Microsystem for Stem Cell-Based Cardiovascular Research

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Abstract The emergence of microsystem offers a tool to analyze the biophysical and biochemical functions of individual cells for biosensing, disease diagnostics, drug screening, and fundamental research. With the rapid expansion and eager need for stem cell-based regenerative medicine, introducing microsystem to stem cell research allows locating, culturing, and reprogramming stem cells under a highly controlled microenvironment, including geometrical restriction, surface biochemistry, extracellular matrix (ECM) composites, and cell–cell interactions. This review focused on the application of microtechnology in stem cell-based cardiovascular research to discuss the creation of biomimetic cardiac microenvironment and its influence on stem cell cardiomyogenic differentiation and stem cell–cardiomyocyte interaction. This review was divided into four sections: three-dimensional tissue construction, two-dimensional cell alignment, single-cell micropatterning and analysis, and development of advanced microsystem "heart-on-a-chip".

Keywords Stem cell biology · Regenerative medicine · Cardiac tissue engineering · Cell patterning · Microsystem · Heart on a chip

1 Introduction

Cardiovascular disease is the leading cause of death in the world, accounting for one third of all deaths worldwide [1].

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Nearly half of cardiovascular diseases are attributable to coronary heart disease, which causes the vital death of 7.2 million people each year [2]. Myocardial infarction (MI) and subsequently ischemic injury are pathologically characterized by loss of functional cardiomyocytes (CMs), which have a very low potential for repair and regeneration. Since the pharmacological agents do not address the fundamental issue of cell loss, there is limitation on the ability of drugs to improve cardiac functions. Heart transplantation is currently the only therapy to treat heart failure, but shortage of donor organ and immune system response make it impossible to solve the huge number of clinical cases. Cellular transplantation ideally realizes the replacement of dysfunctional CMs and scar tissue with new fully functional contracting cells, improving cardiac function and relieving the symptoms of heart failure. Recent advances in the understanding of stem cell biology have reinstated the concept of cellular transplantation for the treatment of MI [3].

Stem cell-based myocardial regeneration has been studied and developed for 20 years [4–6]. Stem cells are defined by their ability to self-renew and differentiate into multiple lineages and are usually divided into two categories according to their differentiation capability: pluripotent stem cells, including embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), and multipotent stem cells residing within adult somatic tissue, also called adult stem cells. ESCs are derived from the inner cell mass of the blastocyst of an embryo; thus, their use is restricted by ethical considerations. iPSCs are generated from fibroblasts by the retroviral transduction of transcription factors, such as Oct3/4, Sox2, KLF4, and c-Myc [7]. The pluripotent stem cells are able to differentiate into CMs by treating with Activin A, BMP4, and FGF [8]. However, the amplification of a self-renewing gene makes iPSCs exhibit high potential of tumorigenesis, which limits their further clinical trials [9]. One promising type of adult stem cells for cardiac tissue regeneration is cardiac stem cells (CSCs), which are isolated from

myocardium with the potential to differentiate into multiple myocardial cell types [10]. However, it is not well understood why endogenous CSCs in the heart cannot more effectively regenerate myocardium after acute infarction compared to the in vitro studies. Bone marrow stem cells, especially mesenchymal stem cells (MSCs), have been reported to participate in cardiac repair as part of the inflammatory response (but not directly differentiating into cardiac cells), which is known to regulate angiogenesis, CM survival, and left ventricular remodeling [11].

The fundamental units of an organism are biological cells, which are involved in the development and maintenance of the organism's hierarchical structures. Communication among neighboring cells, mediated by physical contact and diffusive signaling, regulates normal cellular functions. The functional characteristics of a single cell are determined by its genetic coding and its microenvironment. In vitro cell culture systems are isolated from their original biological context to allow controlled experimentation and analysis for fundamental biological science, drug development, regenerative medicine, and tissue engineering. Recent studies reveal that the microenvironment provided by cell culture models is significantly different from in vivo tissue, which causes deviations in cell response and behavior [12]. Mimicking the in vivo microenvironment in a cell culture, including the spatial arrangement of multiple cell types, is important for restoration of the functions of isolated cells and in vivo relevant cell–cell interactions.

In the natural tissue environment, cells are surrounded by various chemical, mechanical, and electrical cues, which directly regulate the cellular behaviors. Microtechnologies aim at either mimicking these factors to constitute an in vivo cellular microenvironment or isolating individual factors to investigate relevant cellular reactions. To create in vitro research models, which offer control over geometry, cell types, and cell–cell interactions, researchers have developed many cell patterning techniques by borrowing, bending, and building upon the microfabrication techniques of the microelectronic industry. Soft lithography has been combined with photolithography to fabricate elastomeric devices including stamps for microcontact printing, stencile membranes for plasma etching, and microwells. Using the microwell method, researchers are able to regulate the pluripotent stem cell self-renewal and differentiation by controlling embryoid body (EB) size [13]. The use of a microfluidic system has resulted in the accurate discrete localization of specific soluble biomolecules, which play important roles in stem cell differentiation and embryogenesis as well as other biological pathways for wound healing, inflammation, and chemotaxis [14]. Researchers are able to generate the concentration gradient of soluble factors and oxygen with linear, logarithmic, or arbitrary desired patterns

