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Structural Regulation of Myocytes in Engineered Healthy and Diseased Cardiac Model

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ABSTRACT.

The development of cardiac models that faithfully recapitulate heart conditions is the goal of cardiac biomedical research. Among the numerous limitations of current models, replication of the cardiac microenvironment is one of the key challenges, and the effect of mechanical cues remains obscure in cardiac tissue. In this paper, different topological structures in the engineered cardiac models are summarized, and mechanical regulation of myocyte morphology and functional responses are discussed. Microenvironmental cues in vivo are influencing cardiac functions from cellular to tissue levels, and replications of these micro and macro features in the in vitro cardiac

model shed light on cardiac research from a mechanistic point of view. With simple manipulation of topology, both physiological and pathological cardiac constructs can be remodeled to investigate the origin of abnormal cell phenotypes and functional responses in cardiac diseases. The integration of topological guidance with heart-on-chip devices is covered briefly and limitations of the current cardiac constructs are also addressed for future advancements in personalized medicine.

1. Introduction

Heart is a non-regenerative organ that undergoes irreversible damage with deteriorated function. Myocardium, the muscular tissue responsible for heart contraction, consists of cardiomyocytes (contractile cell) and pacemaker cells (conducting cells) embedded in a compliant extracellular matrix (ECM),¹ which provides structural stability, mechanical support, and essential nutrients to cardiac tissues. The highly aligned collagen fibers in ECM provide structural tensegrity and topological guidance for anisotropic cardiomyocytes' growth. The relationship between cells and their surrounding environment are inextricably intertwined. Cardiac cells and ECM undergo constant modulation through a mechanical feedback loop, where cells sense, respond and remodel ECM structures.² In a healthy myocardium, cardiac cells are growing parallelly on ECM and contract regularly but defective ECM leads to maladaptive remodeling of cell structure and impaired function.

To elucidate the link between microenvironment (physio- and pathological) and functional response of cells, in vitro cardiac models have been carefully devised with tailored substrate mechanics. By varying the types of substrate and topology, both physiological and pathological microenvironment of cardiac tissue can be recapitulated in the laboratory. While the matrix

stiffness mimics the extent of collagen deposition,³⁻⁴ the morphology of cardiac tissues can be controlled through topological cues, which alter the cellular organization, gene expression, and eventually leads to abnormal contraction and electrical conduction.⁵⁻⁹ The topological structures can be fabricated through various ways such as contact printing,¹⁰ 3D printing, laser etching, and electrospinning.¹¹ By controlling the sizes, shapes, and fabrication approaches, tissue constructs could be designed with different morphologies and dimensionalities, achieving desired functionalities.

Here we review various structures for recapitulation of in vivo physiological and pathological microenvironment, and how these mechanical cues modulate cell and tissue morphology and functional response. We highlight how micro and macro structures direct remodeling in single and collective myocytes, in both two-dimensional and three-dimension tissue culture. Other factors contributing to more physiological relevant cardiac models such as co-culture and cell source are also briefly discussed.

2. Cardiomyocytes are Mechanosensitive and Mechanotransductive

Cardiomyocyte is commonly accepted as a mechanosensitive and mechanotransductive cell, which regulates its phenotype and functional response in response to the microenvironment. Mechanical information from the extracellular structure is signaled through the cell-ECM interface via mechanoreceptors like integrins and transmitted between cardiac tissue through the cell-cell junction by proteins such as cadherin and catenin.² The mechanotransductive pathways, which involve complex signaling pathways including signaling factor, mechanosensitive units like actomyosin machinery, and stretch-responsive ion channels, represent communication between cell-substrate and cell-cell junction respectively, modulate cellular response towards the environmental cues and intercellular connections.

