization has been demonstrated to enhance blood vessel formation and attenuated apoptosis in transplanted organoids compared to counterparts that had not been prevascularized [119]. In rodents, increased neurogenesis occurs after brain ischemia, and newly differentiated neurons migrate along the vessels to the ischemic area [120]. Moreover, scientists have successfully applied genome-editing technology to establish vascularized human brain organoids [121].

Therefore, current trials to vascularize various organoids include the adjustment of cultivation protocols, the introduction of microfluidic devices, and therapeutic optimization of organoid transplantation in vivo.

6. Next generation drug discovery and personalized medicine

Vascularized organoid-on-chip technology is a novel tool of mimicking organ physiology that enabling therapeutic development of pharmaceutical applications, via preclinical high-content phenotypic screening for novel drug development, advanced disease models of diabetes, cardio-cerebrovascular disease, and 'clinical trials in-a-dish' [122–124] (Fig. 4).

A crucial aspect of success lies in effectively reintegrating these cells or tissues within the recipient organism [125], as well as multiorgan response, such as communications among heart, central and peripheral nervous systems, and the spleen [126] (Fig. 4). Central to this approach is to establish a fast connection with the host's vascular system. To this end, organ-on-chip platform has emerged as an essential platform to explore intricate relationships between vascular, and immune/neural/epithelial cell interactions for studying organogenesis and (patho-) physiological process [127]. One of the research objectives is to optimize transplantation outcomes via regulation of pro-regenerative niche through tissue-specific matrix protein (extracellular matrix; ECM) and formation with the utilization of in vitro cutting-edged platforms (such as bioprinting with ECM-based bioink) to model cellular and cell-extracellular matrix interactions that approximate native tissue microenvironment [128,129], as well as animal models in vivo [130].

Organ-on-a-chip technology was originally developed by the Wyss Institute for biologically inspired engineering at Harvard University [131]. Bioengineered microfluidic chips that co-cultured between different organs are able to emulate potential crosstalk and molecular functions phenotypes of human tissues [132,133]. When organoid-chip technology integrates with each other with robotic microfluidic coupling as models of multi-organ human-body-on-chips, multiple benefit pipelines of drug discovery [134–136], since the chips could be applied as disease models with close human relevance since the system function as a valuable screening tool using human genome to validate drug targets, which could be used to study efficacy and repurposing of drugs, and predictive toxicology [137,138].

Recent breakthroughs have successfully established and monitored formation of endothelial networks around mesenchymal and pancreatic islet spheroids, as well as blood vessel organoids generated from pluripotent stem cells on-chip using advanced microfluidics [76]. Further scientific advancements suggest that integrating human pluripotent stem cell-derived endothelial cells via an open microfluidic vessel-on-chip platform enables the modelling of patient-specific human endothelial tissue via recreating human microenvironment as an alternative to partial animal preclinical testing, and has the potential to become a general tool for animal-free vascular research towards the 3R principles of replacing, reducing, and refining [139,140].

