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Cardiac organ chip: advances in construction and application

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cardiac organ chip; drug; cardiac disease; microenvironment; microfluidic

From the Contents

ABSTRACT

Cardiovascular diseases are a leading cause of death worldwide, and effective treatment for cardiac disease has been a research focal point. Although the development of new drugs and strategies has never ceased, the existing drug development process relies primarily on rodent models such as mice, which have significant shortcomings in predicting human responses. Therefore, human-based in vitro cardiac tissue models are considered to simulate physiological and functional characteristics more effectively, advancing disease treatment and drug development. The microfluidic device simulates the physiological functions and pathological states of the human heart by culture, thereby reducing the need for animal experimentation and enhancing the efficiency and accuracy of the research. The basic framework of cardiac chips typically includes multiple functional units, effectively simulating different parts of the heart and allowing the observation of cardiac cell growth and responses under various drug treatments and disease conditions. To date, cardiac chips have demonstrated significant application value in drug development, toxicology testing, and the construction of cardiac disease models; they not only accelerate drug screening but also provide a new research platform for understanding cardiac diseases. In the future, with advancements in functionality, integration, and personalised medicine, cardiac chips will further simulate multiorgan systems, becoming vital tools for disease modelling and precision medicine. Here, we emphasised the development history of cardiac organ chips, highlighted the material selection and construction strategy of cardiac organ chip electrodes and hydrogels, introduced the current application scenarios of cardiac organ chips, and discussed the development opportunities and prospects for their of biomedical applications.

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Introduction

Cardiovascular diseases are the leading cause of death worldwide, and the effective treatment of diseases of the heart, which is the main organ subject to this pathology, has been a concern. The development of new drugs and strategies for treating cardiac diseases is ongoing.[1, 2] However, the preclinical research in the drug development process has primarily relied on *in vivo* models using rodents. The significant flaw in these drug discovery models is that they cannot accurately predict human responses, such as the physiological differences between animals and humans, leading to varied reactions to the same drug. In addition, many animal studies are poorly conducted, performed and analysed, and researchers' reviews and summaries of evidence from animal studies are methodologically insufficient.[3, 4] Human-based *in vitro* cardiac tissue models can effectively mimic important physiological and functional aspects, facilitating disease treatment and drug development.

In recent years, cardiac organ-on-a-chip technology has gained widespread scientific attention for its potential to achieve physiological similarity *in vitro*, potentially transforming how

scientists and industry test the effects of drugs on human cells and organs (**Figure 1**).[5-7] The concept of the cardiac organ-on-a-chip traces back to the late 1990s with "organ-on-a-chip" technology. It initially focused on simulating the physiological functions of various human organs. Common organ chips include those for the kidneys, lungs, liver, vasculature, and heart. The cardiac organ-on-a-chip is a microfluidic device that combines biology, engineering, and material science and is designed to simulate the physiological functions and pathological states of the human heart by culturing cardiomyocytes (CMs) within a microchip. This technology enables researchers to study cardiac diseases and test drugs in an *in vitro* environment, thereby reducing the need for animal experiments and increasing the efficiency and accuracy of research. The basic framework of a cardiac organ-on-a-chip typically consists of multiple functional units capable of effectively simulating different parts of the heart, such as the atria, ventricles, and cardiac electrical activity.[8] Additionally, the growth and development of cardiac cells as well as their responses under various drug treatments and disease conditions can be observed by regulating the microenvironments of these units.

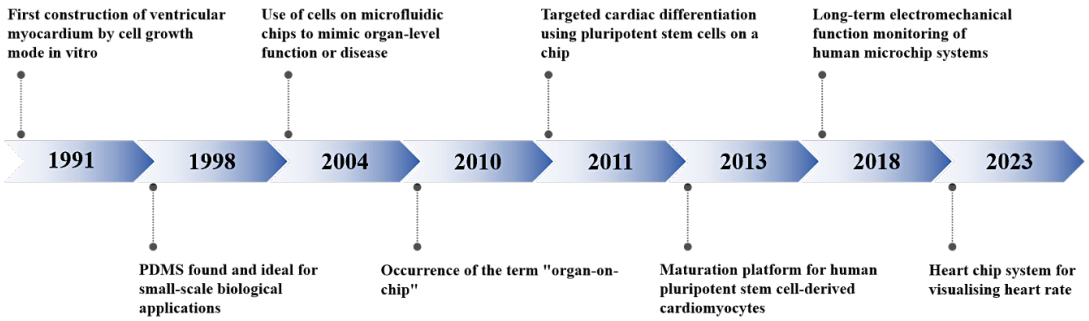

Figure 1. History of the development of cardiac organ chip. Created with Adobe Photoshop 2023. PDMS: polydimethylsiloxane.

One of the earliest studies that used myocardial tissue cell cultures to study diseases was conducted by Rohr et al.[9] who established a culture method for neonatal rat ventricular myocytes that could control the geometry of interconnected growth channels on a chip, resulting in monolayers of myocyte chains with variable lengths and widths. Foundational research on organ-on-a-chip systems has started to emerge, as researchers have sought to replicate the physiological environment found in living organisms using microfluidic technology. In 1998, polydimethylsiloxane (PDMS) was used to construct biochips, and its optical transparency and soft elasticity make it ideal for small-scale biological applications. Since then, PDMS has become a common substrate material for microfluidic chips and has rapidly advanced the field of biological microfluidics.[10]

In 2004, Duffy et al.[10] engineered cardiac function at the organ level using CMs. Electrical stimulation is applied to these cells to simulate natural heart electrical signals and induce to CMs alignment coupling and synchronous contraction. This additional electrical stimulation resulted in a 7-fold increase in the amplitude of synchronised CMs contractions of *in vitro*.[10] By 2010, the term "organ-on-a-chip" had first appeared in the paper of Hub et al.[11] The group constructed a biomimetic microsystem that recreated the functional alveolar-capillary interface of human lungs based on the organ-on-a-chip concept. In addition, with the development of microfluidic technology, scientists have been able to create more refined cardiac models. Cardiac organ chips based on induced pluripotent stem cells (iPSCs) are also being used to better mimic the state of CMs, thereby improving the model authenticity. This advancement has allowed researchers to conduct experiments in environments more closely resembling human physiological conditions.

In 2011, Kattman et al.[12] optimised the cardiac lineage development of iPSC lines from different sources using known factors that control early embryonic development, emphasizing the importance of stage-specific strategies in the differentiation design of iPSCs. However, these induced differentiated CMs were still immature. To overcome this limitation, Sun et al.[13] designed an engineering platform called "biowire", which uses electrical stimulation to generate mature human iPSC-derived CMs. During this period, the electrophysiological properties of cardiac cells and their relationships to drug responses were investigated. As the technology progressed and matured, researchers not only focused on cardiac cell functions but also began to comprehensively consider the interactions between the heart and other organs comprehensively, which led to the gradual development of the concept of multiorgan chips. Oleaga et al.[8] constructed a human four-organ system consisting of heart, liver, skeletal muscle, and nervous system modules. They maintained cell viability and functionality in a serum-free environment using a non-pump system for 28 days. Among these models, designers have noninvasively evaluated the electrophysiology of neurons and CMs, as well

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as the mechanical monitoring of CMs and skeletal muscle cells. This technique ultimately achieves long-term culture and detection of cell functions. To enhance observation and avoid the need for expensive detection equipment and complicated calculations, Sun et al.[14] produced a novel conductive and anisotropic structural colour hydrogel by simply polymerizing non-close-packed colloidal arrays on super-aligned carbon nanotube sheets sheet, promoting the process of organ chips moving towards clinical research (**Figure 1**).