[15]. Microfluidic culture arrays are created to apply reproducible, steady-state conditions to simultaneously investigate experimental variables, such as cell density, medium composition, oxygen concentration, pH value, flow rate, and shear rate [16]. Cardiac cells in the heart are subjected to numerous mechanical forces, which help regulate gene expression and control cellular function. Pneumatic microdevices have been developed to exert mechanical force on stem cells for directed differentiation. Such microsystems have demonstrated myogenic differentiation and directional cell alignment without use of biochemical factors or surface patterning [17]. Microelectrode array technique has been widely used to record the electrical signal from cells for electrophysiological studies in the neuroscience and cardiovascular research. Recent advances in microtechnologies have demonstrated the effectiveness of employing electrical fields to precisely manipulate cell behaviors in wound healing, electrotaxis, neural stimulation, and stem cell myogenic differentiation [18, 19]. Additionally, there are direct cell patterning techniques, like inkjet printing [20], laser printing [21], and other manipulation techniques [22, 23], for controlling the geometry of cell cultures and forming defined cell–cell communications. These techniques are inherently reproducible, able to replicate anatomically accurate in vivo structure, and combined with high-resolution imaging modalities for further biological analysis.

In this review, we focused on innovative microtechnology for cardiac tissue regeneration, in vitro modeling, and single-cell analysis. 3D tissue scaffold was firstly introduced both through novel biomaterial cardiac patch and hydrogel patterning. 2D cardiac alignment to create an in vivo-like cellular structure using different microfabrication methods was discussed in the following paragraphs. Then, the size was scaled down to single-cell micropatterning to study cell behaviors and cell–cell interaction at single-cell level. Lastly, the development of "heart-on-a-chip" was introduced for studying heart development and drug screening in a physiologically complex microenvironment. We highlighted engineered devices that utilized a microsystem for cardiac development and introduced further points of exploration that might be applicable in future research of stem cell-based cardiac tissue engineering.

2 3D Cardiac Tissue Architecture

For regenerating the infarcted area of heart, one of the potent ways is implanting engineered 3D tissue patch to replace the infarcted zone and restore myocardium functions. Native myocardial tissues are organized into parallel cardiac muscle fibers with intracellular contractile myofibrils oriented parallel to the long axis of each cell and junctional complexes

between abutting cells concentrated at the ends of each CM [24]. To optimize the implanted tissue patch to a functionally electrical and mechanical couple with native heart muscle, pre-seeded CMs are patterned on the patch in the end-to-end aligned manner resembling the heart tissue structure (shown in Table 1). CM alignment in three dimensions (3D) could be achieved by electrospinning of aligned biopolymer fibers [25, 26]. CMs self-organized into anisotropy cardiac tissue structure on aligned poly(e-caprolactone)/gelatin (PG) composite nanofibrous scaffolds, which were electrospun to structurally mimic the oriented ECM in myocardium (shown in Fig. 1a). Better alignment could be achieved by shortening the spacing distance between two adjacent fibers [26]. Comparing the random PG fibrous scaffolds, the mechanical properties of aligned scaffolds were closer to the real myocardium [26]. Another approach of obtaining aligned CMs in 3D mode was fabricating scaffold-free multilayer cell sheets. A cell sheet is normally fabricated on a temperature-responsive polymer poly(N-isopropylacrylamide) (PIPAAm), which becomes hydrophilic when the temperature goes lower than its critical solution temperature. Therefore, cells along with ECM can detach from the polymer surface and form a layer of sheet [27]. This technology allows us to obtain a sheet of interconnected cells, fully contacting with their natural extracellular environment. 3D myocardial tissue was fabricated by stacking CM sheets, which were tightly

interconnected to each other through the junctional proteins. The fabricated tissue beat simultaneously and macroscopically and showed the characteristic structures of native heart tissue [28]. The development of this method was the use of iPSC-derived CMs seeded with non-CMs at different ratios for creating cell sheets and bioengineered heart tissue [29]. Immunocytochemical analyses showed the coexistence of CMs and vimentin-positive cells in the cell sheets (Fig. 1b), suggesting that a certain level of non-CMs producing ECM may be necessary for creating cell sheets.

Controlling the differentiation of ESCs, various microscale technologies have been developed for directing the stem cell fate. Microfabricated adhesive stencils were used to pattern ESCs for controlling initial ESC aggregate sizes, which influenced the early differentiation to different germ layers [30]. Using microwell technology, systematically varying the microwell size demonstrated that EBs from intermediate-sized (300 μm) microwells generated the highest percentage of contracting EBs. Figure 1c shows that microwell size influenced the density of CMs in EBs. The smallest microwells (100 μm) had the greatest percentage of CMs in a contracting EB. Therefore, the process of cardiac differentiation of hESCs can be modulated in multiple ways by confined culture conditions using microwells to grow hESCs and form EBs to improve the efficiency of cardiogenesis [31]. Hydrogel patterning has been used to fabricate