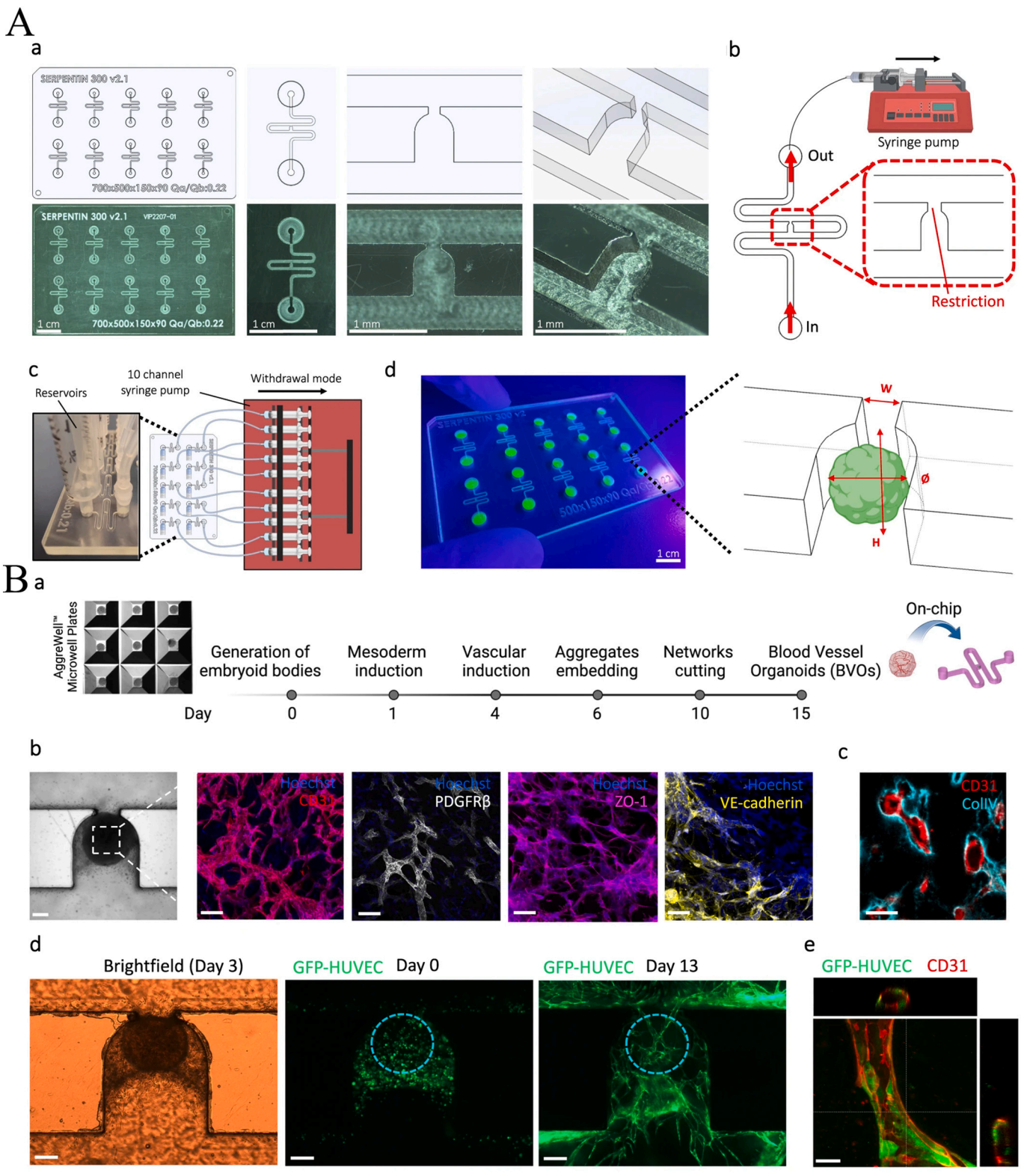

Fig. 3. (A) Brief overview of Device design of microfluidic chip, and schematic three-dimensional view of organoid and cell configurations. (B) Vascularization of Blood Vessel Organoids on-chip [76].

7. Advances of vasculature- and heart-on-chip research models

During recent decades, microfluidic-based technology has developed microscale models recapitulating key physical and biological cues typical of the native microvasculature and myocardium [141–143]. Heart-on-a-chip (HOC) technology is an emerging field where cardiomyocytes among other cell types in the heart can be used to generate functional, beating cardiac microtissues that recapitulate many features of the human heart. HOC models are showing great promise as disease modelling platforms and are poised to serve as important tools in the drug development pipeline [144]. By leveraging advances in human pluripotent stem cell-derived cardiomyocyte biology and