Cardiac-on-a-chip systems with biochemical and physiological control have gradually become powerful tools for simulating the human physiological environment and monitoring the cell status, and their main components are increasingly mature, including microfluidic chips, microenvironmental interventions, and monitoring devices. In the following sections, we elaborate on the main components and applications of cardiac-on-a-chip.

Construction of Cardiac Organ-On-A-Chip

The microfluidic chip is the core component of the whole heart organ-on-a-chip system, which simulates the cardiac microenvironment by precisely controlling the flow of fluid and designing the cavity structure. Additionally, it provides an important platform for evaluating the function of cardiac cells, for the physiological monitoring of cardiac cells and for studying the interactions between cells. In this section, we describe the construction methods of microfluidic chips and the application of related materials, focusing on the selection of electrode materials based on the electrical properties of CMs as well as potential hydrogel culture systems.

Microenvironment construction methods

Current three-dimensional (3D) printing technology enables the use of various materials, such as resins, polysiloxanes, and cell-laden hydrogels, to manufacture microfluidic chips and biomimetic tissues. These materials are crucial for constructing cardiac models that simulate physiological conditions. Researchers at Boston University have developed a novel cardiac chip platform using two-photon polymerisation technology, which can generate 3D printed structures at the scale of biological tissues and with sub-micrometer resolution, making it well suited for simulating natural environments.[15] This 3D bioprinting constructs microfluidic chips in a layer-by-layer fashion, allowing for the creation of complex 3D structures. This integrated manufacturing method can complete the fabrication of microfluidic chips in one step, preventing assembly errors. Although 3D printing technology has fundamentally changed the construction of cardiac chip platforms, challenges remain in replicating the complex structures and functions of the human heart. Ongoing collaboration among researchers, clinicians, and engineers is essential to overcome these challenges and leverage the full potential of 3D-printed cardiac chip systems in clinical and research settings.

Photolithography is a process that transfers patterns onto substrates through selective exposure, using a light source (such as ultraviolet light) to irradiate photosensitive materials with a mask.[16] This process results in a chemical reaction that transfers the pattern from the mask onto the material, forming the desired microstructures; it boasts high resolution, potentially reaching the nanoscale, but the equipment costs can be high. Soft lithography is a micro-nanofabrication method based on elastomeric materials, offering efficiency, low cost, and the ability to create 3D structures, especially for topological structures and materials with which that traditional photolithography struggles. This technology uses polymeric elastomers as masks, stamps, or templates to fabricate nanoscale structures. During the construction of cardiac organ-on-a-chip devices, soft lithography can be used to create microfluidic devices and other miniature components. This technique not only achieves high-precision patterning but also meets the diverse needs of biomedical applications.

Thermoforming involves pressing heated moulds into thermoplastic materials (such as polymethyl methacrylate) under high temperature and pressure to form microstructures. This method is suitable for mass production but may lead to the deformation of microfluidic chips due to the high temperatures involved. In biomedical applications, thermoforming is employed to manufacture microfluidic chips and other micro-nano functional components. The development of these technologies provides various possibilities for constructing cardiac organ-on-a-chip devices, enhancing their applications in cardiovascular disease research and drug screening with greater precision and effectiveness.

Cell origin and transformation

The cardiac organ-on-a-chip is an advanced *in vitro* model that can replicate the physiological and functional characteristics of the human heart. These chips integrate various cell types, including CMs, fibroblasts, and endothelial cells, to mimic the complex interactions within cardiac tissue. The development of cardiac chip technology is driven by the demand for more accurate drug testing and disease research models, providing a promising alternative to traditional cell culture and animal models.

iPSC-derived CMs: These cells form the core of the heart chip, providing the contraction functionality required to mimic cardiac tissue. iPSC technology allows for the generation of patient-specific CMs, enhancing the physiological relevance of the model.[17] Fibroblasts and endothelial cells: The inclusion of fibroblasts and vascular endothelial cells is crucial for replicating the structural and functional environment of the heart. These cells participate in paracrine signalling and direct intercellular interactions, which are essential for normal cardiac function. The vascular endothelium is integrated into the chip to simulate the blood flow and shear stress conditions that the heart experiences *in vivo*. It plays a significant role in drug testing by affecting the permeability and action of drugs on CMs.

Microtissue construction: Microtissues refer to cell clusters cultivated in microfluidic chips that can be either two-dimensional (2D) or 3D in nature (**Figure 2A** and **B**). The 2D microtissues typically refer to single layers of cells growing on a flat surface, whereas 3D microtissues mimic a more realistic 3D structure of heart tissue (**Figure 2C**). [18] Techniques such as encapsulation in N-isopropylacrylamide gel can create stable cardiac microtissues, which can be used for scalable workflows in various applications.[19]

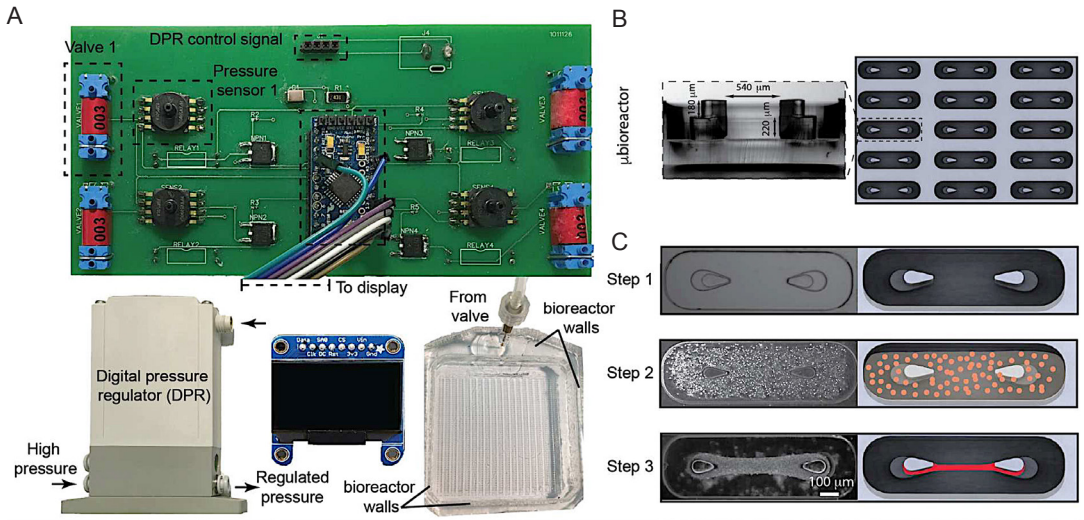

Figure 2. Microfluidic platform. (A) Custom-designed electronic circuitry. (B) Schematic of the bioreactor platform with an array of bioreactors for large-scale production of cardiac tissues along with the cross-sectional view. (C) Schematic and images of the cardiac tissue formation. Reprinted from Parsa et al.[18] Copyright 2017 The Royal Society of Chemistry.

