

## REVIEW ARTICLE

## Regulatory effect of anisotropic structure on cardiomyocyte maturation

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**Abstract**

Cardiovascular disease (CVD) is the leading cause of death worldwide. Due to the limited regenerative capacity of the adult heart, treatments based on human pluripotent stem cells (hPSCs) have become the focus of a great deal of research. Human pluripotent stem cell-derived cardiomyocytes (hPSC-CMs) can provide an ideal cell source for CVD model construction, cardiac tissue repair, and drug cardiotoxicology research. However, the immaturity of hPSC-CMs seriously restricts its clinical application. The maturation of cardiomyocytes depends on the orderly arrangement of myofilaments and the increase of the expression of connexin, so the geometric regulation of bioengineering substrate is one of the keys to the maturation of engineered myocardial tissue. This review focuses on the key indicators of anisotropic structures provided by biomaterials to improve the maturation characteristics of cardiomyocytes, so as to promote the maturation of cardiomyocytes, and looks forward to the development direction in this field.

**Keywords:** *Cardiovascular disease, human pluripotent stem cells, cardiomyocytes, bioengineering, anisotropic structure*

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Cardiovascular disease (CVD) is regarded as one of the leading causes of death worldwide. Although cardiovascular science and preventive medicine have made remarkable progress in half a century, CVD still has a high mortality and high morbidity worldwide (1). Among them, the most common CVD is myocardial infarction (MI), which is caused by ischemic necrosis of cardiomyocytes caused by coronary artery embolism. When the adult heart is injured or sick, the injured downstream cardiomyocytes reshape the heart wall, resulting in the gradual deposition of heterogeneous fibrous tissue to form dysfunctional fibrous scar, which, to a certain extent, will lead to coronary artery ischemia and anoxia, thus affecting the normal function of the heart (2). Due to the extremely limited ability of regeneration of adult cardiomyocytes after injury, the methods of heart transplantation are also limited by serious shortage of donors and cardiac rejection. Currently, there is an urgent need for a heart regeneration therapy that can fundamentally solve the problem to restore myocardial function after MI (3).

In the past two decades, many studies have explored a variety of suitable sources of cardiac cells for cardiac regeneration therapy, including primary cells, cell lines, and undifferentiated human pluripotent stem cells (hPSCs). Primary cells are cells isolated directly from tissues and can maintain a phenotype relatively similar to their *in vivo* counterparts. However, the life span and proliferation capacity of human primary cells are extremely limited, which limits the use of primary cells, and there are problems with commercially available and fully certified sources of primary human cardiomyocytes. The common cell lines of cardiomyocytes are AC16 and HL-1, which can proliferate and differentiate under specific culture conditions and exhibit well-organized ganglion structure and the ability to maintain contractile function after transmission (4, 5). Therefore, the standardized use of cell lines has a great advantage in the identification of cardiac tissue regeneration models and used in most *in vitro* models (2). Because cardiomyocytes derived from embryonic stem cells and pluripotent stem cells can effectively differentiate into cardiomyocytes, it provides great

potential for human heart tissue regeneration, and the treatment of human embryonic stem cells (hESCs) and human-induced pluripotent stem cells (hiPSCs) has been proved to have the ability to produce new cardiomyocytes (6). Based on the current research background that stem cells can treat a variety of diseases, differentiating hPSCs into cardiomyocytes is a promising method for cardiac regeneration therapy.