Table 1 Summary of methods for in vivo-like CM alignment

Categories of CM alignment	Methods of alignment	Substrates or materials	Patterned/coated proteins	Biological assays	References
3D CM alignment	Electrospinning of aligned biopolymer fibers	Poly(ε-caprolactone)/gelatin, polymethylglutarimide	N/A	Implant patch	[25, 26]
	Cell sheet	Poly(N-isopropylacrylamide)	N/A	Implant patch	[27]
	Hydrogel patterning	Gelatin methacrylate hydrogels, fibrin-based hydrogel matrix	N/A	Implant patch	[32, 33]
	Mechanical stretching by microcantilevers	3D collagen matrix	Collagen	Mechanical contraction	[34]
	Accordion-like honeycomb wells scaffold by excimer laser microablation	Poly(glycerol sebacate)	N/A	Implant scaffold	[35]
2D CM alignment	Cell scraper and gravity flow	Collagen aligned by cell scraper on a tilted cell culture dish	Collagen	Myofibrillogenesis	[37]
	Microabrasion technique	Unidirectional abraded PVC coverslip	Fibronectin	Optical mapping	[39]
	Microgrooves	PDMS membrane, photocrosslinkable chitosan	Laminin	Localization of electrical and mechanical couplings	[40, 41]
	Microcontact printing	Glass coverslip, PDMS membrane, polyacrylamide-based hydrogels	Laminin, fibronectin	Calcium handling, electrical conduction, mechanical stretching	[42, 47, 61]
	Microfluidics	Silicon, glass	Collagen, hyaluronic acid	Cell contraction	[43, 44]
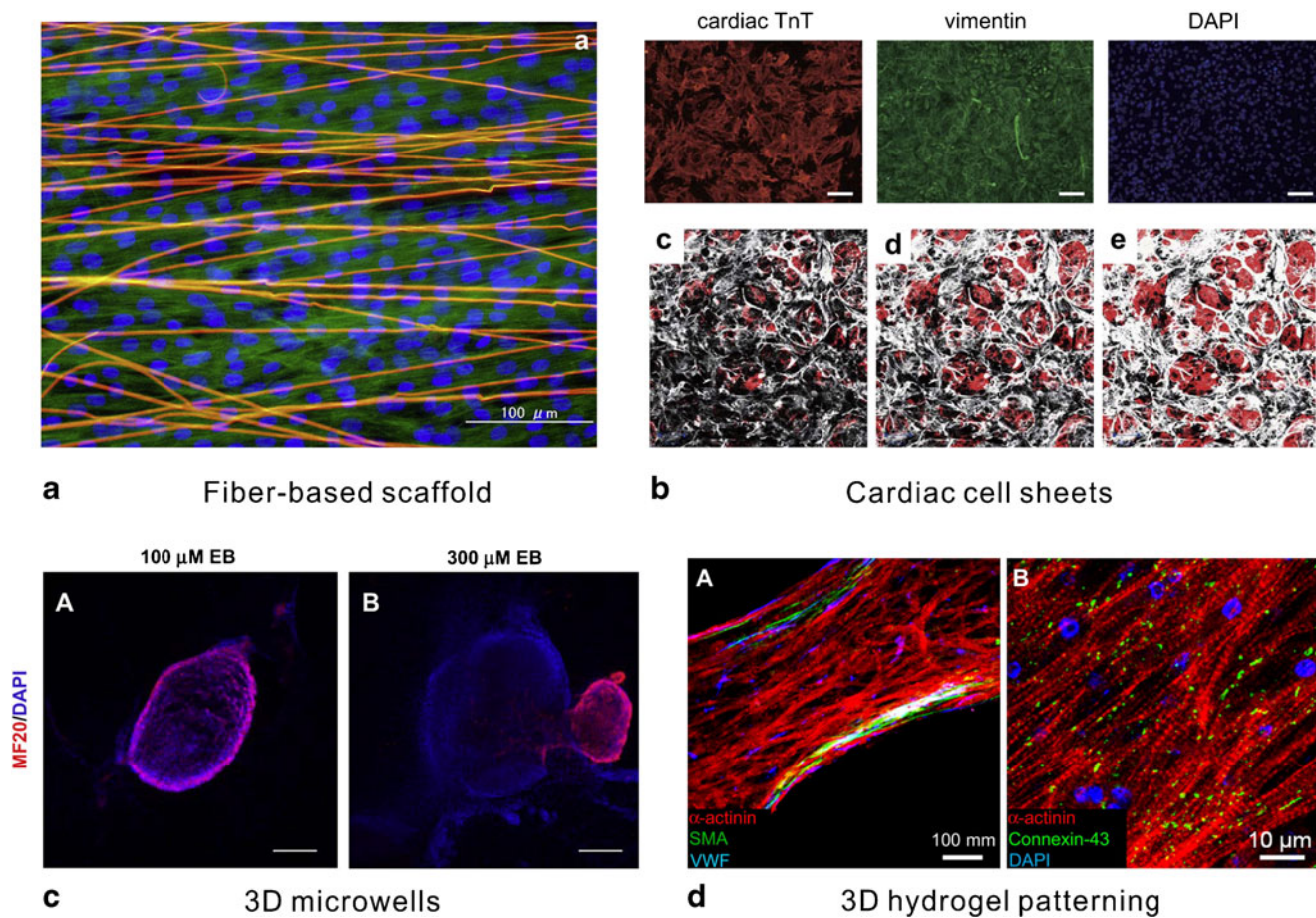
Fig. 1 3D cardiac tissue architecture for heart repairing. **a** Electrospinning of aligned biopolymer nanofibers to mimic the oriented ECM in myocardium for 3D CMs alignment [25] (with reprinting permission from Elsevier, Biomaterials), **b** cardiac cell sheets with iPSC-derived CMs and non-CMs to replicate native heart tissue [29] (with reprinting permission from Elsevier, *Biomaterials*), **c** 3D microwells to control EB sizes to improve the efficiency of cardiogenesis [31] (with reprinting permission from Elsevier, *Biomaterials*), **d** microfabricated fibrin-based hydrogel matrix was created as a 3D cardiac tissue patch with controllable size and architecture [33] (with reprinting permission from Elsevier, *Biomaterials*)