Construction of microfluidic systems

The cardiac chip platform was developed using microfluidic chip manufacturing methods, allowing for precise control of the microenvironment, including fluid flow and mechanical forces.[20-22] The cardiac organ-on-a-chip can employ controlled fluid flow within the cell culture environment to regulate the nutrient supply and the removal of metabolic waste, thereby simulating the *in vivo* microenvironment of the heart. Additionally, microfluidic technology is employed to achieve mechanical stimulation, such as cyclic stretching, to optimise the contractile stress and maturity of CMs. The fluid circuit board represents a significant innovation, enabling automated and modular control of microfluidic flow, which is crucial for maintaining the physiological conditions necessary for cardiac tissue culture.[23] Polymer materials, such as PDMS, polyacrylic acid, and polystyrene, have been used as biocompatible substrates for microdevices (**Figure 3A**). These polymers combine the advantages of silicon/glass systems and offer a wide variety, repeatable deformation, and low cost, making them more suitable for mass production.[24, 25] As a result, they have become ideal materials for microchip fabrication. Among many polymers, PDMS is the most popular material used in chip manufacturing due to its good oxygen permeability and cost-effectiveness (**Figure 3B**). Lee et al.[26] used a parylene layer to cover polyurethane fibers, creating ultrasoft biointegrated electronic devices for monitoring the dynamic beating of cardiac cells. Lind et al.[27] synthesised various viscoelastic ink materials for 3D printing chips, which can integrate multiple functions, structures, and biological materials, thereby improving the efficiency of model fabrication. Two teams successfully employed a 3D microfluidic analytical device for simultaneous detection of three biomarkers for acute myocardial infarction and developed a universal method for 3D printing elastomers.[28, 29] Mohammadi et al.[30] described a method that relies on microfabrication of elastomers, which are able to assemble 2D arranged cell sheets in layers into functional conical ventricles (**Figure 3C** and **D**). A typical microfluidic chip includes 2D scaffolds, 3D scaffolds, porous scaffolds, and bioprinting. These structures typically utilise microfluidics to mimic *in vivo* environments such as blood flow or mechanical stimuli. For drug testing and research: they can both be used for cardiac drug screening and cardiac disease research.

Components of the cardiac microfluidic chips

The manufacturing methods for "chips" originated from the preparation of computer microchips and they have gradually improved. Cardiac chips can involve various micromanufacturing techniques, such as micromoulding, laser etching, and injection moulding, depending on the materials used. These technologies enable precise micropatterning in tissue engineering environments and can be integrated with microsensors for analysing biochemical factors, cell migration, and fluid pressure. First, the choice of materials for cardiac microfluidic chips is critical in simulating the physiological characteristics of human organs, affecting their applications in drug testing, disease modelling, and personalised medicine. Materials with good biocompatibility and mechanical properties, such as elastomers and hydrogels, etc., are gradually being developed.

Substrate materials for microfluidic chips

The materials used in cardiac chips have been continuously optimised over the past decade. The earliest materials used for chip construction were silicon/glass. These materials have been gradually replaced due to their poor electropermeability, permeation of gases and poor biocompatibility. These systems are notably impermeable to oxygen, allowing them to be used in combination with normoxic/hypoxic conditions to simulate pathological conditions for *in vitro* research. However, owing

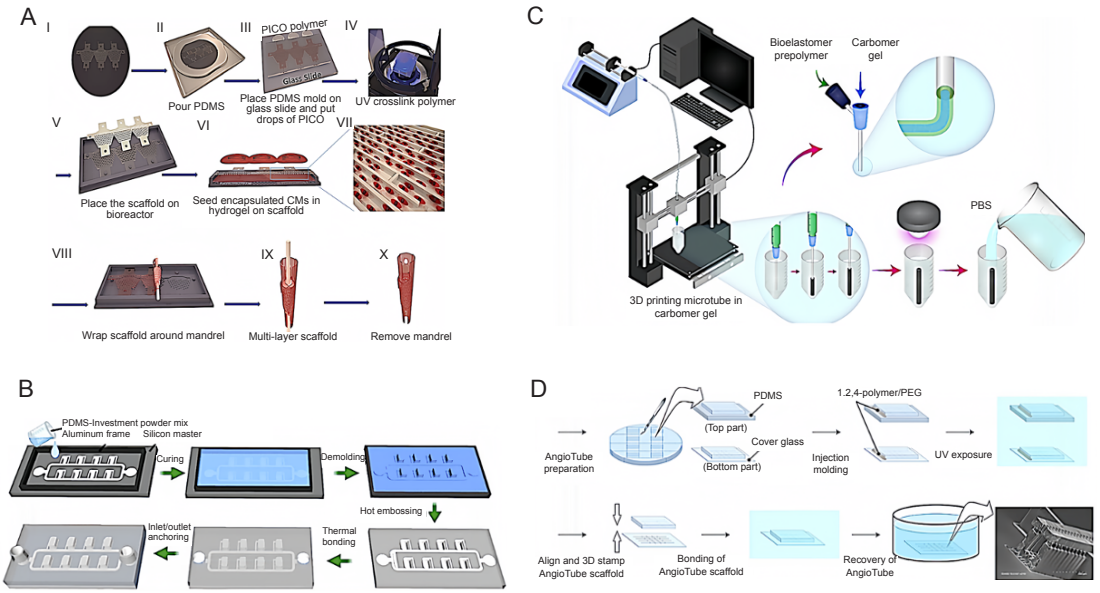

Figure 3. Fabrication of heart-on-chips and cardiac tissue scaffolds. (A) Assembly of a conical cardiac ventricle through (I, II) soft lithography of PDMS master molds, (III–V) replica molding of PICO, (VI, VII) and CMs cultured on the scaffold and (VIII–X) scaffold wrapping using a mandrel which is later removed. Reprinted from Mohammadi et al.[30] Copyright 2022 Wiley - VCH GmbH. (B) Application of a reinforced PDMS mould for hot embossing of COP to produce microfluidic chips. Reprinted from Qin et al.[25] Copyright 2022 Royal Society of Chemistry. (C) 3D printing of bioelastomer prepolymers using a co-axial needle to create vascular tubes. The carbomer was used as a supporting bath. Reprinted from Savoji et al.[29] Copyright 2020 American Chemical Society. (D) Fabrication of AngioTubes through the casting of 1,2,4 prepolymer into PDMS moulds to create the top and bottom layers of the tubes, which are then aligned and 3D-stamped. Reprinted from Lai et al.[24] 3D: three-dimensional; CM: cardiomyocyte; PBS: phosphate buffered saline; PDMS: polydimethylsiloxane; PEG: polyethylene glycol; PICO: polycarbonate; UV: ultraviolet rays.

to their high costs and complex manufacturing processes, they have not been widely used. Researchers have gradually developed polymer polymerisations such as PDMS, polyacrylic acid, and polystyrene as biocompatible substrates for the construction of microfluidic chips.[25] These polymers combine the advantages of silicon/glass systems and offer a wide variety, repeatable deformation, and low cost, which make them more suitable for mass production and cast them as ideal materials for microchip fabrication.