In order to achieve a good therapeutic effect, it is very important to obtain high-quality cardiomyocytes. However, the main limitations of cardiomyocytes from the above sources are their disorganized, functionally immature, fetal-like phenotype, so physiologically these sources are not equivalent to natural adult heart tissue (7). There is evidence that human pluripotent stem cell-derived cardiomyocytes (hPSC-CMs) have immature gene expression characteristics (8), ultrastructural phenotype (9) and electrophysiological characteristics (10), and abnormal  $\text{Ca}^{2+}$  cycle (11). In order to solve this problem, many recent studies have explored ways to improve cardiomyocyte maturation. At this stage, the main methods to promote cardiomyocyte phenotypic maturation are co-culture (12), mechanical stimulation (13), electrical stimulation (14), 3D culture (15), and extracellular matrix interaction (16). These methods are backed up by extensive research demonstrating that they can improve the functional performance of cardiomyocytes. It is proved that it can improve the functional performance of cardiomyocytes (15). In this review, we will focus on the anisotropic structure provided by biomaterials to enhance the phenotypic maturation of cardiomyocytes for myocardial tissue repair and introduce the anisotropic structural characteristics and functions of micro- and nanoscale surface patterns and scaffolds. We also focus on the important contribution of this anisotropic structure in promoting the function and structural maturity of cardiomyocytes derived from hESCs and hiPSCs.

### Anisotropic structure

Currently, there is a plethora of anisotropic structures designed using biomaterials. Many studies have shown that these structures can regulate the behavior, directional arrangement, growth, and proliferation of cells, including cardiomyocytes (17, 18). Due to the nano/micron characteristics of the size of natural biological tissues and cells, micro/nanopattern technology is very important for the development of new functional biomaterials. Natural materials such as bones, ligaments, shells, and scales have great potential to meet the complex and multifunctional requirements of the body. The recent development of biomaterials has also focused on the design of biomimetic materials, which can trigger specific cellular responses and guide the formation of new tissues by changing the structure and size of materials. Polymer-based micro/

nanopatterns have been widely used in these fields because polymer-based materials offer the advantages of low cost, good biocompatibility, high optical transparency, and high impact strength (19).

Based on the current research, the anisotropic structures used to regulate cardiomyocytes can be divided into anisotropic micro/nanoarray and anisotropic micro/nanofiber scaffolds. The study can promote the directional arrangement of the cytoskeleton in the cell by designing the structural model of the optimal size so as to further promote the development of myofibril tissue and sarcomere length, which is also a sign of cardiomyocyte maturation.

#### *Anisotropic micro/nanoarray*

The micro/nanoarrays are used to promote the directional alignment and maturation of cardiomyocytes mainly in the form of grooves and micropillars. When cardiomyocytes are inoculated into this anisotropic structure, they can form muscle fibers, improve sarcomere tissue and intercellular connexin expression and contraction intensity (20), and regulate cell morphology and anisotropic conduction patterns (21). Micro/nanoarrays are typically fabricated using materials such as silicon (Si), polystyrene (PS), and polydimethylsiloxane (PDMS). Nowadays, many studies on the growth and regulation of cardiomyocytes on micro- and nanopattern microcolumns made of different polymer materials have been reported (22, 23). In the existing research, the most popular material for manufacturing models is PDMS, which is a transparent, elastic, and biocompatible polymer material, and its properties have a great influence on the function of the anisotropic topology (24). Although PDMS has good performance for the fabrication of 500 nm or larger structures, its inherent softness makes it inaccurate in the preparation of nanoscale anisotropic structures, resulting in uneven distribution of cells inoculated on the surface patterns. In order to solve this problem, the material can be converted to harder properties by using UV curing polysiloxane or by adding polymer curing materials such as polyurethane acrylate (PUA). Based on these improved methods, PDMS is now being used to create high-density surface patterns down to the 20 nm scale (25).