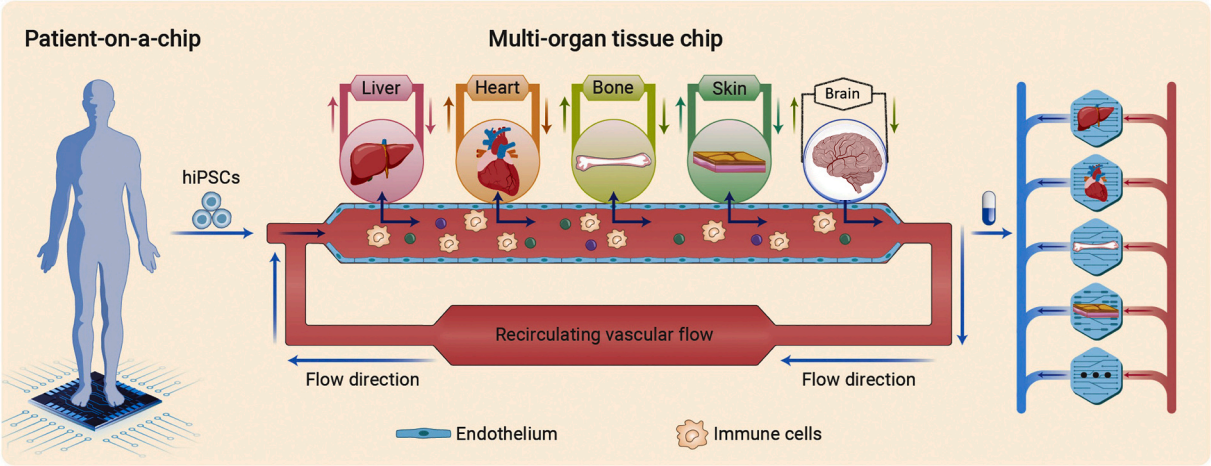

Fig. 4. Human specific induced pluripotent stem cells (hiPSCs)-derived multiorgan tissue chip as patient on-a-chip and potential 'clinical trials in a dish' model for drug testing (https://cn-bio.com/physiomimix/organ-on-a-chip).

microfabrication technology [145,146], diseased HOCs are highly tuneable and can be generated via different approaches such as: using cells with defined genetic backgrounds (patient-derived cells), adding small molecules, modifying the cells' environment, altering cell ratio/composition of microtissues, among others [147,148] (Figs. 2 & 3).

Recent breakthroughs have achieved on cardiovascular on chip models. Studies have successfully established perfusable, and endothelialized microvasculature-on-a-chip modelling featuring an interpenetrating-polymer-network hydrogel that recapitulates the stiffness of blood vessel intima, basement membrane self-deposition and self-healing endothelial barrier function, thereby enabling real-time visualization, with high spatiotemporal resolution, of microvascular obstruction and endothelial permeability under physiological flow conditions [149]. In the meanwhile, a higher throughput "heart-on-chip" which utilizes a semi-automated fabrication technique has been well-established [150,151].

Induced pluripotent stem cells (iPSC) can differentiate into multiple cell types, including cardiomyocytes and have tremendous potential for drug discovery and regenerative therapies [152–154]. Despite the line-to-line variability of gene expression in the undifferentiated state of embryonic stem cells and iPSC, the variance narrows significantly in lineage-specific iPSC-derived cardiac progenitors, and these progenitor cells can be isolated and used for transplantation without generation of unwanted cell types [155]. Furthermore, combination of patient-derived and genetically engineered iPSCs with tissue engineering uncovered insights into mitochondrial roles in cardiac development and disease and elucidated the pathophysiology underlying the cardiomyopathy [151]. Simultaneously, well-characterized human pluripotent stem-cell-derived ventricular cardiomyocytes with key electrophysiological features of native human ventricle, have emerged as a revolutionary tool to assess preclinical drug-induced arrhythmogenicity [156]. In the meanwhile, patient-specific iPSC–derived cardiomyocytes offer a unique avenue for studying primary cardiac abnormalities and uncovering pathogenic mechanisms that underlie sporadic congenital heart diseases [145]. To mimic the cardiac environment more accurately, further studies using the Biowire II platform, engineered cardiac tissues with an epicardial outer layer and inner myocardial structure have been successfully constructed [157]. Further studies have successfully and reproducibly applied implantation of conductive hydrogels via intramyocardial injection with facilitated electrical pacing of iPSC-cardiomyocytes followed by gelation within the heart tissue, thereby preserving the structure of the myocardium with no signs of encapsulation, injury or implant-related complications in any of the animal assays [158,159].