Electrode materials for cardiac organ chips

The heart exhibits typical electrophysiological characteristics. Therefore, electrical pulse interventions on organs-on-a-chip are essential. These interventions can more precisely enhance the structure and function of CMs and facilitate the formation of synchronised beating tissues, thereby simulating a microenvironment of CMs that is closer to physiological conditions. Integrating electric fields with cardiac chips enables precise control over cell behaviour, migration, and differentiation. The choice of electrode material significantly impacts spontaneous beating in cells, with common types including conductive polymers, metal electrodes, and microelectrode arrays. Conductive polymers (such as polypyrrole, polyaniline, polythiophene, and its derivative poly(3,4-ethylenedioxythiophene)) are widely studied because of their high specific capacitance and good conductivity. In addition, graphene and carbon nanotubes also possess excellent conductivity and mechanical properties and can be incorporated into flexible materials such as hydrogels to create conductive scaffolds that enhance electrical coupling and synchronised contraction between CMs. Metal electrodes such as platinum and gold offer good conductivity and chemical stability and are commonly used to manufacture microelectrode arrays. Microelectrode arrays can integrate multiple electrodes at a small scale, allowing for high-density recording of electrical signals from cells or tissues and providing a larger contact area and a more physiologically relevant electrophysiological environment, thus improving signal quality and resolution. These technologies demonstrate significant potential in the diagnosis and treatment of heart and brain health. Zheng et al.[31] applied micro/nano 3D bioelectronics technology within the field of cardiac electrophysiology. Through 3D micro/nano fabrication techniques, they developed passive extracellular nanoelectrode arrays and active field-effect transistors, combined with advanced membrane penetration strategies, enabling high-quality intracellular action potential recordings from CMs. This technology allows for accurate, long-term, and high-throughput recordings of intracellular action potentials, extracting rich information on ion channels, which can be used for studying the mechanisms of cardiac diseases and evaluating drug toxicity.

Hydrogel system for construction of the cellular microenvironment

Hydrogels are widely used in cell culture because of their similar properties relative to those of the extracellular matrix (ECM), good biocompatibility, appropriate stiffness and suitable permeability. Hydrogels have been identified as the preferred choice for the constructing *in vitro* cell/tissue culture models. These materials have tunable properties for adequate simulation of the extracellular microenvironment, including pH value, biochemical factors, substance interactions, and structural properties. Additionally, a culture matrix constructed with hydrogels is fully involved in cell adhesion, proliferation, migration, differentiation and cell-matrix interactions. During the development of organ chips, a variety of hydrogels have been developed, including natural-based hydrogels, synthetic-based hydrogels, and composite hydrogels. Among them, natural materials have become the main materials for constructing organ chips because of their inherent biocompatibility, which is exhibited by the composite of multiple natural materials or the composite of natural materials and polymers. These hydrogels include fibrin,[32] gelatin,[33] collagen I,[34] matrigel,[35] hyaluronic acid,[36] poly(ethylene glycol) diacrylate, and polycaprolactone[36] (**Figure 4**). As a natural material with biodegradable and cell-promoting properties, fibrin is the preferred material for constructing cardiac organ chips. Occhetta et al.[37] reported a dynamic microtissue model *in vitro* based on fibrinogen, aprotinin and Ca^{2+} to simulate cardiac scar formation. Gelatine molecules obtained through collagen hydrolysis possess excellent biocompatibility, making them another option for constructing cardiac organ-on-a-chip platforms. Furthermore, methacrylated gelatine has also been used for the construction of heart organs-on-a-chip, with the advantage of possessing enhanced mechanical properties. Nawroth et al.[38] reported a novel biocompatible laser etching method that enables large-scale on-demand micropatterning via gelatine hydrogels for organ-on-a-chip applications. Fu et al.[39] reported a strategy for constructing heart chips using methacrylated gelatine elastic hydrogels, which serve as scaffolds to promote CM adhesion and growth. Elastic methacrylated gelatine hydrogels have several advantages in organ-on-a-chip applications; they promote CM adhesion and growth. Additionally, these hydrogels enhance the periodic contraction frequency of CMs, providing greater flexibility and sustained expansion and contraction capabilities.

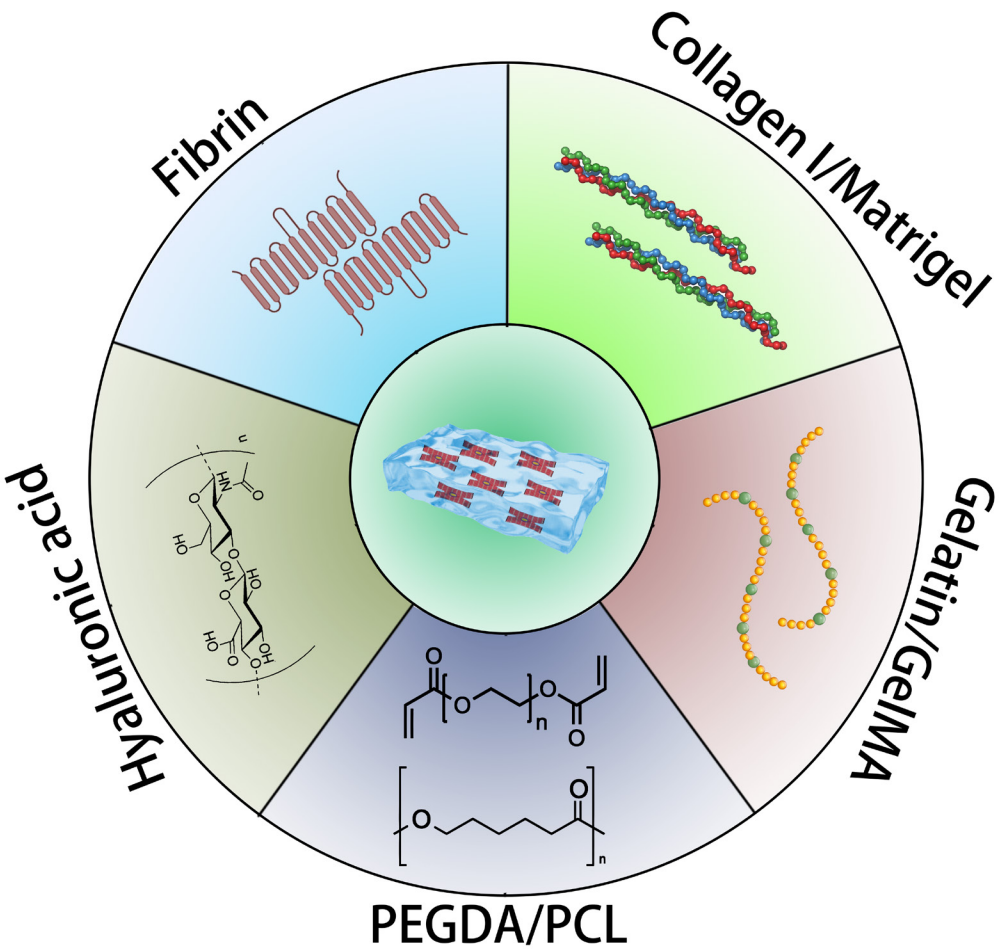

Figure 4. Cardiac organ chip hydrogel construction material. Created with Adobe Photoshop 2023. GelMA: methacrylated gelatine; PCL: polycaprolactone; PEGDA: poly(ethylene glycol) diacrylate.