Since the changes in the mechanical environment are received by mechanosensing transcellular proteins and feedback to cytoskeleton and nucleus,² a series of modulation in cytoskeleton and gene expression were triggered to balance between mechanosensitivity and structural remodeling.¹²

2.1 Extracellular mechanosensation through cell-substrate interaction

Mechanical signal conveyed by ECM is one of the dominant factors controlling heart form and function. The constant systole and diastole lead to bending of myocardium during morphogenesis and this active tension development is also critical to render the final cell shape and orientated myofibrillar architecture.¹²

Information on the underlying matrix is conveyed to cells through mechanical signals, which are first picked up by integrin at the cell membrane. The activation of mechanosensitive signal transducers like focal adhesion kinase, thereafter translate the mechanical signals into biochemical cues, that regulate transcription and cellular activities. Multiple proteins in cardiomyocytes were demonstrated to serve as mechano-sensors to activate different stress-related responses. For instance, melusin, which interact with integrin,¹³ senses myocardial wall stress;¹⁴ muscle LIM protein (MLP) induces hypertrophy in cardiomyocyte via calcineurin-NFAT pathway;¹⁵ and titin, a sarcomeric protein, involves in strain sensing in cardiomyocyte.¹⁶

Since mechanosensitive protein complexes associate with certain cytoskeletal structures, these architectures endow myocytes with noise filtering function to have selective mechanosensation and mechanotransduction with respect to their orientation, source, and contraction frequency.¹² It is the primary mediator between structure remodeling and external stimuli, and also serves as a channel to disseminate the mechanical signals to the myocyte body.

2.2 Inter- and intra-cellular mechanosensation

Cadherin, a transmembrane receptor, is linked with terminating actin through catenin. It responds to tugging forces intercellularly and reinforces the intercellular junction correspondingly.¹⁷⁻¹⁸ The gap junction channels in cardiomyocytes were shown to link closely with adherent junctions, as the expression of connexin 43 and N-cadherin correlate spatio-temporally.¹⁹ Mechanical stretch in vitro induces an increase in the expression of connexin 43 and also a concomitant increase in conduction velocity.²⁰

On the other hand, elastic titin proteins serve as unique mechanosensors in myocyte as it is highly deformable, high sensitive, and unique linked to sarcomeric elements (Z disc and M band). When cells are mechanically loaded (e.g. stretched), titin proteins were reported to be stretched with activation of titin-bonded muscle ankyrin repeat proteins (MARPs), which activate transcription and signalling.²¹

This mechanical feedback can be implemented in a cardiac model in vitro to understand the influence of microenvironment. For example, appropriate substrate stiffness was found to promote the maturation of induced pluripotent stem cell (iPSC) derived cardiomyocytes and embryonic cardiomyocytes.²²⁻²³ At a physiological relevant stiffness, cardiomyocytes exhibited more mature phenotype, electrophysiological conduction, and higher expression of late cardiac markers.^{22, 24}

3. Topological-Mediated Changes at the Cellular Level

3.1 Myocyte Shape in vivo is a Hallmark of Cardiac Status.

Adult cardiomyocyte has a typical length of ~120-140 μm and a diameter of 15-30 μm in healthy myocardium.²⁵⁻²⁶ Myocyte shape is a hallmark of maturation and pathological status in cardiac tissue (Figure 1). While neonatal myocyte is isotropic, mature cardiomyocyte adopts a cylindrical shape with an aspect ratio (AR) of approximately 7:1.¹² The dimension and aspect ratio may vary in a defective heart with a maladaptive microenvironment. For example, the length of cardiomyocytes was 40% longer in an ischemic heart with slightly changed width, significantly increasing the length/width ratio by almost 50%.²⁷

Figure 1. Illustration of myocyte and ECM architecture under physiological to pathological conditions. Scale bar: 50 μm . Reproduced with permission from ref ²⁸. Copyright 2019, The Author(s).