a 3D cardiac tissue scaffold, in which CMs exhibited aligned cellular morphology. Microengineered 3D gelatin methacrylate hydrogels demonstrated that cells with intrinsic potential to form aligned tissues in vivo would self-organize into functional tissue in vitro if confined in the appropriate 3D architecture [32]. The 3D cardiac tissue patch was also created using fibrin-based hydrogel matrix generated by soft lithography technique with controllable size and architecture [33]. CMs differentiated from ESCs and CPCs were seeded into this highly engineered hydrogel to yield highly aligned CMs and robust intercellular coupling with rapid action potential conduction (22–25 cm/s) and significant contractile forces (up to 2 mN; shown in Fig. 1d). The cardiac microtissue, a mixture of collagen matrix and CMs, was aligned by stretching 3D collagen matrix between two microcantilevers, which were able to simultaneously constrain CM contraction and report contraction forces in real time [34]. A sophisticated 3D scaffold

for myocardium replacement was created by excimer laser microablation with an array of accordion-like honeycomb wells. This structure was developed in biodegradable poly (glycerol sebacate) with high porosity, elasticity, and controllable stiffness [35].

## 3 2D Cardiomyocyte Alignment

Standard cell culture models with confluent and nonstructured monolayers are of limited use for cardiac research as they do not preserve the important structural and functional properties of the myocardium. Lack of CM alignment is a substantial deviation from the normal phenotype demonstrated by the irregular alignment of sarcomeres in CMs grown on traditional planar substrates [36]. In the early studies on CM anisotropic properties, the cardiac cells were aligned on a thin collagen surface coating that was spread using a cell scraper

and polymerized while slowly being poured within slightly tilted dishes [37]. Although this method was successfully applied in the 10-cm culture dish, it was difficult to apply when scaled down to a 2-cm-diameter coverslip. Therefore, micropatterning technology has been widely used for establishing in vitro culture models and investigating the fundamental and pathological characteristics of CMs under an in vivo mimicked microenvironment [38].

Microabrasion technique was firstly introduced to create aligned CMs with anisotropic sarcomere structure by unidirectional abraded polyvinyl chloride (PVC) coverslip with 12- or 25-µm parallel lines. Sharing the similar concept of cell alignment by surface topography, polydimethylsiloxane (PDMS)-based microgrooves were fabricated using soft lithography method with controllable depth and space. It was found that thinner microgrooves produced better alignment and lower connexin 43 and N-cadherin expression of CMs [39]. Microgroove patterns were also achieved by fabricating photo-crosslinkable chitosan to obtain cellulous material for CM alignment between two chitosan lines [40] (shown in Fig. 2a). Microcontact printing method can successfully deposit lines of ECM proteins on the glass coverslip or PDMS membrane, and CMs form stable anisotropic monolayer or aligned strands after a few days of culturing [41]. Printing laminin lanes onto mechanical tunable polyacrylamide-based hydrogels resulted in aligned cardiac myofibers by seeding CMs and aligned myotubes by seeding myoblasts [42] (shown in Fig. 2b). In this study, both cardiac myofibers and skeletal muscle myotubes were part of an array composed of independent, spatially, and dimensionally controlled individual fibers. Microfluidics was used to spatially apply different chemical solutions to rush through the channels and leave a patterned substrate for cell seeding. The chemical solutions were either cytophilic [43] to form cell-adherent patterns or cytophobic [44] to form cell-repelling patterns.