Intervention and monitoring

Mechanical and electrical interventions in cardiac chips, which mimic the physiological conditions of the heart, are crucial for advancing cardiac tissue engineering. These interventions enhance the maturity and functionality of *in vitro* cardiac tissues, providing valuable insights for drug testing and disease modelling. Electrical stimulation is essential for evaluating the physiology of cardiac tissues (**Figure 5A**).[40] Conductive hydrogel pillars have been used to facilitate non-invasive electrophysiological studies, allowing for continuous monitoring of cardiac tissues[41,42] (**Figure 5B**). Indium tin oxide electrodes provide a biocompatible and semi-transparent solution, promoting calcium transients and the propagation of calcium waves within cardiac tissues.[43] A microbioreactor platform has been developed to apply controlled electric fields and current densities, thereby enhancing the beating characteristics of cardiac microtissues.[44] Additionally, fluidic medium connectors can serve as electrodes in novel cardiac chip systems for monitoring electrical activity and metabolic functions.[45] Through the application of electrical stimulation to simulate the electrophysiological characteristics of the heart, electrodes are installed on the chip and connected to an electrical pulse generator. Mechanical stimulation involves applying pressure or deformation to microtissues to mimic physiological activity in the heart. Mechanical stimulation is often combined with electrical cues, which are vital for replicating the native environment of the heart. Microbioreactors apply cyclic uniaxial strain to cardiac microtissues, simulating physiological conditions.[44] The integration of atomic force microscopy with microelectrode arrays allows for real-time recording of contractile dynamics and electrical events, providing deeper insights into the mechanical properties of cardiac tissues.[46] In summary, constructing cardiac chips is a complex process that requires interdisciplinary knowledge and technologies, including bioengineering, cell biology, materials science, and microfluidics. Through continuous optimisation of designs and materials, cardiac chips can more accurately simulate the physiological and pathological states of the heart, providing powerful tools for research and treatment of cardiovascular diseases.

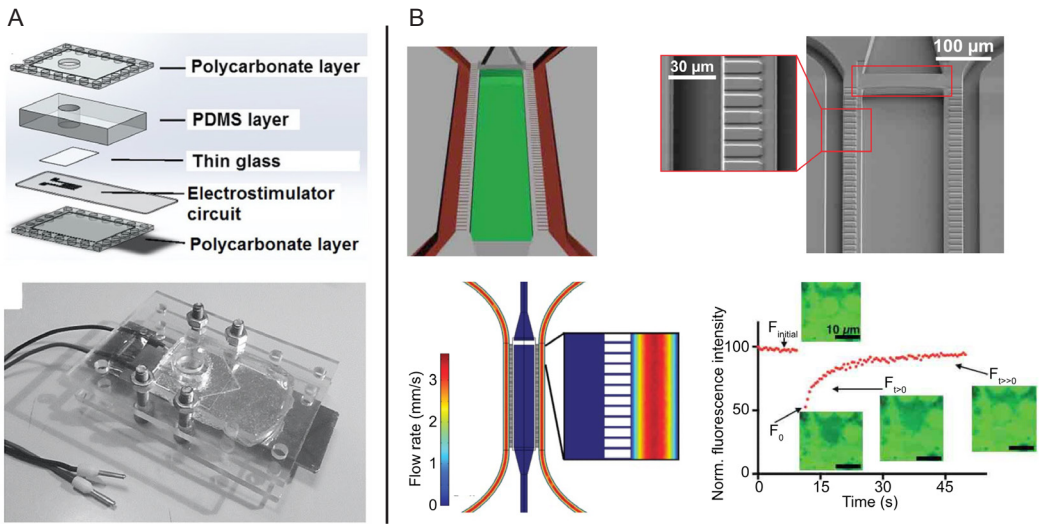

Figure 5. (A) Diagram of the electrical stimulator assembled parts. Reprinted from Aragón et al.[40] (B) The cardiac microphysiological system. Reprinted from Mathur et al.[42] PDMS: polydimethylsiloxane.

The Application of Cardiac Organ Chips

The current drug development model fails to meet the needs for treating many cardiac diseases, highlighting the urgent need for more precise human models to simulate the effects of drugs on the heart.[47] Traditional cell cultures and animal models have limitations in reflecting human physiology, leading to significant discrepancies in clinical trial results. Therefore, new tools such as microphysiological system (MPS), organ-on-chip technology, and multiorgan microdevices are increasingly attracting attention to enhance our understanding of cardiac diseases, accelerate drug development, and promote personalised medicine. Furthermore, in the realm of translational medicine, cardiac organ-on-chip technology has greatly assisted in disease modelling, drug screening, personalised medicine, and education and training. In summary, cardiac organ-on-chip technology, as an emerging research tool, holds vast application potential. It not only drives the advancement of cardiac research but also improves the efficiency of drug development, ultimately providing better treatment options for patients.[48]

Drug development

Currently, drug development is inefficient and expensive, typically requiring 10–15 years and about approximately $5 billion. Preclinical development and clinical research account for the majority of expenses, but existing animal models and cell lines struggle to predict human responses accurately, resulting in a success rate of only 5%, leading to significant resource waste. Adverse drug events cause many emergency visits each year, particularly those related to cardiac and liver issues.[49] Kidney toxicity is especially concerning, as unpredicted drug-induced injuries increase the risk of patient hospitalisation.

There is an urgent need for new *in vitro* systems to enhance predictions regarding drug safety and efficacy. Organ-on-a-chip technology can simulate the human microenvironment and biological characteristics, providing a more reliable method for drug testing, with cardiovascular chips being particularly important in cardiovascular drug development (**Figure 6**).[50,51] By combining microcantilever technology and flexible sensors, researchers can better monitor the response of cardiac tissue to drugs.[52] In summary, organ-on-a-chip technology offers a new approach to drug development that can improve drug efficiency and reveal new research pathways.

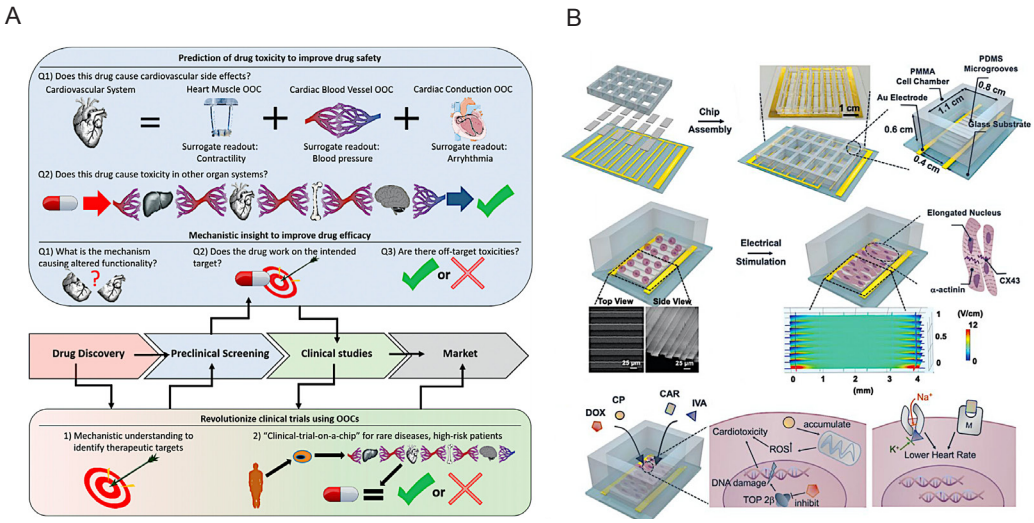

Figure 6. (A) The use of organ-on-a-chip can disrupt drug development at multiple points: mechanistic studies of drug action, preclinical trials of drug toxicity and efficacy, clinical studies using patient-specific organ-on-a-chip for models of patient diversity, and the development of a "clinical-trial-on-a-chip" to discover therapeutic options for rare diseases. Reprinted from Ronaldson-Bouchard et al.[50] (B) Schematic for drug screening enabled by the heart-on-a-chip platform. Reprinted from Ren et al.[51] Copyright 2020 Wiley - VCH GmbH. CAR: carbachol; CP: cyclophosphamide; CX43: connexin 43; DOX: doxorubicin; IVA: ivabradine; M: muscarinic; OOC: organic-on-a-chip; PDMS: polydimethylsiloxane; PMMA: polymethyl methacrylate; ROS: reactive oxygen species; TOP 2β: topoisomerase II-β.