3.2 Microscopic Structures Regulate Myocyte Shape, Intracellular Remodeling, and Corresponding Functional Responses.

Fabrication of microstructures provides a way to mimic ECM morphology in vitro.²⁹⁻³¹ Through structural designs, myocytes could be manipulated to assume any shapes, thereby altering cellular structures and developing different functional responses.³²⁻³⁵

Geometrical cue is crucial in directing neonatal myocyte growth. For instance, planar substrate renders myocytes pleomorphic shape with disorganized myofibril and z line,³³⁻³⁴ but micropatterns encourage the remodeling of myocyte phenotype and intracellular structures (Figure 2a, b). It introduced higher tension on myocyte by assisting it to occupy a larger area ($2500 \mu\text{m}^2$) than on a flat substrate ($900 \mu\text{m}^2$), with a denser myofibrils network throughout the cell volume.^{34, 36-37} Shape dictates the intracellular stress field and directs the myofibrils organization to radiate along the direction of maximal tensile stress, (e.g. corner-to-corner on square, triangle, and star patterns).³⁸⁻³⁹

Figure 2. Effect of microenvironment on myocyte phenotype and functional response. a) Topological effects are varied in shapes, aspect ratios, and sizes of a cardiac construct. b) Shape of single myocyte dictates intracellular structure and stress field. First column: Sarcomeric α -

actinin architecture in triangle (A), square (F) and circular (K) myocytes. Second and third column: DIC images showing full relaxation (B, G, L) and full contraction (C, H, M) of micropatterned myocytes. Forth column: Contractile traction map of triangle (D), square (I) and circular (N) myocytes Scale bar: 10 μm . Reproduced with permission from ref ³². Copyright 2011, Grosberg et al.. c) Traction analysis as a function of cell aspect ratios at peak systole. The greatest traction stress is achieved at the aspect ratio of healthy myocytes (7:1). Reproduced with permission from ref ⁴⁰. Copyright 2012, American Society for Investigative Pathology. Published by Elsevier Inc.

Increasing the AR to normal physiological AR 7:1 and beyond, the myofibril architecture in myocyte was changed from corner-to-corner diagonal radiation to parallel alignment to the long axis, thereby altering the cell's functional response.³³⁻³⁶ Owing to enhanced myofibril alignment and sarcomere activities, contraction in myocytes was directed along the longer axis and the total contractile force and mechanical output increased with increasing ARs.³⁶ Beyond AR 7:1, high anisotropy in cells disrupted myofibril structure, as manifested by fewer z lines, deteriorating the functional output.⁴¹ Hence, the optimal functional response was achieved at physiological myocyte shape with AR 7:1 (Figure 2c), with the greatest movement of myofibrils leading to maximal mechanical output, in agreement to the physiological condition in healthy myocardium.^{38, 40}

Besides mechanical communication, electrical conduction at the intercalated disc, or more specific gap junction, also varies with the morphology of myocytes. Utilizing the linear correlation between the intercellular electrical conductance g_j and Connexin 43 immunosignal, conduction velocity was found to increase with increasing ARs.⁴² Action potential is the highest at AR 7:1 with optimal gene expressions, which were downregulated in other pathological ARs.⁴³ These

observations evidence how physiological AR (7:1) remodel intracellular organizations to generate optimal cellular functions.

Meanwhile, myocytes from different origins display cell line-specific remodeling processes and functional responses. For instance, it was found that murine-induced pluripotent stem cell-derived cardiomyocyte had a more matured and organized myofibrillar architecture than human-induced pluripotent stem cell-derived cardiomyocyte under the same culturing condition⁴⁴ and single neonate myocyte exhibited stronger traction on the two ends as compared to embryonic stem cell developed myocyte (ES-CM) and induced pluripotent stem cell-derived myocyte (iPS-CM).^{39, 45}

Figure 3. Cell line-specific mechanical analysis in myocyte cell pairs. a) homogeneous and heterogeneous cell-cell coupling with different cellular structures and contraction profiles. Scale bar: 10 μm . b) Schematic illustration of disparate stress distributions arising from structural remodeling. Reproduced with permission from ref⁴⁵. Copyright 2016, Aratyn-Schaus et al.