Micro/nanoscale arrays can facilitate the directional arrangement of cardiomyocytes and intercellular interconnections by being designed as anisotropic structures of grooves and micropillars. Therefore, the design of its structure in terms of size and shape is particularly important. It is reported that the width and depth of the groove are two key parameters in the design process. Studies so far have shown that when the depth increases from 0.2 to 69 microns wide, more cells attach and proliferate along the grooves. In terms of width, when close to cell size, cells not only grow more directionally along the grooves, but

also their genes related to regeneration and cytoskeleton development are significantly upregulated. Daniel Carson et al. proposed a nanogrid culture array composed of nanogrooves with a width range of 350–2,000 nm. By studying the effects of different nanostructures on the structural development of hiPSC-CMs in vitro, it was found that the tissue and structure development of cardiomyocytes depended on the characteristics of nanogrooves. Grooves in the size range of 700–1,000 nm can promote the maturation of cardiomyocytes by arranging cardiomyocytes (26). According to Rao et al., iPSC-CMs showed an organized sarcomere structure when cultured on a grooved substrate compared with cells cultured on a flat substrate. Based on the effect of the microcolumn structure on the tissue in the sarcomere cells, the distributed columns can form an anisotropic structure to guide the directional growth of cardiomyocytes. Through immunostaining of sarcomere  $\alpha$ -actin, it was observed that hiPSC-CMs could spread and adhere to the microcolumn structure with anisotropic arrangement within 24 h (27). Thus far, the reported diameters of PDMS microcolumns range from submicron to 10 microns, the ratio of column height to diameter can reach about 20, and the spring constant of the column is from 0.06 to 4,000 nNm<sup>-1</sup> (equivalent bulk modulus  $E_{eq}$  is 10 Pa –3 MPa), which is much wider than that achieved by natural or synthetic hydrogels (28). Ribeiro et al. mentioned that the stable covalent binding of laminin to microcolumns can increase the contraction rate and time of cultured cardiomyocytes, and thus improve the contractility of newborn mouse cardiomyocytes (29).

#### *Anisotropic fiber scaffold*

Anisotropic fiber scaffolds have the characteristics of large surface area-volume ratio and porosity and are easy to process. Many literatures have shown that cardiomyocytes inoculated on anisotropic fiber scaffolds can be used to simulate the anisotropic structure of cardiomyocytes and thus promote their growth and directional arrangement and play a great role in promoting their development and maturation. The morphology of anisotropic fiber scaffolds, such as pore diameter and shape, fiber thickness, fiber alignment, and porosity, can guide cell response. In other words, the scaffold structure appears to affect cell attachment, shape, proliferation, or migration patterns (30). Biodegradable synthetic polymers are often used to make fiber scaffolds, among which poly(glycolic acid) (PGA) (31), polylactic acid (PLA) (32), and polycaprolactone (PCL) (33) are the three most commonly used synthetic polymers. These synthetic polymers have advantages because of their mechanical properties and can be customized to meet the needs of clinical degradation, elasticity, and biocompatibility. In addition, these materials are inexpensive and readily available. Among the various

methods of fiber scaffold fabrication, electrospinning is an effective technique for the production of anisotropic oriented fiber scaffolds and an efficient method for the preparation of micro- and nanoscale fibers. The development of fiber scaffolds which can simulate the anisotropic structure of natural myocardial tissue and replicate its biological function is undoubtedly beneficial to myocardial regeneration. Because of their similarity to fibrous Extracellular matrix (ECM) protein in aspect ratio and size, arranged fiber scaffolds, especially nanofiber scaffolds, can perfectly simulate the anatomical structure of fibrous ECM protein and provide a clear anisotropic structure, thus effectively promoting the arrangement and growth of cells and finally tissue reconstruction. Appropriate micron or nanometer fiber scaffolds are designed to guide the arrangement of multilayer cells and provide structural support for the synchronous contractile activity of cardiomyocytes. Taking PCL as an example, some studies have shown that directional PCL fiber scaffolds can induce the directional arrangement of anisotropic cells in hPSC-CMs. This is mainly due to the excellent mechanical properties and tensile strength of PCL, and when PCL binds to natural polymers such as gelatin or collagen, the scaffolds can show significantly improved biocompatibility (33). Han et al. studied the culture of hPSC-CMs on Matrigel-coated anisotropic (directional alignment) and isotropic (randomly alignment) PCL fiber scaffolds for 2 weeks and tissue culture polystyrene (TCPS) as control. The results showed that directional electrospinning fiber scaffold could induce the directional arrangement of anisotropic cells in hPSC-CMs (34).