Drug discovery

Owing to genetic and microenvironmental differences, different patients respond similarly to medications, highlighting the need for precise assessments of treatment effects. By integrating primary cells from healthy and patient donors, organ chip, effectively evaluate individual patient responses within a simulated human pathological and physiological environment. Ronaldson-Bouchard et al.[50] developed a beating cardiac-on-a-chip system that can generate functional 3D microcardiac tissues. This device was equipped with two compartmentalised PDMS microchambers separated by a PDMS membrane. The top compartment was subdivided into a central channel and two side channels. CMs and human iPSC-derived CMs were suspended in a matrix of fibrin gel in the central channel, and the culture medium was replenished through the side channels to generate a 3D cell construct. Upon pressurizing the bottom compartment, the PDMS membrane deformed, thereby compressing the 3D cell construct. This microfluidic platform could be used to evaluate the cardiac MPS by assessing the effects of drugs on cardiac cells.

Drug screening

An important application of cardiac chips is drug screening, as certain drugs may cause cardiac damage or heart failure. Therefore, studying drug-induced cardiotoxicity has become particularly necessary. By using efficient and precise *in vitro* models, such as "hearts on a chip", it is possible to screen drugs effectively and assess cardiotoxicity. This technology can simulate the microenvironment of vascular tissue, allowing for drug compound screening under a wide range of blood flow conditions. Yan et al.[53] used bioprinting technology to create vascular networks within hydrogels, forming 3D vascular beds through ultraviolet rays crosslinking, which enables fine-tuning of mechanical properties and ECM signalling to target specific vascular regions, leading to the rapid, controllable, and economical generation of complex 3D structures. These technologies help simulate different vascular regions within the human body. Ren et al.[51] invented a cardiac chip for high-throughput drug screening, selecting clinically approved doxorubicin and cyclophosphamide as model drugs while exploring potential cardioprotective treatments such as ivabradine and carbamazepine. Mathur et al.[41] created a 3D micro-organised cardiac chip to study the cardiotoxicity of various drugs (such as antibiotics, antidiabetic agents, and anticancer drugs).

Toxicological tests

Cardiovascular safety is a major reason for drug recall; for example, Vioxx (Rofecoxib) was associated with tens of thousands of heart attacks and sudden deaths, leading to significant compensation claims against Merck. The Development of suitable *in vitro* cardiac models for drug testing

poses challenges, primarily due to the limited availability and proliferative capacity of human CMs. Consequently, the pharmaceutical industry typically employs mathematical models and specific human ion channel-overexpression cell lines, along with animal models, to assess cardiovascular safety. Although human induced iPSCs and embryonic stem cells can provide many CMs, they often exhibit foetal characteristics due to insufficient maturity, raising questions about their relevance in drug testing. Mozneb et al.[54] developed a multilineage, fully-integrated, cardiovascular organ-chip that can enhance iPSC-endothelial cell and iPSC-derived cardiomyocytes functional and genetic maturity, model endothelial barrier permeability, and demonstrate long-term functional stability. With the increasing efficacy of drug development, multiorgan chip technology has gradually gained attention. These chips simulate the functions of multiple organs (such as the intestines, liver, skin, and kidneys) for more comprehensive drug toxicity testing. For example, researchers have developed a cardiac-liver-skin tri-organ system to analyse the effects of acute and chronic substances on cardiac and liver functions. The "human body chip" aims to reflect the physiological state of the entire human body on a single platform, facilitating studies on drug pharmacokinetics and pharmacodynamics. These human chip platforms may become important tools to replace animal models and have the potential to change the pharmaceutical industry fundamentally, although their complexity also presents numerous technical challenges.[55]

Heart disease model

Disease modelling is crucial for understanding the causes of diseases and drug development. Recent studies have shown that patient-derived induced iPSCs can provide personalised cell sources for generating target organs or tissues, or preserving the patient's pathogenic genes, thus advancing personalised disease modelling and drug screening (**Figure 7**).[48, 56-58] For example, human blood-brain barrier chips constructed from patient iPSCs exhibit significant differences in barrier characteristics between different patients. Using iPSC-derived CMs, researchers have been able to model cardiovascular diseases, screen potential drugs, and assess their effects. Additionally, some researchers have demonstrated the practicality of patient-specific long QT syndrome variant type 3 iPSCs in evaluating pharmacological responses to proposed drugs and improving therapeutic outcomes. Furthermore, combining iPSCs with organ-on-a-chip technology enables the creation of "organoids on chips", which serve as powerful research tools. Researchers have used biological scaffolds to manufacture heart chips and explore the electrophysiological characteristics and calcium transients of CMs. The heart and vascular system together form the circulatory system, and heart chips allow for in-depth studies of disease models such as arrhythmias and myocardial infarctions. In addition, endothelial cells can be encapsulated in microfluidic channels to form artificial vascular models, enabling real-time observation of drug effects on blood vessels.

