iPSC-derived Cardiomyocytes

Introduction

Responsible for 17.3 million deaths annually, cardiovascular disease (CVD) is the leading cause of death worldwide[1]. Part of the high CVD mortality rate is due to the limited regeneration potential of heart muscle cells, or cardiomyocytes. As such, damaged cardiomyocytes cannot be readily replaced or repaired by the body. Cardiomyocytes that have suffered adverse events (like myocardial infarction) limit the overall function of the heart, which eventually succumbs to congestive heart failure[2]. With the number of annual deaths expected to surpass 23.6 million by 2030[3], biomedical researchers and healthcare providers are eager to rise to the challenge of preventing CVD onset and progression. To this end, induced pluripotent stem cell-based disease models and regenerative medicine approaches have shown great promise. Generated from mature cells that have been genetically reprogramed to a pluripotent stem cell state[4], induced pluripotent stem cells (iPSCs) can be readily expanded and induced to specialize or differentiate into cardiomyocytes in vitro. This capacity has made iPSCs advantageous in developing CVD models and novel treatments that allow for the study of early onset cardiovascular diseases and treatment of heart damage and dysfunction.

In an iPSC patient-specific disease model, patient cells are harvested and genetically reprogrammed to produce disease-specific iPSC lines. iPSC-derived cardiomyocytes (iPSC-CMs) are considered ideal for disease modeling and clinical applications as they possess a robust proliferation capacity that is ideal for scalability and may be readily employed where other pluripotent cells like embryonic stem cells are prohibited. Moreover, use of the patients' own cells in clinical applications can reduce the risk of immune rejection following transplantation as they are autologous. Herein is a brief overview of iPSC-CM differentiation, disease modeling, and potential clinical applications.

Methods

Differentiation and Characterization of iPSC-CMs

Newly established or purchased iPSC lines should be checked for pluripotency and karyotypic normalcy. The hallmarks of pluripotent cells include expression of particular genomic and proteomic markers (e.g., OCT4, SOX2, NANOG), high alkaline phosphatase activity, and the capacity to form benign tumors comprised of tissues from all three germ layers (for more information on hPSC pluripotency click here). Prior to cardiogenic induction, iPSCs are maintained in a pluripotent state—as pluripotent cells have the most efficient differentiation capacity. iPSCs are routinely maintained in a reliable pluripotency maintenance medium such as NutriStem® hPSC XF (Fig 1.) to sustain iPSC quality. When iPSCs are ready to differentiate, pluripotency maintenance medium is replaced by a basal medium (such as RPMI) supplemented with cardiac induction factors. Various cardiac induction factor formulas have been reported[5,7]. Cultures may be prepared for cardiogenesis in an 80-90% confluent monolayer configuration[5], by forced aggregation in a 96-well plate to form embryoid bodies (EBs)6, or resuspension and spontaneous formation of EBs in non-adherent culture conditions[7]. Successful cardiomyocyte differentiation should be measured by a combination of the following: presence of visible contractile clusters in culture, electrophysical recordings of cardiomyocyte contraction action potentials, immunofluorescent detection of cardiomyocyte-specific proteins (e.g., α-actinin and cardiac troponin), and/or PCR/qPCR measurement of cardiac marker (e.g., Tbx5 and α-myosin heavy chain) expression.

Figure 1: Induced pluripotent stem cells (iPSCs) are maintained in a reliable pluripotency maintenance medium like NutriStem® hPSC XF to maintain pluripotency in cultures.

Cardiac Disease Modeling Using iPSCs

Patient-derived iPSCs offer an advantage over traditional in vivo cardiac disease modeling as the reprogrammed cells incorporate the complex genetics associated with cardiac disease. In this way, mutations associated with cardiac disease may be translated to structural and functional aberrations observed in cardiomyocytes (Fig. 2). In vitro disease modeling allows for investigation of pathological mechanisms as well as evaluation of therapeutic options (e.g., pharmaceutical efficacy, etc.). Distinctions between healthy and diseased cardiomyocytes are typically made using cardiac functional assessments in addition to structural (e.g., organelle morphology, sarcomere structure) and molecular (e.g., aberrant signaling pathways) evaluations. To date, arrythmias[8], cardiomyopathies[9], cardiometabolic disorders[10], and multifaceted cardiac diseases[11] with yet-to-be-defined genetics have been modeled in vitro using iPSC-CMs.