In the existing complex anisotropic scaffolds, nanofibers or micropores independently provide structural clues for the deposition of cytoskeleton and extracellular matrix. Although nanofiber scaffolds can well simulate the fiber structure of natural ECM, they often have low porosity, which limits the uniform infiltration of cells. In contrast, microporous scaffolds provide more space for cell infiltration and growth, but they lack in vivo topological guidance. Therefore, it is believed that the future research on the development of nanofiber-microporous hybrid scaffolds by embedding nanofibers into micropores can make full use of their advantages and maximize the space for cell infiltration and growth.

#### **Regulatory effect of anisotropic structure on cardiomyocytes**

Although hPSC-CMs have great potential in the treatment of MI, their immature fetal-like phenotype limits their physiological functions, such as contractility, intracellular calcium transient, and electrophysiology. The main reason is that their myogenic fiber arrangement differs from that of adult cardiomyocytes, as well as the content and organization of myosin so that the anisotropic structure

can regulate the direction of cardiomyocyte growth to orientate them and thus promote cardiomyocyte maturation to some extent.

hPSC-CMs have emerged as the most suitable source of cells for the manufacture of functional heart tissue. However, due to the lack of global standards for defining and evaluating the maturity of CMs and cardiac tissue, scientists have been comparing the differences between hPSC-CMs and adult CMs in terms of morphology, intracellular calcium transport, gene expression, and electrophysiology. The transcriptional, biochemical, and functional changes of cardiomyocytes through changes in electrophysiology, calcium transport, and contractility are inherent in the development from fetal state to adult state (18). Therefore, the maturation of cardiomyocytes *in vitro* can be evaluated by analyzing these characteristics.

#### *Formation and arrangement of cellular sarcomere*

Orderly arrangement of cells is essential for controlling the biological function of cells. Numerous studies of cells grown on micron- and nanometer-sized anisotropic structures have shown that cell morphology is extremely sensitive to spatial constraints and topographical order (26). A growing number of studies have highlighted the sensitivity of morphology and gene expression to micron and nanometer grooves, microcolumns, and micropores. A large number of studies have been conducted to assess the maturity of hPSC-CMs, and consistent results show that after differentiation, these cells are more similar to fetal CMs than adult CMs. Compared with the random arrangement of hPSC-CMs, adult CMs are anisotropic with high density and neatly arranged myofibril structure, which contributes to the efficiency of electrical conduction and contraction (35). In contrast, hPSC-CMs and early fetal CMs are randomly arranged and distributed, and myofibril structure density is lower. Through microscopic observation and analysis, all prominent myofibril areas can be seen in adult CMs, including I band, A band, M line, and H band, as well as Z disc (36), which represents the end of sarcomere. In contrast, hPSC-CM sarcomere lines are mostly difficult to identify, usually only Z plates or I bands, because immature sarcomeres are not organized to provide different areas of myofibril. Also, mature cardiomyocytes have prominent T tubules and high tissue ultrastructure. In adult CMs, T tubules are very important for regulating ion flux and are also the key to excitation-contraction coupling and synchronous triggering of endoplasmic reticulum (ER) calcium release, so T tubule is considered as an indicator of CM maturation (37).

Sarcomere is the basic contractile unit of muscle tissue. The optimal sarcomere length in adult cardiomyocytes is 2.2  $\mu\text{m}$ . In many reports, the maturity of cardiomyocytes is determined by measuring whether the sarcomere length

reaches adult sarcomere length (38). Many studies have shown that anisotropic topology can promote the growth and directional arrangement of hPSC-CM sarcomere. At the same time, some literatures have shown that it can promote the formation of T tubules. It has been reported that the formation of T tubules can represent the signs of cell maturation to a certain extent (39). Based on the aforementioned characteristics, we can make a judgment in the study of cardiomyocyte phenotype and anisotropic structure on the degree of cardiomyocyte maturation.