Both single and myocyte pairs have demonstrated cell-line dependency in electrical and mechanical couplings.^{39, 42, 45-46} Rectangular patterns with different ARs regulated the shape of myocyte pairs and their intercellular junctions. By pairing up myocytes both homogeneously (pair of same cells) and heterogeneously (pair of different cells), disparate functional responses were reported (Figure 3).⁴⁵ Neonate myocyte, ES-CM, and iPS-CM pairs exhibited different calcium level and traction profile. While neonate pair displayed the highest contraction ($\sim 0.55 \mu\text{N}$) in homogeneous cell pairs (ES-CM $\sim 0.25 \mu\text{N}$ and iPS-CM pairs $\sim 0.30 \mu\text{N}$), it deteriorated in heterogeneous pairs and a similar trend was also found in Ca^{2+} level. Notably, traction stress appeared at cell-cell interface in the heterogeneous pairs, indicating re-assembly of focal adhesion to dissipate excessive force and mitigate mechanical imbalance.^{39, 45}

3.3 Stiffness as Another Critical Mechanical Factor Governing Myocyte Structure.

Besides AR, substrate stiffness is another major factor determining cell structure and function. While actin architecture was shown to be only dependent on the myocyte shape, the microtubule underwent enhanced polymerization on a stiffer substrate, leading to increased elastic modulus in myocyte.³⁵ At stiffness exceeding 35 kPa, discontinuities started to emerge in the myofibril organization,³⁸ and remodel intracellular structures such as FAC, microtubule, and thereby changing myocyte's functional output.³⁸

Hence, the combination of stiffness and AR needs to be carefully adjusted to recapitulate *in vivo* conditions. On a physiologically relevant substrate (stiffness < 30 kPa), the optimal functional performance was achieved at AR 7:1, whereas a lower aspect ratio is preferred in diseased conditions.^{35, 38} High stress with low work output is a key characteristic of cells residing on stiff substrates. This is probably attributed to the presence of more parallel myofibrils in myocyte with low AR, so that cell could pull on a larger area of matrix and generate higher systolic stress.

Therefore, as compared to the engineered cardiac system relying on rigid substrates, hydrogel materials hold high potential due to their high biocompatibility and low modulus.⁴⁷⁻⁴⁹ The modulus of hydrogel could be easily tuned from a few kilopascals to megapascals, which matches with the modulus of tissues. In addition, it is largely made up of water, rendering the material with good permeability of nutrients.

4. Structure-Regulated Morphology and Functional Response of Cardiac Tissue

4.1 Micropattern-Induced Remodeling in Two-Dimensional Myocyte Monolayer

Nanoscale topography drastically enhances cell viability on myocytes. It was discovered that simple roughening on the surface promotes cell adhesion and with the introduction of micropattern, cell orientation, structure as well as functional responses can be tuned.⁵⁰ Micropatterns in building a 2D biological model can be fabricated through a variety of methods, such as laser cutting,⁵¹ laser photopatterning,⁵¹ microabrasions,⁵² and contact printing.⁵³⁻⁵⁴ Through controlling the pattern design, myocytes monolayer with varying morphology can be achieved. These micropatterns could recapitulate both physiologically relevant and pathological relevant microenvironments *in vitro*, remodeling the structures of cell sheets and tuning its functional output.

Figure 4. Cardiac construct in vitro. a) Cardiac monolayer with and without geometrical guidance. Reproduced with permission from ref⁴¹. Copyright 2020, Morris et al. b) Monolayer with parquet pattern to mute effect of gene expression. Scale bar: 10 μm (upper), 100 μm (lower) and 25 μm (insets). Reproduced with permission from ref⁵⁵. Copyright 2016, Biophysical Society. Published by Elsevier Inc. c) Examples of 3D cardiac constructs. Reproduced with permission from ref⁵⁶. Copyright 2019, Elsevier Ltd. Reproduced with permission from ref⁵⁷. Copyright 2019, Acta Materialia Inc. Published by Elsevier Ltd.