By mimicking the heart muscle tissue, the physiological characteristics of CMs can be conveniently and precisely investigated via these in vitro aligned cardiac models. Calcium handling was measured on the anisotropic monolayer of aligned CM lines, and the variations of calcium concentration during systole and diastole in anisotropic monolayer were significantly different from those in the isotropic monolayer [41]. Researchers also found that conduction velocities and action potentials were faster and more similar to adult mouse myocardium in CMs aligned as cardiac muscle fibers versus those grown in randomly oriented cultures [45]. The stem cell bridge model in the aligned cardiac muscle fibers was used to determine the electrical conductivity of mesenchymal stem cells [46]. The aligned cardiac muscle fibers were created by lithographic methods
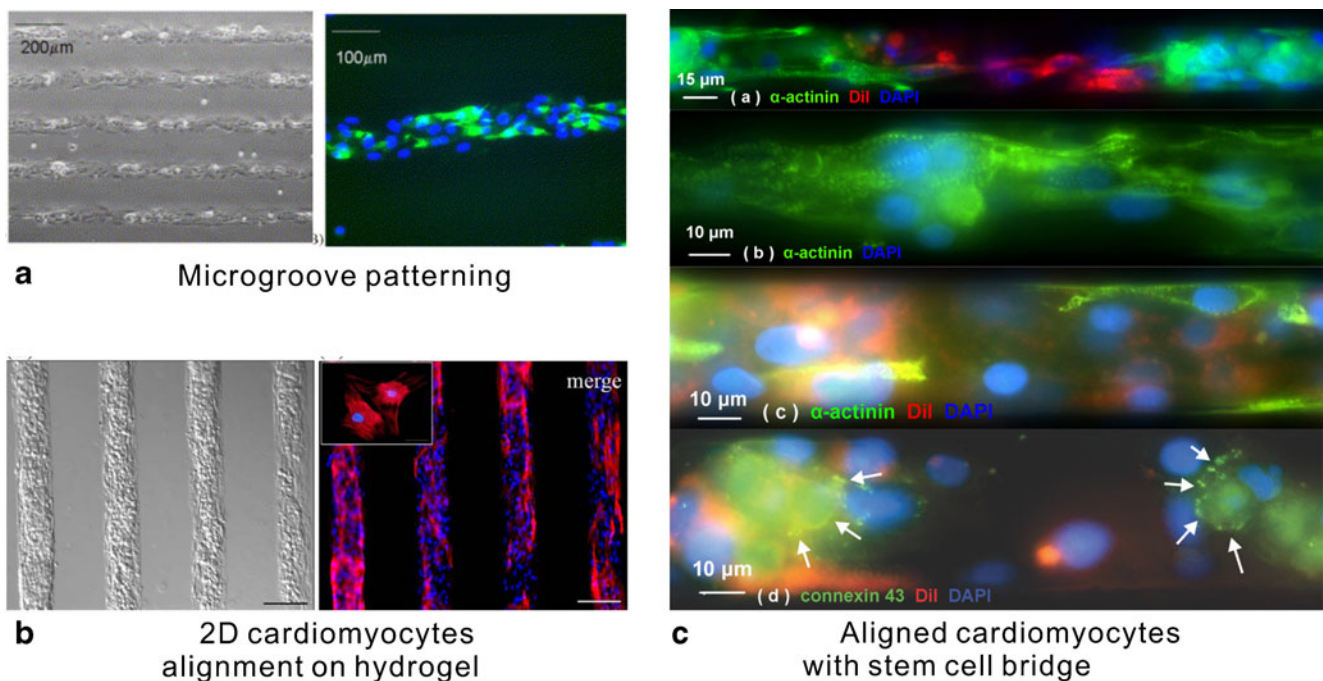

Fig. 2 2D cardiac cells alignment for in vitro cardiac modeling. **a** 2D CMs alignment by microgroove method [40] (with reprinting permission from Elsevier, *Biomaterials*), **b** 2D CMs alignment by microcontact printing method on mechanical tunable polyacrylamide-based hydrogels [42] (with reprinting permission from Springer, *Biomedical Microdevices*), **c** MSC bridges created inside aligned cardiac muscle fibers on a MEA chip to assess their electrical conductivity [46] (with reprinting permission from Royal Society of Chemistry, *Lab on a Chip*)

on the multielectrode array (MEA) chips for long-term electrical recording. Portions of fibers were dissected and filled up by mesenchymal stem cells using laser-guided cell micropatterning technique (shown in Fig. 3c). Since the width of the fiber was smaller than the cardiac electrotonic space constant, electrical propagation along the fiber was pseudo-1D. This allowed the microscopic tracking of electrical propagation along a distinct linear path without ambiguity about the exact pattern of electrical signal propagation. Stem cell bridges exhibited higher and more stable conduction velocities than fibroblasts, which indicated that stem cells had higher electrical compatibility with native cardiac muscle fibers than cardiac fibroblasts. To study the mechanism of cardiac hypertrophy, aligned CMs cultured on deformable elastomers were statically stretched in the transverse or longitudinal direction. Aligned CM strands stretched in a transverse direction had a higher expression level of connexin 43, N-cadherin, and atrial natriuretic factor than the longitudinal stretch and random culture modes [47].