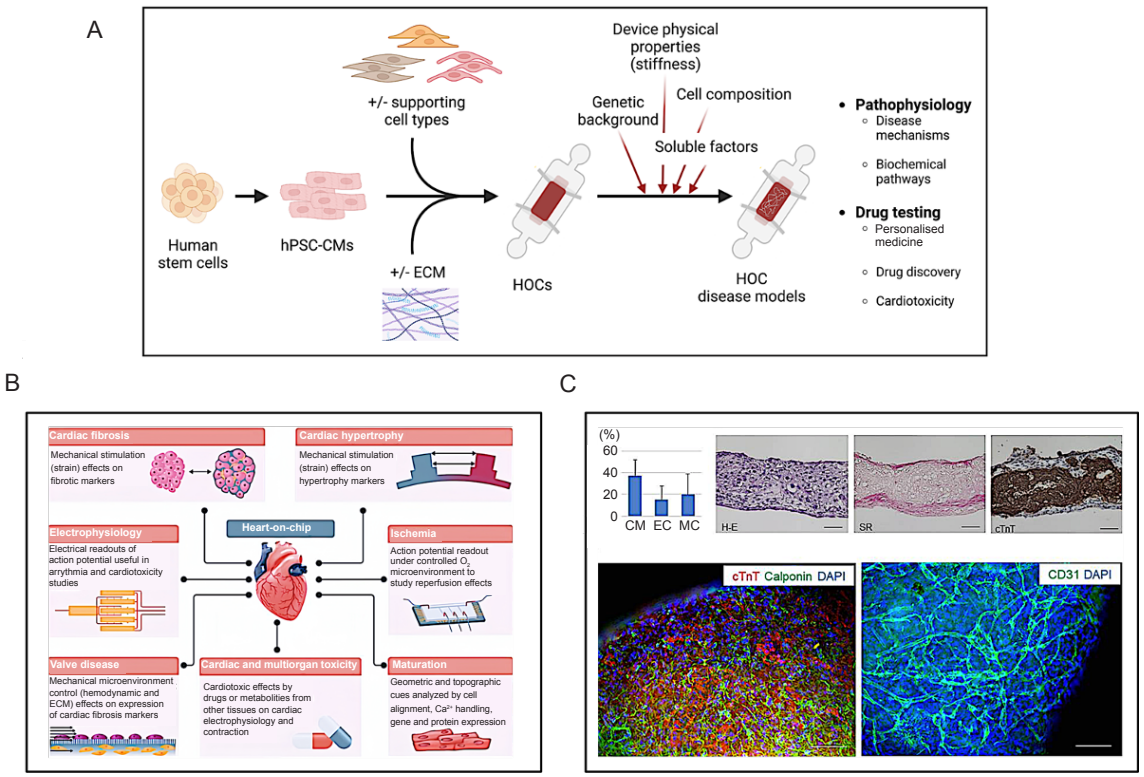

Figure 7. (A) Disease modelling using the heart-on-a-chip. Reprinted from Mourad et al.[57] Copyright 2023 American Heart Association, Inc. (B) Heart-on-a-chip devices can recapitulate cardiac functions *in vitro* and integrate sensing units to monitor the cells in culture. Reprinted from Paloschi et al.[48] (C) Preparation of 3D cardiac microtissues. Scale bars: 100 μm. Reprinted from Abulaiti et al.[58] 3D: three-dimensional; CM: cardiomyocyte; cTnT: cardiac isoform of troponin-T; DAPI: 4′,6-diamidino-2-phenylindole; EC: endothelial cells; ECM: extracellular matrix; H-E: haematoxylin-eosin; HOC: heart-on-chip; hPSC-CM: human induced pluripotent stem cell-derived cardiomyocytes; MC: mural cells; SR: sirius red.

Arrhythmia

Arrhythmia is a significant cause of global morbidity and mortality, accounting for 10–15% of all deaths. Although most arrhythmias are caused by acquired cardiac diseases, hereditary channelopathies and cardiomyopathies disproportionately affect children and young adults. The mechanisms of arrhythmia are complex and involve anatomical structures, ion channels, regulatory proteins, and the interactions among the cardiac conduction system, CMs, fibroblasts, and immune cells.

Animal models of arrhythmia are not only important tools for studying the molecular and cellular mechanisms underlying arrhythmias but also powerful means to explore cardiac mechanisms as a whole and test the effectiveness of therapeutic interventions.[59] Recently, Visone et al.[60] proposed a pharmacological dynamic model for evaluating arrhythmic events. The uHeart is a microphysiological model of the human myocardium that incorporates induced iPSC-CM and dermal fibroblasts embedded in fibrin. After mechanical training, the uHeart achieved synchronised contractions of the cardiac microtissue within a week, and recorded field potential signals were recorded through an integrated electrical system. This model was used to test 11 known compounds that can cause QT interval prolongation or arrhythmic events. These compounds act on single or multiple cardiac ion channels, effectively analysing the changes in electrical parameters of the uHeart before and after drug administration, including the contraction cycle, field potential duration, field potential amplitude, and detection of arrhythmic events. The results indicate that the uHeart can successfully predict clinical outcomes, particularly QT interval prolongation, with a sensitivity of 83.3%, specificity of 100%, and accuracy of 91.6%. Within the clinical maximum plasma concentration range, cardiotoxic concentrations of drugs were detected, making uHeart a suitable preclinical tool for cardiotoxicity research.

Myocardial infarction

Acute myocardial infarction, also known as myocardial infarction, is a life-threatening condition caused by the acute blockage of coronary arteries, leading to inadequate blood supply to specific areas of the myocardium and resulting in myocardial necrosis. This disease is primarily caused by various factors, including coronary artery atherosclerosis, arrhythmias, and respiratory failure, which lead to insufficient blood supply, myocardial hypoxia, and excessive oxygen consumption by the myocardium. Additionally, owing to the poor regenerative capacity of the myocardium, tissue repair after ischaemia is often accompanied by an increase in scarring, further exacerbating myocardial fibrosis. However, preclinical animal experiments have failed to reflect human physiological characteristics effectively, making the lack of clinically relevant treatment methods a significant issue. To address this challenge, Chen and Vunjak-Novakovic[61] developed a chip-based ischaemia-reperfusion injury model that uses flexible pillars to enable human induced iPSC-derived CMs to attach and align (**Figure 8A**). In this system, researchers have explored four therapeutic strategies: ischaemic preconditioning, intracellular pH normalisation, minimisation of mitochondrial permeability transition pore opening, and reduction of oxidative stress levels. Ischaemic preconditioning was found to increase cell survival after reperfusion, potentially through the activation of survival-promoting kinases during preconditioning. Moreover, normalisation of the intracellular pH and minimisation of mitochondrial permeability transition pore opening also improved cellular outcomes postreperfusion to varying degrees. Matsumura et al.[62] present the first large animal study to achieve functional and geometric improvements in the treatment of myocardial infarction by using a relatively rigid fully synthetic hydrogel designed for intra myocardial injection, and to better characterise the physiological pathways affected by its implementation to facilitate successful clinical translation.

Heart failure

Heart failure may stem from cardiac fibrosis, which is the accumulation of fibrotic scar tissue. By modulating the number of fibroblasts and the collagen concentration in microenvironments, researchers successfully generated a cardiac fibrosis model on a chip. These cardiac models provide an important platform for exploring the pathological mechanisms of cardiac fibrosis and for identifying effective treatment methods. Arrhythmias—changes in heart rhythm caused by abnormal electrical activity—are also significant contributors to heart failure. Arrhythmias and related cardiovascular diseases have been modelled using cardiac chip technology. Mathur et al.[49] constructed a model of *in vitro* arrhythmia on a chip, observing electrophysiological signals and contractility associated with arrhythmias, as well as studying responses to specific drugs. Additionally, cardiac chips have been used to simulate other cardiovascular diseases, such as hypertension, hypotension, and hypertrophy, thus offering broader application prospects for related research.

Myocardial hypertrophy

The main function of the heart is to maintain the coordinated operation of surrounding organs to meet their needs under both normal and stress conditions. In cases of increased preload or afterload, the heart and its individual CMs often undergo hypertrophy to accomplish this task. This phenomenon is known as cardiac hypertrophy. Initially, cardiac hypertrophy increases contractility by increasing the number of sarcomeres in parallel. Additionally, according to Laplace's law, an increase in left ventricular wall thickness reduces the stress on the left ventricle, thereby enhancing the efficiency of the heart. Cardiac hypertrophy is also accompanied by qualitative changes, specifically alterations in gene expression that affect metabolism, contractility, and the survival of CMs.[63] Currently, existing *in vitro* models have limitations in elucidating the mechanisms underlying volume overload-induced cardiac hypertrophy. With the advancement of cardiac organ-on-a-chip technology, *in vitro* models of cardiac hypertrophy are gradually being developed. Parsa et al.[18] developed a high-throughput μ-bioreactor that constructs models of cardiac hypertrophy due to volume overload by applying pneumatic (non-contact) loading to cardiac tissue. In this system, real-time chip analysis enables the study of the effects of mechanical stress on cardiac hypertrophy. These results indicate that this

platform is reusable and generates stable and reproducible cardiac μ-tissue. The heterotypic and homotypic μ-tissues produced in this device underwent pneumatic loading under various conditions, with real-time analysis of their tissue phenotype. Concentrated loading of cardiac tissue effectively reproduced the pathological volume overload observed in natural cardiac tissue (**Figure 8B**). Under sustained volume overload conditions, μ-tissues can induce pathological cardiac remodelling associated with the upregulation of foetal gene programs, resulting in a dose-dependent response.