An integrated microfluidic system with high-precision fabrication has been successfully generalized to expand the accessible spectrum of organ-on-a-chip models towards structurally and biomechanically sophisticated tissue systems [160]. Furthermore, integrated with a QRS complex wave detection architecture for signal preprocessing and a two-layer deep-learning network for post-processing, artificial intelligence (AI)-enabled chips have become remarkably attractive solutions for portable monitoring and classification systems [161–165]. Furthermore, AI-driven, real-time software is able to recognize and assign specific AF electrical patterns, specifically spatio-temporal electrogram dispersion [166,167]. Advanced tissue technologies of blood-brain barrier organoids, in combination with an artificial intelligence (AI) algorithm, have been successfully developed to increase the throughput and the reliability of histo-morphologic evaluations of apoptosis as high-throughput toxicity readouts in drug development [168]. AI-guided ablation of spatio-temporal dispersion areas in addition to PVI is superior to PVI alone in eliminating AF at 1-year follow-up in patients with persistent and long-standing persistent AF [169].

Also, a series of pioneering studies have successfully developed deep learning models for organoid analysis via predicting gene expression [170,171], visualized and quantitative analysis for high-throughput drug screening [172,173], predictable reliable real-time identification of cell fates of in situ organoids [174,175], or quantifying morphogenetic organizations of organoids at multi-scales [176], which is promising for novel therapeutic drugs and clinical applications [170,174,177,178]. Therefore, to combine microfluidic cardio-cerebrovascular system-on-chip with AI and deep learning systems will boost the transformation of personalized precision medicine and drug therapy of cardio-cerebrovascular diseases into the clinic.

8. Conclusions and outlook

Drug discovery and human disease modelling of cardio-cerebrovascular system face challenges in recapitulating cellular complexity and animal-to-human translation due to the limitations of conventional 2D cell culture and animal models. The tremendous development of tissue vessel-chip models constructed with human cells offers a new approach to bridge the gap between preclinical research and translation of angiogenic therapy as interesting alternatives to preclinical animal models. Along with innovative regenerative surgery, tissue engineering and regenerative medicine fuse together, and are promising to fuel the personalized medicine and clinical translation (Graphical abstract).

Critically, the future development of functional bioengineered vascularized tissues aided with biomaterials and smart AI platform that is compatible with cell-specific functions as well as manufacturing

technology. Therefore, advanced research models with multisystem input of vascularization and neurogenesis may enable the development of transformative drug discovery as essential disease modelling tools of multiorgan system. Additionally, armed with cutting-edge-research platforms (e.g. high-throughput multi-omics analysis and 3D bio-printing) to test effects of drug actions from diverse perspectives, and mimicking key signaling governing cellular and cell-extracellular matrix interactions during cardio-cerebrovascular development, vascularized organoid-on-chip model of cardiovascular system paves a new avenue of integrated human biology to study and quantify changes in the vascular architecture and function during vascular and organ development, or upon drug treatment, thereby enabling translation of cardio-cerebrovascular disease conditions into the clinic via novel drug development.

CRediT authorship contribution statement

Xi Chen: Writing – review & editing, Writing- original draft. **Weiping Lin:** Writing – review & editing, Writing- original draft. **Micky Daniel Tortorella:** Conceptualization, Validation, Revision, Supervision.

Ethical statement

Not required.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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