Go to the Mattresses
A New Method for Human-Induced Pluripotent Stem Cell-Derived Cardiomyocyte Maturation
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Human-Induced Pluripotent Stem Cells
The ability to reprogram adult mammalian somatic cells to a pluripotent state has proven to be a paradigm shifting discovery. In 2007, this reprogramming technology was translated to the engineering of human-induced pluripotent stem cells (hiPSCs) using skin fibroblasts by both the Yamanaka (transduction of c-Myc, Oct3/4, SOX2, and Klf4) and Thomson (transduction of Oct3/4, SOX2, Nanog, and Lin28) laboratories. Importantly, human donors could be fully phenotyped, and their hiPSCs, which retain their genetic blueprint, could be isolated and used to study human disease mechanisms in vitro. Given the potential use of these cells in understanding and treating disease, Feaster et al, in this issue of Circulation Research, optimize the culture conditions to promote the differentiation of hiPSC-derived cardiomyocytes.

Article, see p 995

Our current understanding of the mechanisms that govern heart development and the pathophysiology of cardiovascular diseases have emerged primarily from genetic research models, such as the mouse and zebrafish. These genetic animal models, however, are limited in their ability to recapitulate the phenotype of patients with cardiovascular disease. Therefore, efforts in the past focused on the isolation of adult human cardiomyocytes from either an endomyocardial biopsy or from a surgical or pathological specimen. The yield from these samples was low, and the ability to obtain tissue from normal subjects was limited and not without risk.

Novel insights into cardiogenesis, gained from the study of embryonic stem cells, have been instrumental in the development of differentiation protocols that efficiently direct hiPSCs to a cardiac fate. These differentiation protocols include the exposure to distinct signaling factors to direct the hiPSCs to a cardiac fate over a 2-week period. Refinement of these protocols has now resulted in reproducibly obtaining a relatively pure cardiomyocyte preparation (75%–95% cardiomyocytes). Although the ability to direct hiPSC populations to a relatively uniform pool of cardiomyocytes, the size, shape, molecular profile, and physiological responses to pharmacological stimuli of these cardiomyocytes remain immature or fetal-like. For example, the hiPSC-derived cardiomyocytes (differentiated for 20–30 days) are spherical in shape, are small, have poor subcellular organization with variable Z-disc alignment (typically referred to as Z-bodies), and have contractile properties but no t-tubule formation.

The hiPSC-derived cardiomyocytes express a fetal molecular program, use glucose for metabolism, and have an embryonic calcium signaling mechanism. Furthermore, their response to adrenergic pharmacological stimuli (ie, 1 μmol/L isoproterenol) is limited (typically resulting in only a doubling of the beating frequency). In contrast, adult human cardiomyocytes are cylindrical, rod-shaped, a size of ≈100 μm in length by 25 μm in diameter, a mature molecular program with a high myofibril density, aligned Z-discs, and a mature t-tubule system. Although previous gene transfer strategies have been successfully used to promote differentiation, the impaired maturation of the hiPSC-derived cardiomyocytes has been an obstacle for physiological and pharmacological analyses.

Feaster et al present an innovative methodology that rapidly promotes the maturation of hiPSC-derived cardiomyocytes and allows the assessment of contractile performance at the single-cell level. hiPSCs were initially cultured using a conventional cardiac differentiation media, and then at approximately day 30 of cardiac differentiation, cells were dissociated and replated as single hiPSC-derived cardiomyocytes for 5 to 7 days on a thicker mattress of undiluted Matrigel and compared with those cultured on diluted Matrigel (1:60 dilution) or freshly isolated adult rabbit cardiomyocytes. These mattress-supported hiPSC cardiomyocytes assumed a rod shape and had increased sarcomeric length, and essentially all of the cardiomyocytes displayed spontaneous contractile activity compared with the smaller and circular-shaped control hiPSC cardiomyocytes (Figure). The contractile kinetics and the excitation–contraction coupling, including the intracellular calcium release from sarcoplasmic reticulum, were comparable between the mattress-supported hiPSC cardiomyocytes and freshly isolated adult rabbit cardiomyocytes.

In addition, the interrogation of the molecular program associated with the mattress-supported hiPSC cardiomyocytes revealed increased expression of cardiac troponin I (cTnI or TNNI3 [troponin type 3 (cardiac)]) and a corresponding decrease in the fetal isoform (ssTnI [troponin type 1 (skeletal, slow)] or TNNI1 [troponin I type 1 (skeletal muscle, slow)]) compared with the unsupported hiPSC cardiomyocytes. This stoichiometric reduction of ssTnI (TNNI1 gene) has previously been shown to correlate with maturation of the cardiomyocyte. Using this Matrigel mattress–supported preparation,
Excitation–contraction coupling was evaluated from single-cell cardiomyocytes in response to selected pharmacological agents. These pharmacological agents altered cardiomyocyte contractility by impacting changes in myofilament calcium sensitivity. Collectively, this methodology now allows single-cell hiPSC cardiomyocytes to undergo rapid physiological assessment of both calcium handling and contractility, which should be valuable for the development of high-throughput screening strategies of agents for drug discovery, disease modeling, and assessment of cardiotoxic agents.

**Future Initiatives and Impact**

As is evident with each advancement, new questions and challenges emerge from the research. The study by Feaster et al provides a major advance for the hiPSC cardiomyocyte field and clearly demonstrates an accelerated maturation of the cardiomyocyte using the Matrigel mattress (Figure). Future studies will need to compare the physiological (excitation–contraction coupling) and the molecular program of the Matrigel mattress–supported hiPSC cardiomyocytes with freshly isolated adult human cardiomyocytes. It will be important to examine whether the hiPSC cardiomyocytes ever achieve the status of a fully mature cardiomyocyte. If the Matrigel mattress does not support the single-cell preparation long term, then efforts will need to be focused on the identification of other extracellular matrices or patches. Moreover, comprehensive proteomic and molecular analyses of the mattress-supported hiPSC cardiomyocytes compared with freshly isolated adult human cardiomyocytes will allow investigators to determine the age of the cardiomyocytes and will prove to be a valuable discovery reagent for the field. Although the ability to interrogate single-cell hiPSC cardiomyocytes is an important advance for the field, further focus will be necessary to uniformly direct the differentiation to a specific lineage (i.e., atrial, nodal, ventricular cardiomyocytes, etc.), and this new information will further enhance the use of this model to decipher the mechanisms of disease modeling and drug discovery.

In summary, the translational field of cardiovascular research has been impacted with the discovery of hiPSC cardiomyocytes. With the release of the iconic film, The Godfather in 1972, the world was introduced to the concept of going to the mattresses. In the article by Feaster et al, the authors have gone to the mattresses both literally and figuratively by developing a new, readily available method to accelerate the maturation program to generate single, beating, and increasingly mature hiPSC-derived cardiomyocytes. This simple and inexpensive, yet, elegant technique will serve as a step toward un harnessing the power of hiPSC-derived cardiomyocytes and will facilitate the use of these cells as a potent tool to enhance our understanding of disease mechanisms and for drug discovery as a platform for new therapies.

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**Disclosures**

None.

**References**


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