4 Single-Cell Micropatterning

Single-cell micropatterning offers a tool to analyze the biophysical and biochemical functions of individual cells under a highly controlled microenvironment to determine the variations among the individual cells within a heterogeneous cell population. The patterned cell array also allows the development of parallelized systems for high-throughput analysis and detection. Compared to CM alignment for mimicking heart muscle tissue, single CM micropatterning is extensively involved in exploring myofibrillogenesis and its relationship with extracellular factors. By microcontact printing ECM protein on the coverslip to shape single CM into the predesigned patterns [48–51], researchers found that not only cell shape was defined but also the cytoskeleton was under reconstruction into the predicted architecture (shown in Fig. 3a). It was noticed that the spatial configuration of ECM played a key role in regulating the other three factors: cell shape [48], sarcomere orientation [49], and nuclear morphology [51].
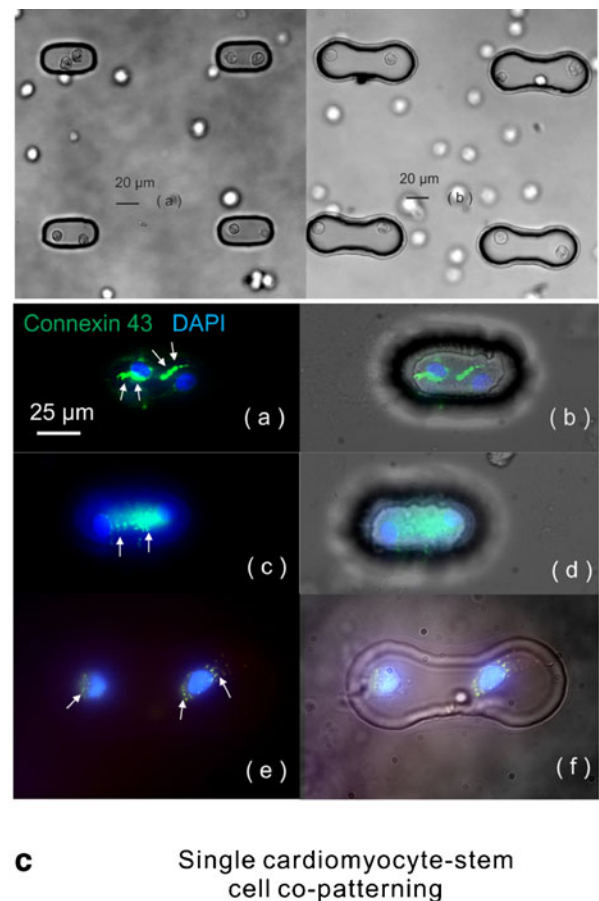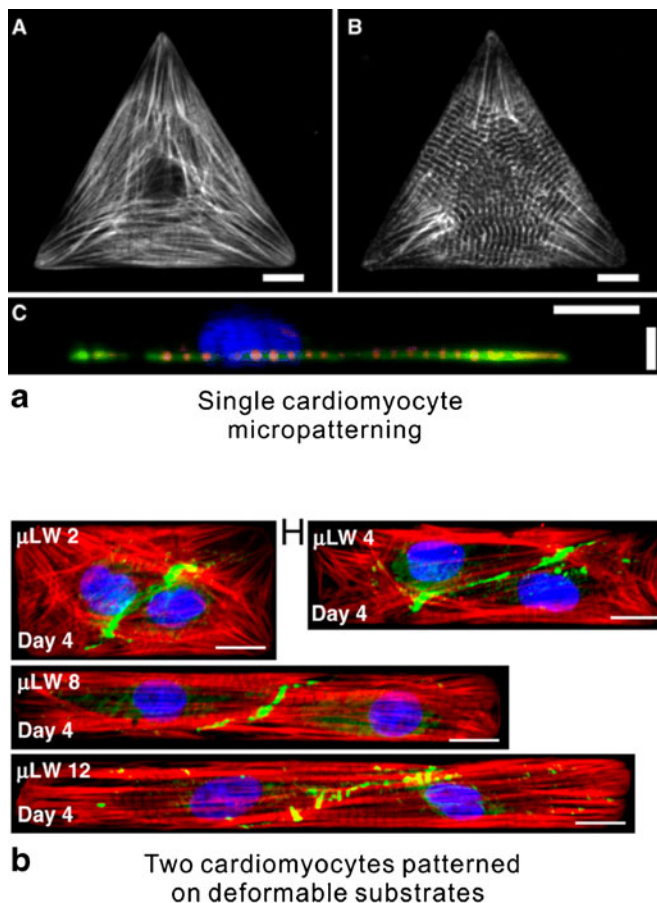
Fig. 3 Single-cell micropatterning and analysis for cardiac research. **a** Single-cell micropatterning by microcontact printing fibronectin island for modeling CM sarcomere orientation and myofibrillogenesis [50] (with reprinting permission from Springer, *In Vitro Cellular & Developmental Biology*), **b** two CMs micropatterned on deformable substrates to map their traction stresses [52] (with reprinting permission from the National Academy of Sciences, *PNAS*), **c** individual MSCs and CMs were laser-patterned into biochips to study their electrical coupling and stem cell protective effect under defined contact modes [59] (with reprinting permission from Springer, *Cellular and Molecular Bioengineering*)