Myocytes could form an integrated sheet with high anisotropy under the effect of line micropatterns (Figure 4a). While line width is governing the anisotropy of myocytes, gap size determines its relevance with in vivo microenvironment. Minimum line width above 10 μm is required for the cell sheet to develop healthy cell-cell junctions and synchronized contraction.⁵⁸ If the gap size is too large ($>25 \mu\text{m}$), the cell sheet could barely maintain confluence and exhibited weak synchronization simulating pathological conditions.⁵²

In contrast to unpatterned myocyte sheets, these patterned monolayers manifest denser microtubule organization and generating higher contraction force.^{41, 51, 59} The conduction velocity of electrical impulses is also enhanced as a result of high anisotropy. Parameters such as tissue confluence, alignment, and myocyte shape are potential mediators of action potential.⁶⁰ Under the effect of line pattern, high global anisotropy and high aspect ratio of myocytes increased longitudinal conduction velocity (LCV) in the myocyte monolayer, but decreased conduction velocity in the transverse direction,⁵² highlighting the significance of cell sheet morphology in the regulation of cellular organization and overall functional response.

Realizing the widely extended effect of microenvironment, one may start to question the origin of these distinct functional outputs. Topology-modulated gene expression was initially suspected to be the reason but later proved unrelated by Knight et. al.⁵⁵ They muted the downstream effect of gene expression by designing parquet patterns with transiting orientations (Figure 4b) and demonstrated its irrelevance in the mechanotransduction process. Their findings elucidated the relationship between global myofibril organization and net cardiac contractile force, with the exclusion of other interferences. However, how these distinctive tissue responses were activated remains elusive.

4.2 Three-Dimensional Cardiac Construct Has Superior Properties over Myocyte Monolayer

Although 2D micropatterning technique serves as a powerful tool for biomechanical studies, it fails to reproduce the *in vivo* cardiac microenvironment. Three-dimensional (3D) structure is essential to cardiac morphogenesis and functions. For instance, 3D cardiac patches have shown superior properties and advanced functional maturation than 2D cardiac sheets under the same culturing conditions.⁶¹ To build reliable 3D cardiac constructs., a range of advanced biofabrication techniques such as lithography, 3D bioprinting, and laser cutting was employed with

different accuracies and resolutions.,^{11, 57, 62} Many different micro and macro structures were studied and analyzed, from simple microgrooved and micropegged patterns⁶³⁻⁶⁵ to complex 3D microlattices and fibrils.^{57, 66-67} To maintain the integrity of the 3D cardiac construct, the materials used need to be mechanically strong, nutrient permeant, structurally stable. PDMS⁶⁷⁻⁶⁸, PCL⁵⁷, and PVDF⁶⁶ are reported to construct 3D tissue structures such as patches, fibers, and lattices. These structures guide cell-ECM dynamic communication and shed light on the understanding of morphologically controlled cardiac remodeling and function. Cardiomyocytes phenotype was found to trigger a series of Ca^{2+} handling modifications on microgrooved pattern, including elevated diastolic and systolic Ca^{2+} levels, increased diastolic Ca^{2+} rise rate, and steeper restitution of Ca^{2+} transient duration.⁶⁹⁻⁷⁰ 3D cardiac wire was developed by Xiao et al. to mimic the native cardiac bundle and simulate the drug perfusion process.⁶¹ With electrical stimulation to regulate cardiomyocyte contractions, good coupling was achieved in the bundle, as manifested by stronger expression of Connexin 43.

Besides microscale patterns, cells could sense structural cues over hundreds of microns to align sarcomere structures and improve contraction, owing to upregulation of excitation-contraction coupling related genes.⁷¹⁻⁷² Moreover, geometrical design regulated the propagation of action potential through different conduction velocities in longitudinal and transverse directions, which was also utilized to simulate reentrant arrhythmia.⁷²⁻⁷³ These large format cardiac patches (in dimensions of several millimeters) are useful as implantable scaffolds for preclinical animal trials and personalized regenerative medicine, with good scalability and reproducibility.⁷⁴

4.3 Co-Culture of Cardiomyocyte with Other Cell Types

To maintain proper heart function, many different cells are working in coordination, and co-culture of cardiomyocytes with these cells yields interesting synergistic effects in the cardiac model, offering a more reliable and thorough understanding of heart functions *in vivo*.