Connexin-43 (Cx-43) is a gap junction protein responsible for electrical coupling between cells, which is usually used to evaluate the phenotype of cardiomyocytes. This is very important for regulating the electrical coupling of mammalian cardiomyocytes, so Cx-43 distribution is usually regarded as a key indicator of hPSC-CM maturity. Ribeiro-Rodrigues reports show that hiPSC-CMs inoculated on nanofibers can communicate well with each other through the participation of Cx43 and contribute to the synchronous function of cardiomyocytes (40). In addition, the cross-fringe pattern between  $\alpha$ -actin ( $\alpha$ -actin), myofibrillar, troponin-I (cTnI), and Cx-43 is also often used to indicate the functional electromechanical coupling between cardiomyocytes and to verify the phenotypic differentiation of hPSC-CMs to mature CMs (38).

#### *Electrophysiological characteristics*

Due to the immature electrical coupling of the hPSC-CMs, very asynchronous contraction occurs, while the adult CMs are excited only when it provides electrical stimulation and contracts synchronously (41). Through excitation-contraction coupling, the cardiac action potential (AP) causes a coordinated contraction of the CMs, which pumps blood forward to the peripheral tissue. Patch clamp is usually used to detect the representative AP trajectories of hPSC-CMs inoculated on anisotropic structures. The AP amplitude and action potential duration at 50% (APD50) and 90% (APD90) repolarization levels, respectively, found that the anisotropic topology shows a considerable improvement in its functional properties such as resting membrane potential and conduction velocity. Compared with adult CMs, the depolarization speed of fetus and PSC-CMs decreased by 6 to 50 times (35). In their study on the role of cardiac tissue alignment in regulating electrical function, Zhong et al. showed that the alignment of CMs has a significant effect on electromechanical coupling and contractile force under the adjustment of anisotropic structure, and concluded that anisotropic structure is an effective factor for CMs to regulate electrical stability (42). The research of Liu et al. mainly promotes the maturation of hPSC-CMs by making grooves that make the regular arrangement of hPSC-CM muscle fibers and contract in the same direction (43).

### *Intracellular calcium transport*

The  $\text{Ca}^{2+}$  cycle, which acts as a second messenger during the excitation-contraction coupling of the heart, is an integral part of cardiomyocyte biology as it is absolutely essential for the proper maintenance of cardiac function. The change of intracellular  $\text{Ca}^{2+}$  concentration in cardiomyocytes will lead to cardiac systolic dysfunction and arrhythmia. There is evidence that cardiomyocytes that lack mature  $\text{Ca}^{2+}$  transport characteristics are a major obstacle to their use in treatment, perhaps because  $\text{Ca}^{2+}$  transport plays a key role in the phenotype of hereditary (44) and acquired cardiomyopathy (45). The influx of  $\text{Ca}^{2+}$  into cardiomyocytes usually occurs in L-type voltage-gated channels, and the activation of  $\text{Ca}^{2+}$  ion channels triggers the release of  $\text{Ca}^{2+}$  stored in the sarcoplasmic reticulum (SR), resulting in the contraction of  $\text{Ca}^{2+}$ -induced  $\text{Ca}^{2+}$  release (CICR) in cardiomyocytes. During development, the number of low T-type calcium channels expressed in early fetal CMs increased gradually from early fetus to adulthood.