Two single CMs were end to end patterned together to establish an in vitro culture model for studying their mechanical and electrical coupling at single-cell level. Two CMs were patterned onto polyacrylamide gels with physiological stiffness (13 kPa) or pathological stiffness (50 kPa) to mimic health or fibrosis heart, and their traction stresses were mapped using traction force microscopy. As shown in Fig. 3b, cell–cell junction had a sigmoid-like contour, similar to yin–yang interfaces observed in migrating endothelial cells and diagonal interfaces in patterned myocyte pairs. People found that with increasing elastic modulus, peak systolic displacement decreased, force generation increased, and focal adhesion size at the longitudinal ends increased, which indicated that the intercalated disc required mechanical reinforcement during fibrosis process [52]. A similar design with micropatterned CM pairs was used to study the relationship of electrical conduction and gap junction formation [53, 54]. The electrical conductance was measured using a dual patch voltage clamp, and the connexin43 expression level of the patched cell pairs was determined by quantitative confocal microscopy. Eventually, they concluded that there was lineage relationship between electrical conductance and fluorescent intensity of connexin 43 immunostaining. The micropatterned CM pairs provided a tool for high-throughput analysis of CM-to-CM crosstalk at single-cell level. A multiple CM network was created on an agarose microchamber array chip, which can be used for cell-based drug screening [55]. By administrating the drug haloperidol on the cell network, the results showed that the community size of the cell network played an important role to maintain the stable cellular model.

To study cell-to-cell communication between stem cells and CMs at single-cell level, heterotypic cell micropatterning is achieved to allow for obvious observation of cell–cell crosstalk and sufficient samples for biological statistics. Paired trapezoid-shaped fibronectin islands were created by microcontact printing method for micropatterning one MSC and one CM onto one paired island [56]. Through analyzing the confocal images of connexin43 and N-cadherin distributions on the boundary of a heterotypic cell pair of MSC and CM, they found that around 38 % of cell pairs exhibited junctional distribution of junctional proteins and more than 50 % with diffuse type. Combining microwell method and laser–tweezers technique, MSC–CM interaction was studied by controlling their contact mode at single-cell level (shown in Fig. 3c). Two types of biochips were fabricated to promote or prevent cellular contact between single MSCs and CMs. Cell viability study indicated that MSCs in contact-promoting biochips rescued the contacted CMs in an unhealthy microenvironment, and the protective effect of MSCs appeared to be dependent on direct cell-to-cell contact [57]. In the contact-promoting biochips, MSCs and CMs formed close contact with a continuing boundary, and MSCs were capable to

crosstalk with CMs and acquired cardiac-like electrophysiological properties [58]. Connexin 43 staining in the contact-promotive/preventive biochips showed a junctional or diffusive feature between the single MSC and CM [59].
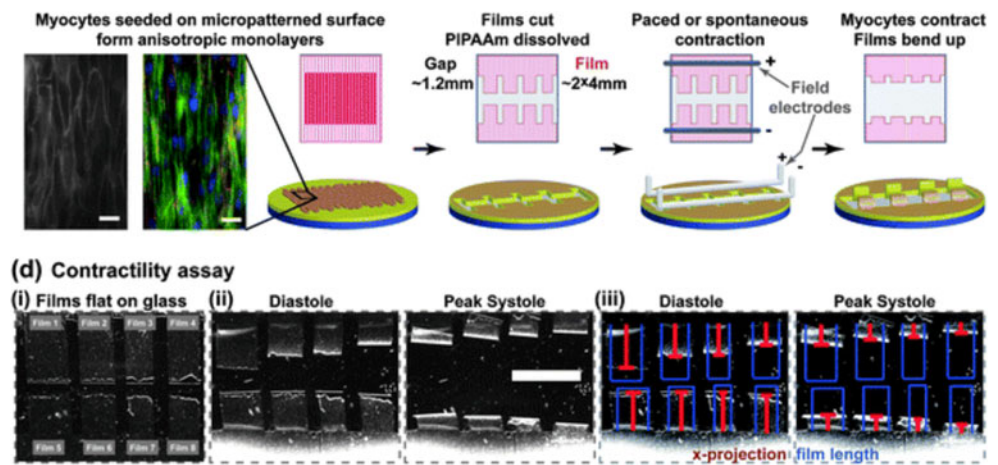
5 Heart-on-a-Chip