The most commonly co-cultured cell is cardiac fibroblast, which is abundant in the myocardium and responsible for ECM construction.^{62, 68, 72, 75} It was speculated that fibroblast cells communicate with cardiomyocytes to assist in electrical conduction in the myocardium and influence cardiomyocyte phenotype.⁷⁵⁻⁷⁷ Co-culture of cardiomyocytes with cardiac fibroblast cells was found to promote myocyte maturity and enhance the synchronization as well as overall electrophysiological properties through enhancement of overall cell-cell communication.^{76, 78-80} This cardiac construct displayed better myocyte spreading, improved orientation index, and cell-ECM interaction as compared to cardiomyocytes-only construct. In addition, heart is extensively vascularized to support the high metabolic activity of constant contraction. The coculture of cardiomyocyte with epithelial and stromal cells on the crossly patterned network was found to promote maturation of myofilaments.⁸¹

4.4 Engineered Model of Cardiomyopathy

In healthy myocardium, cardiomyocytes align parallel with collagen fibers to provide structural stability and strength for normal cardiac function. Cardiomyopathy, on the other hand, is often associated with extensive cellular and extracellular remodeling in the myocardium. Pathological stimuli induce maladaptive remodeling in cardiac tissues, which adversely changes the cardiac microenvironment and subsequent cellular functions (Figure 5). Fibrosis, a common heart disease, develops from either impaired or hypertensive cardiac tissue. Over-deposition of collagen secreted by myofibroblast remodels the ECM structure and changes its stiffness and organization.⁸²⁻⁸³ Cardiomyocytes residing on the diseased ECM became shorter, less anisotropic,

and displayed deteriorated contractility, impulse propagation, and many other defective functional responses.⁸⁴ Besides, mechanical overload also induces morphological changes in the heart and dysregulates individual myocyte shape, such as concentric hypertrophy with thickened ventricular wall and reduced myocyte anisotropy,⁸⁵ which impair tissue contractility and could lead to eventual heart failure.

Figure 5. Schematic illustrating changes in myocyte and ECM during pathological remodeling of myocardium, leading to deteriorated contraction function.

Many cardiac diseases are associated with abnormal remodeling of cell and tissue morphologies, which can be recapitulated by microstructures in cardiac models in vitro. Advancements in micropattern fabrication and biomaterial have provided many strategies to

simulate and study cardiac diseases from two aspects: tissue alignment and substrate stiffness. Abnormal ECM remodeling induces drastic changes in tissue morphology with myocardial infarction being a good example, in which cardiomyocytes located at the infarct border zone completely lose their alignment and anisotropy. These pathological features can be replicated in vitro through substrate design, producing weakly aligned cardiomyocytes on a stiff substrate (>40 kPa).^{24, 86} For example, rectangular micropatterned protein was used to analyze Barth syndrome⁸⁷ and 3D microtissue was built to model titinopathies⁸⁸, involving extensive myocyte architecture remodeling, disrupted sarcomere, and stress generation.

5. Integrated Heart-on-Chip Platform

Upon successfully recapitulating the cardiac morphology in vitro, extending the simple cardiac construct to a complete organ-on-chip device is a key milestone for cardiac research. Heart-on-chip could meet both the basics research needs (*e.g.* study of physiological and pathological tissue) and translational research investigations (*e.g.* drug screening). The miniature heart-on-chip model based on microfluidic technology holds a high potential for advancing personalized medicine, as it promotes the testing of human cells due to the low volume of culturing cells required.