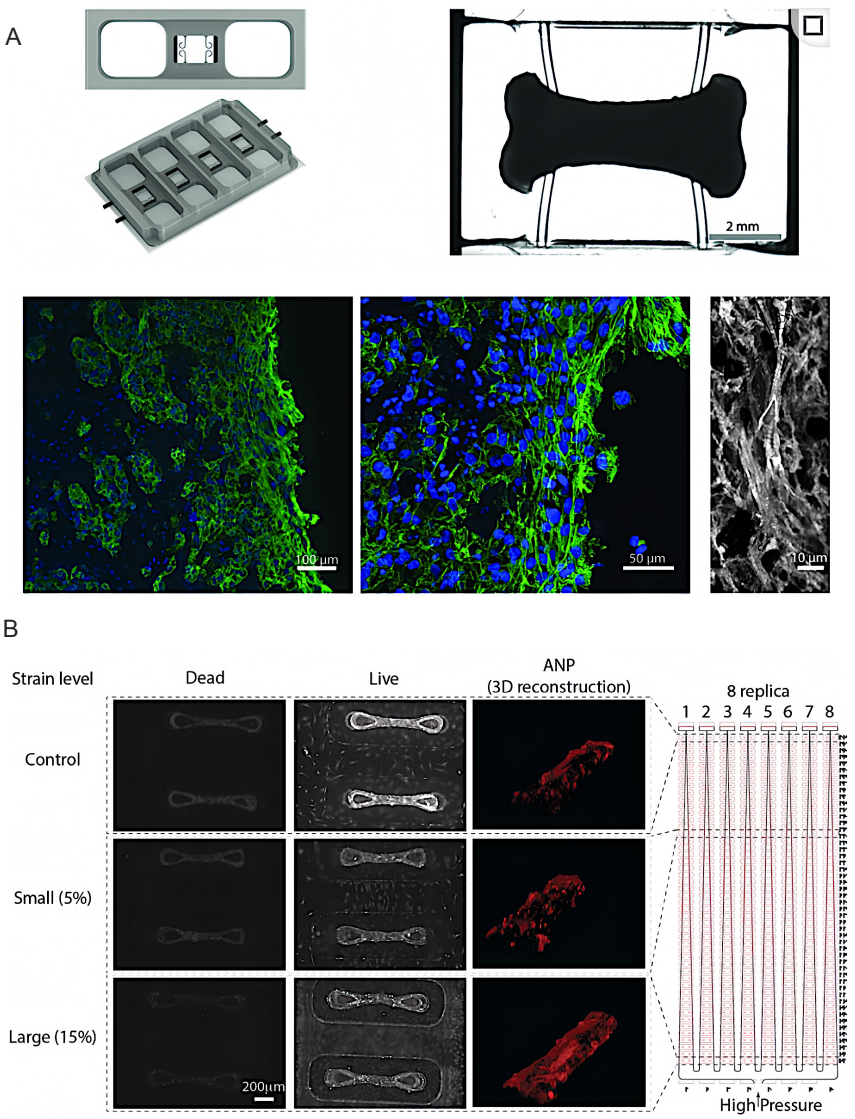

Figure 8. (A) Overview of the bioreactor, immunofluorescence images demonstrating the presence of aligned and cross-striated iPS-CMs along the edge of the construct. Reprinted from Chen and Vunjak-Novakovic[61] Copyright 2019, Mary Ann Liebert, Inc. (B) Dose-dependent hypertrophic response. Reprinted from Parsa et al.[18] Copyright 2017 The Royal Society of Chemistry. 3D: three-dimensional; ANP: atrial natriuretic peptide; iPS-CMs: induced pluripotent stem cell-derived cardiomyocytes.

Cardiac fibrosis

Cardiac fibroblasts are among the main cell types in the heart and play a crucial roles in maintaining healthy cardiac function. When the heart suffers damage, such as in myocardial infarction, the production of profibrotic and inflammatory cytokines, combined with increased mechanical load, causes resting cardiac fibroblasts to transform into myofibroblasts. These myofibroblasts generate tension by forming stress fibres containing actin in the cytoplasm. In normal healing processes, the activation of myofibroblasts is transient and reversible, as the ECM tension gradually recovers and mechanically bears the load again. However, under conditions of increased mechanical load or chronic injury, myofibroblasts may remain persistently activated, leading to excessive ECM deposition that renders cardiac tissue stiff and affects contractile function, resulting in CM injury and dysfunction, ultimately causing cardiac fibrosis. As the mechanisms underlying fibrosis are not yet fully understood, exploring the potential relationship between biochemical and biomechanical factors is crucial for developing new therapeutic strategies for cardiac fibrosis.[64]

Kong et al.[65] successfully reconstructed increased ECM stiffness using photopolymerisable hydrogels, simulating the mechanical load experienced by cardiac fibroblasts under profibrotic conditions. Research has indicated that cyclic mechanical loading and exposure to biochemical factors (such as transforming growth factor-β) can more accurately replicate the microenvironment of cardiac fibrosis. Additionally, non-microfluidic devices use cardiac tissue models that combine human induced iPSC-derived CMs and fibroblasts to simulate the characteristics of cardiac fibrosis.

Limitations

This review emphasises the development history, construction composition, and applications of cardiac organ chips, aiming to showcase the current research progress of cardiac organ chips to related researchers. However, it does not provide detailed information on the design specifics of cardiac organ chips, including high-throughput designs, substrate construction standards, and case analyses of preclinical applications, among others. We will continue to follow the development of cardiac organ chips.

Future Prospects

Over the past two decades, cardiac chip platforms have demonstrated their immense potential in drug discovery and development. In the next ten years, these platforms will achieve significant advancements in functionality, integration, automation, and personalised medicine to meet the needs of preclinical models. Cardiac chips can simulate multiple organ systems, becoming crucial tools for disease modelling and precision medicine, thus advancing drug screening and discovery. Future chip platforms should support multiorgan integration and feature plug-and-play capabilities. By using materials such as healthy tissues, tumour cells, and induced iPSCs, personalised organ chips can be created. Moreover, incorporating AI technology will enable automated data analysis, accelerating the exploration of disease mechanisms. Although most organ chips are currently still predominantly handmade, there is a need to improve the standardisation and high-throughput of production processes, considering the use of advanced manufacturing methods. Chips based on patient-derived materials (e.g., decellularised ECM) significantly impact precision medicine, with iPSCs providing potential cell sources to help construct personalised organ chips. Our ultimate goal is to create human-on-a-chip systems using human cells to replace animal experiments. The new U.S. Food and Drug Administration regulations eliminate the mandatory requirement for animal testing, marking a significant transformation in the fields of biomedical research and drug development. This change may promote the development of organ-on-a-chip technologies, reduce ethical controversies, and accelerate the research and development process. This advance requires enhancing the feasibility and stability of the systems while maintaining their cellular characteristics. Improvements in materials, standardisation of manufacturing and screening processes, and the introduction of biocomputer-aided design will be necessary to optimise the design and development of new interconnection technologies, enabling the connection and functional recreation of multiple organ chips. A modular approach will be central to the future design of human chip systems.

Author contributions

Conceptualisation, resources and supervision: XQ, LW, and ZL; literature research and analysis; writing - original draft and figure preparation: JLi, QL, JLiu, and CZ; writing - review & editing: HH; and project administration: YZ. All the authors approved the final version of the manuscript.

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Conflicts of interest statement

The authors declare that they have no competing interests.

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