The development of a microsystem currently is focusing on creating an organ-like microenvironment for studying organ development and drug screening. Creating a multicellular architecture with a highly controlled cellular microenvironment makes it possible to study complex interactions between different compartments inside the whole organ-on-a-chip microsystem [60]. A heart-on-a-chip microsystem was created by microcontact printing to align CMs on a temperature-sensitive polymer PIPAAm. This heart chip, composed of eight individual 2D membranes as layers of heart muscle tissue, can be used for real-time data collection and analysis during pharmacological intervention [61] (shown in Fig. 4a). The average systolic and diastolic stress can be measured on the chip during the spontaneous and synchronous systole and diastole of the heart muscle tissue. Chronotropic effect with frequency-rising drug, epinephrine, obtained from this heart chip can recapitulate previously reported epinephrine dose–response curves from isolated atria strips of adult rats [62].
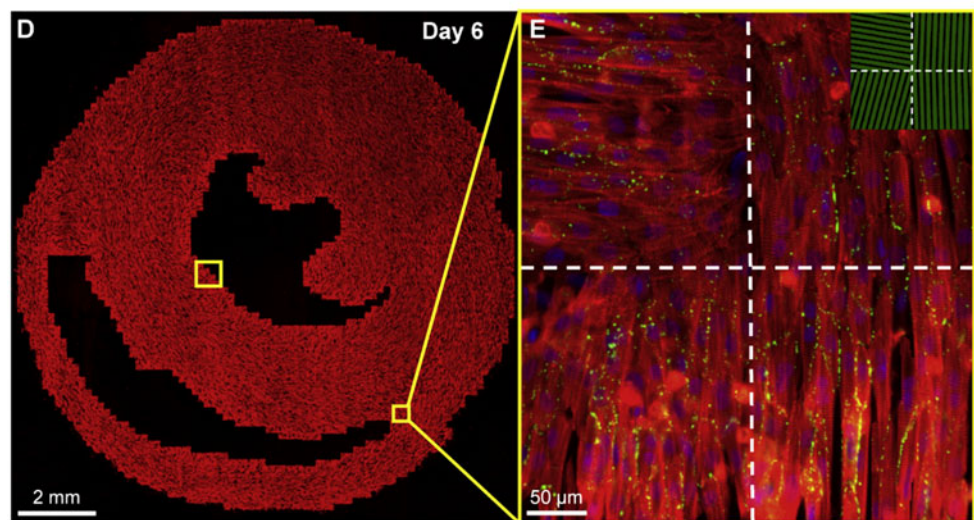
Through multicellular level of micropatterning, a 2D stem cell bridge was created on a MEA-based heart chip, which can directly measure the local activation time and membrane potential in situ of cells. Microabrasion technique was used to align the MSCs to control the bridge direction relative to the edge of the CM area. The electrical conduction of MSC bridges perpendicularly aligned to the edge of the CM area was higher than the parallel aligned and random nonaligned bridges, and the orientation of connexin 43 distribution was corresponding to the alignment of MSCs and the direction of electrical propagation [63]. This heart chip can be used to mimic stem cell transplantation into anisotropic 2D monolayer, which provided us better understanding of the electrical compatibility of aligned MSCs on the engineered tissue patch to match the orientation of muscle fibers in the infarcted area before implantation. A ring-shaped closed-circuit microelectrode was developed to record the extracellular field potential of a line-up of CM network in an agarose microchamber. This microsystem was used as a cardiac toxicity assay to determine lethal arrhythmia, QT prolongation, ventricular tachyarrhythmia, and fibrillations [64].

Using diffusion tensor magnetic resonance imaging (DTMRI) technique, people were able to replicate the pattern of CMs in vitro to the realistic cross-sectional tissue structure of the ventricle [65, 66] (shown in Fig. 4b). The

directions and position of heart muscle fiber were represented as primary eigenvector component, which can be printed into photomask for lithographic microfabrication and microcontact printing. With this method, CMs can be patterned into exactly the same structure of a heart muscle layer of interest, and electrical propagation within the patterned CMs can be similarly contoured using optical mapping. It has been utilized to investigate the development of conduction block inside the patterned CM monolayer, and specific anatomical features of the ventricular walls can act as independent and important contributors to conduction failure and arrhythmogenesis [67].

6 Perspectives

Cardiovascular diseases, especially MI, are the leading causes of patient morbidity and mortality globally, and stem cell-based cardiac tissue engineering aims to regenerate the cardiac tissue for transplantation. In native tissue, cells are surrounded by 3D organized extracellular matrix and neighboring cells, which interact to provide complex chemical and mechanical signals. Taking the advantage of microtechnology, researchers are able to create the 3D engineered heart tissue resembling the native heart structure. These in vitro 3D tissues generated from pluripotent stem cells have been found with similar mechanical and electrical properties to the in vivo heart tissue. On the other hand, 2D cell patterning methods, though not able to create a 3D micro-environment, provide a highly controlled cellular model to investigate the cellular behaviors, cell–cell interaction, and self-organization. These 2D patterning systems for cell alignment and single-cell analysis can be used as standardized tools for stem cell research with uniform size and shape, high-throughput and reproducible fashion. Furthermore, "heart-on-a-chip" platforms with aligned CMs have

proposed to be used for understanding, studying, and developing new strategies for treating cardiovascular diseases. These platforms will contribute as: (1) in vitro cardiac models mimicking the native aligned cardiac structure and providing a vision on how cardiac diseases influence tissue functions, (2) standardized in vitro models allowing for controlled modulation of various parameters (e.g., cellular composition, environmental factors) and amenable to high-throughput drug screening and toxicity testing, and (3) cellular assays for assessing the survival and integration of various transplanted stem cell populations (MSCs, CSCs, ESCs, iPSCs). The microsystems, which aim to replace the animal models, can be moved beyond the academic prototypes to the commercial laboratories in the pharmaceutical, biotechnology, chemistry, and environmental safety industries.

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