The heart-on-chip model could essentially feedback two basic characteristics in cardiac tissue: contraction and electrical conduction. Integration of signal detection and monitoring was achieved to provide one-stop feedback on cardiac tissues^{67, 89} and the device could also characterize the effect of cardiotoxic drugs on the cardiac construct.^{56, 90-91, 92} In more complex design, Kamei et. al. developed a body-on-chip device with the integration of heart, liver systems, and a blood circulation loop. In their work, liver cancer cells were cultured in the liver chamber and an anti-cancer drug was introduced to study its side effect on cardiomyocytes in the heart

chamber. Such integration involving multiples tissues offers valuable insights on systemic toxicity.⁹³

Additionally, timely feedback on the recorded signals is always necessary. Incorporation of wireless control and feedback loop in the heart-on-chip device simplifies the research procedure and offers an accurate and timely reflection on the tissue condition. A large-scale, integrated, and smart cell-culture platform (LISCCP) was developed for adherent cells to monitor their culturing conditions (e.g. pH, temperature) and cell status (e.g. cell density) digitally and provide real-time feedback in the computer.⁹⁴ Zhang et.al. integrated a heart-on-chip device with commercially available Google Glass and achieved bi-directional Glass-device interfacing. Acting as both a signal receiver and a controller, Google Glass could monitor physical and physiological parameters (such as temperature, morphology, and pH) of the chip in real-time.⁹⁵

A heart-on-chip device fills the gap between in vivo and in vitro conditions, serving as an excellent platform for cardiac research and drug discovery. With incorporation of topological regulation, both physiological and pathological cardiac conditions can be studied thoroughly for better clinical treatment.

Figure 6. Prospective challenges for future advancements in cardiac constructs. Reproduced with permission from ref ⁹⁶. Copyright 2016, Acta Materialia Inc. Published by Elsevier Ltd. Reproduced with permission from ref ⁹⁷. Copyright 2016, Elsevier Ltd. Reproduced with permission from ref ⁹⁸. Copyright 2016, by the American College of Cardiology Foundation. Published by Elsevier.

6. Conclusion and Outlook

Advancement in biofabrication and biotechnology has offered countless strategies to study the physiological and pathological state of cardiac tissue. Spatial and temporal control of microenvironment can be achieved, and tissue morphology can be recapitulated through various techniques. These mechanically modified structures regulate phenotype and functional response of cardiomyocytes and cardiac tissues, hence are essential in cardiac models. With the integration of microfluidics, sensors, and digital controls, heart-on-chip device is a brilliant platform for

cardiac research and offers a one-stop service for real-time characterization of cardiac functionalities, monitor of culturing environment, and wireless control.

Despite current achievements, there are still many issues to be addressed (Figure 6). Replication of ECM environment remains a key challenge as no available material could simulate the viscoelastic property of ECM and understandings on 2D and 3D tissue constructs are still lacking. Other than microenvironment, cell source in the cardiac model is another issue. As cardiomyocytes are postmitotic, human iPS-CMs are the best candidate for research and personalized medical treatment but they are immature with weaker contractile phenotypes.⁹⁹ Hence, the introduction of additional mechanical or chemical cues to promote maturation of myocytes is critical for reliable cardiac research and disease treatment. In addition, current studies on cardiac physiology are limited to solely electrical or mechanical coupling of cardiomyocytes, but these two effects are highly coupled *in vivo*. Integration is necessary to study the mechanical-electrical coupling in the myocardium and its functional principle. Lastly, 4D tissue modeling, in which 3D tissue constructs are responsive to external stimuli such as magnetic and temperature, provide a new perspective for future cardiac research. These new approaches hold potential in designing combinatorial models integrating mechanical, physiological, and electrical factors.

ASSOCIATED CONTENT

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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ABBREVIATIONS

ECM, extracellular matrix; iPSC, induced pluripotent stem cell; AR, aspect ratio; FAC, focal adhesion complexes; ES-CM, embryonic stem cell developed myocyte; iPS-CM, induced pluripotent stem cell-derived myocyte.

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