Normally, in adult CMs, the AP propagates downward along the transverse tube, which is the deep protuberance of the sarcolemma, allowing the AP to spread effectively. These APs cause the activation of L-type calcium channels along the transverse tube, which leads to calcium release from SR by binding to the ryanodine receptor (RyR) co-located with L-type calcium channels. Sarcoplasmic reticulum stores large amounts of calcium through calcium-treated buffer protein calcification protein (CASQ2). When the myofilament undergoes cross-bridge cyclic contraction, this intracellular calcium release leads to excitation-contraction coupling. After the contraction is completed, the intracellular calcium is called sarcoplasmic reticulum calcium ion ATPase (SERCA) with a special structure brought back to the SR. Calcium is transported out of the cell by sodium-calcium exchanger (NCX). On the contrary, hPSC-CMs have an underdeveloped calcium transporter structure. They have no transverse tube, which greatly affects the efficiency of calcium transport and excitation-contraction coupling. The SR of hPSC-CMs is underdeveloped, and the contents of SERCA, RyR, and CASQ2 are generally low. In terms of calcium kinetics, because hPSC-CMs have fewer CASQ2, RyR, SERCA, and L-type calcium channels, immature calcium treatment is more dependent on sarcolemmal calcium influx (most likely from T-type calcium channels and NCX) and the release of a small amount of calcium from SR through IP<sub>3</sub>-regulated calcium channels (46).

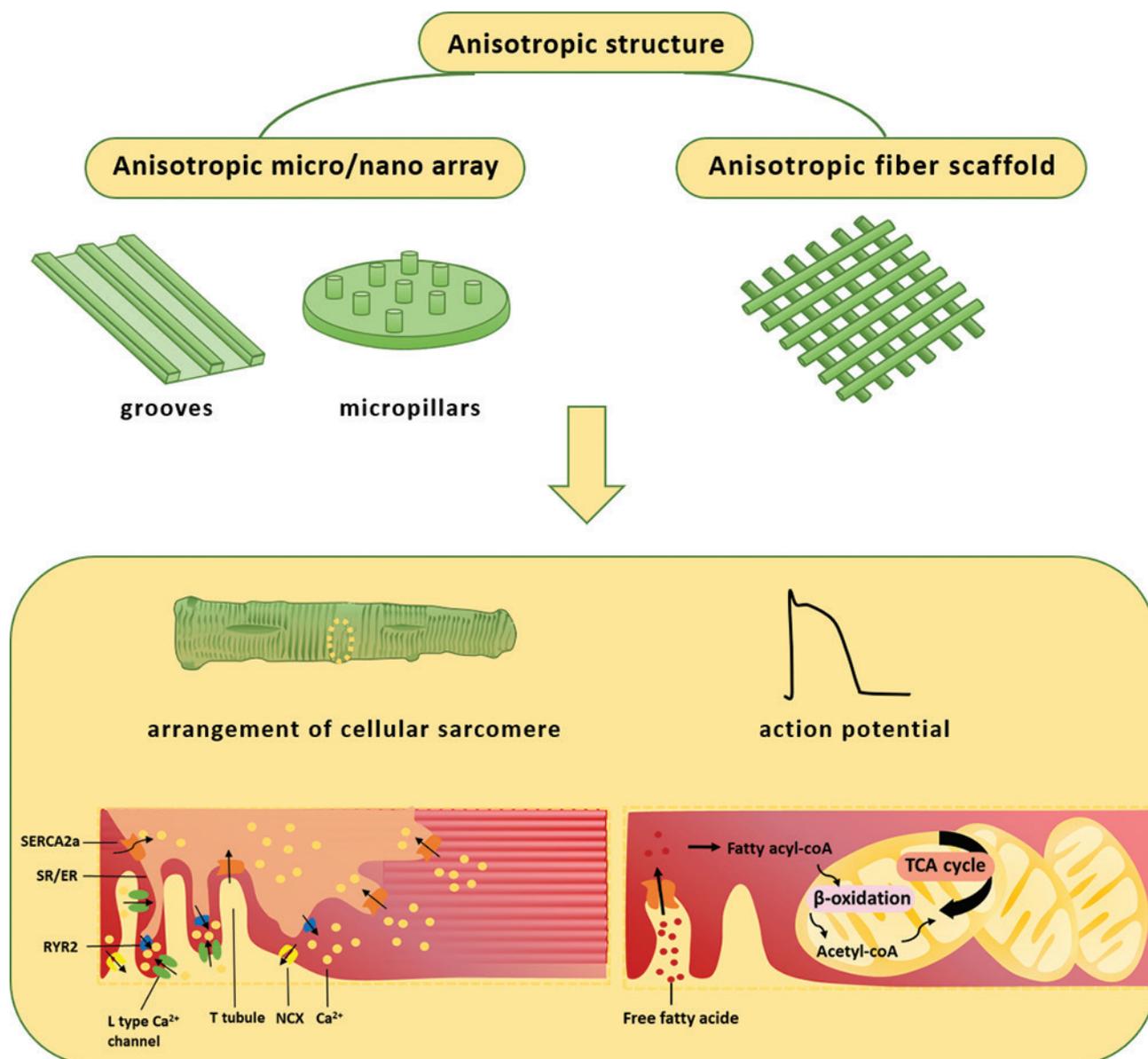
According to the characteristics of  $\text{Ca}^{2+}$  influx and release in cardiomyocytes, Ronaldson-Bouchard has tried to explore the degree of maturation of cardiomyocytes in the regulation of calcium transport (47). Nifedipine, a calcium channel blocker on the myocardial membrane, inhibits the influx of extracellular calcium ions. After

addition, it is found that the calcium signal decreases, but due to the release of calcium ions by ER/SR in mature cardiomyocytes, there is still a weak signal. At the same time, verapamil, a slow channel blocker of calcium influx, was used to prevent extracellular calcium from entering the cell. Due to the lack of activation of calcium ions, RyRs in SR could not release calcium ions in SR, so the calcium signal gradually disappeared. Then caffeine was added as a RyR agonist on the ER to stimulate it, making the SR release calcium ions again, thus restoring the calcium signal. It also shows that mature cardiomyocytes have functional intracellular calcium stores. In addition, toxic carotene, which is a SR calcium pump inhibitor, will block the reflux of calcium, so that the calcium ion in the ER gradually decreases and the calcium signal changes obviously. Mature cardiomyocytes no longer have calcium outflow even under the action of caffeine. However, the main sources of calcium in immature myocardial cytoplasm are not ER and SR, so there is no significant effect. In the case of only caffeine, the signal of mature cardiomyocytes will suddenly increase due to the influence of agonists, indicating that there are a lot of RyRs on SR/ER, and the cardiomyocytes are in a more mature state. From the effects of these drugs that inhibit the CICR of cardiomyocytes, we can infer that the characteristics of calcium transport are a strong proof in judging cardiomyocyte maturation. The functional  $\beta$ -adrenergic receptor system depends on intracellular calcium storage and co-localization of Cav1.2 channels and T tubules, so many literatures believe that the comprehensive response to  $\beta$ -adrenergic agonists is an indicator of phenotypic maturity (48).

Many literatures have reported that hiPSC-CMs cultured on anisotropic topology have faster calcium cycle rate and higher contraction frequency than cells cultured in plane. Including the transient amplitude of  $\text{Ca}^{2+}$ , the time to reach the peak, and the exponential decay constant ( $\tau$ ), it is proved that it can promote the arrangement of cells and increase the speed and amplitude of calcium cycle. In the study of Rao et al., PDMS grooves promoted the arrangement of cells and the formation of more organized sarcomere. The  $\text{Ca}^{2+}$  cycle characteristics of hPSC-CMs cultured on these grooves changed significantly, the peak amplitude shortened, and responded to caffeine. The release of more  $\text{Ca}^{2+}$ , from the SR, indicated that the  $\text{Ca}^{2+}$  cycle of SR was improved, and the anisotropic topology promoted the maturation of cardiomyocytes to some extent by improving calcium transport (27).

### *Metabolic assay*

In vivo, CM maturation is characterized by a shift in metabolism from glycolysis to priority fatty acid oxidation. hPSC-CMs shows the plasticity of metabolic substrates and can be regulated to promote the  $\beta$ -oxidation



*Fig. 1.* The schematic image of the effects of anisotropic structure on the maturation of human pluripotent stem cell-derived cardiomyocytes (hPSC-CMs).

of fatty acids. Yang's team has shown that compared with glucose-rich cultures, galactose-containing fatty acid cultures can improve the structure, function, and metabolic maturation of hPSC-CMs to prevent lipotoxicity (49). The difference in energy production between native CMs and hPSC-CMs is common. Adult CMs have a mature oval mitochondrial network, accounting for about 35% of the cell volume, and are arranged along the sarcomere direction, providing sufficient ATP for contraction. hPSC-CMs have immature mitochondria. Cristae are significant surface area folds that provide effective cellular respiration for mitochondria, which do not exist in hPSC-CMs, but are densely distributed in adult CMs (50). In adult CMs, most of the energy production is produced through

oxidative metabolism, while hPSC-CMs mainly use glycolysis with low efficiency. From a developmental point of view, metabolic maturation occurs after birth when cardiomyocytes are exposed to higher energy requirements and oxygen and fatty acids. In addition to its role in energy production, metabolism also regulates cellular processes. Therefore, the researchers promote the maturation of mitochondria of cardiomyocytes inoculated on these structures by using anisotropic topologies, but the specific mechanism of promoting maturation is not clear.

#### Summary and prospect

Thus far, although hPSC-CMs have been used in cardiac regeneration therapy, there are significant limitations at

present, the most important of which is phenotypic immaturity. Therefore, promoting maturation of hPSC-CMs is a challenge in this field, which determines whether it can be used in myocardial regeneration therapy and clinical environment. Researchers have made considerable progress in myocardial regeneration therapy by constantly innovating ways to promote the maturation of hPSC-CMs. Also, there has been a lot of evidence that anisotropic topology can promote embryonic stem cells and pluripotent stem cell-derived cardiomyocytes to obtain adult cardiomyocyte gene expression, significant ultrastructural tissue, functional contraction mechanism, and significantly improved electrophysiological characteristics.

In the past two decades, the continuous development of micro/nanomanufacturing processes and the synthesis of functional biomaterials have been successfully developed and applied to biological and biomedical researches, and a variety of micro/nanoengineering functional biomaterials have been produced. The summary of the effects and mechanisms of anisotropic structure on the maturation of hPSC-CMs is illustrated in Fig. 1. This improvement of cardiomyocyte phenotype is very important for promoting the application of hPSC-CMs in CVD modeling, drug discovery and development, and regenerative medicine. Advances in hPSC biology and the development of new materials and biomanufacturing methods have made it possible to regenerate specific cardiac tissue in patients with CVD, which in turn opens up the possibility of realizing the promise of personalized medicine. Thus, while the ultimate goal is to achieve phenotypic maturation of pluripotent stem cell-derived cardiomyocytes, we can further study how anisotropic structures drive cardiomyocyte maturation in vivo. As a result, significant advances in pluripotent stem cell-derived maturation have been made toward more clinically relevant models of cardiac regeneration.

The characteristics of cardiomyocyte maturation can be proved not only by a single index but also by a number of complex and interrelated characteristics, such as structure, electrophysiology, calcium transport, and gene expression. Therefore, when we explore ways to promote cardiomyocyte maturation, we need to analyze its multiple characteristics in order to achieve omnidirectional maturation of cardiomyocytes so as to be closer to adult myocardium and achieve myocardial regeneration. Similarly, through the combination of various methods of promoting maturity, we can strive to develop the best way to effectively produce large-scale mature hPSC-CMs and promote the development of CVD treatment.

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