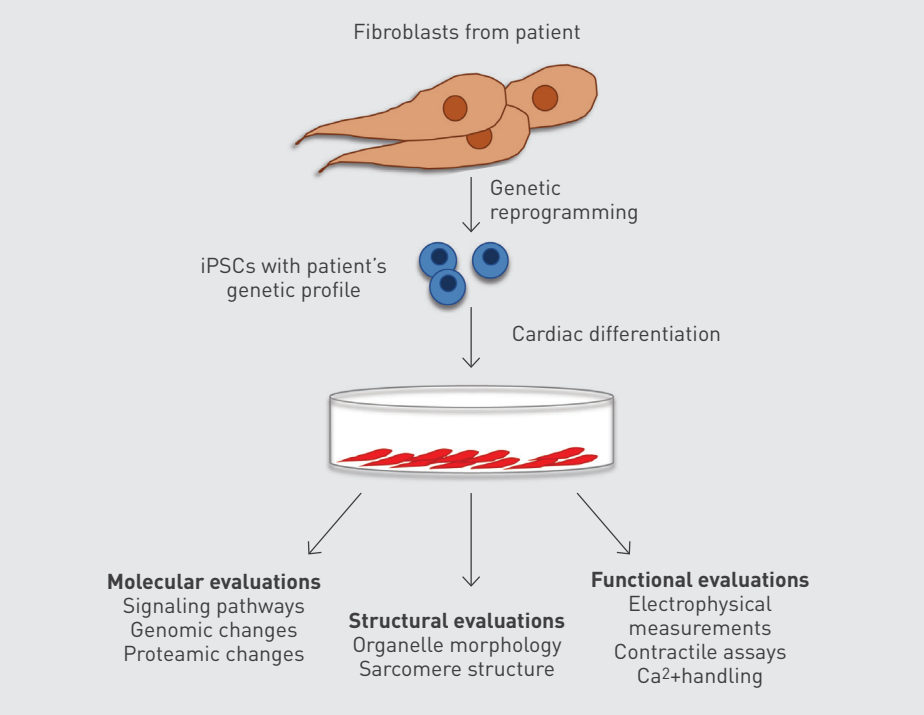

Figure 2: Cardiac disease models are achieved by genetically reprogramming a patient's cells to a pluripotent stem cell state. When exposed to the correct factors, these induced pluripotent stem cells (iPSCs) may be driven to differentiate into cardiomyocytes that possess the patient's genetic profile. This allows for in vitro disease modeling that may be further explored via molecular, structural and functional evaluations.

Cardiac Functional Tests

Electrophysiological Measurements

Electrophysiological measurements evaluate the electrical impulses that become irregular in diseased cardiomyocytes. Multielectrode arrays (MEA) measure voltage across a population of cardiomyocytes to determine duration of the electrical depolarization and repolarization cycles known as QT intervals[12]. The Patch clamp approach, on the other hand, monitor individual ion currents (e.g., INa, ICa) and may be useful in evaluations of ion channel function[13]. While both techniques have been successfully employed in iPSC-CMs, limitations of each approach should be considered in the context of study design and data evaluation.

Contractile Evaluation

One of the notable in vitro iPSC-CMs hallmarks is the active contraction of cardiomyocytes in the dish. Video microscopy and motion tracking software may be used to capitalize on this feature and quantify the beating frequency, amplitude, and kinetics of contractile cardiomyocyte clusters[14]. Contractile evaluations allow for measurement of abnormal contractile mechanics related to cardiac disease.

Calcium dependent Assays

Cyclical Ca^{2+} release from and reuptake by the sarcoplasmic reticulum drive the excitation-contraction coupling process are responsible for cardiomyocyte contraction. As such, calcium-sensitive Fluo-4 and Fura-2 dyes[15,16] may be used to detect the initial Ca^{2+} release events that ultimately produce contractile beating clusters.

Clinical Applications of iPSC-CMs

Unlike other organs, the heart possesses a reduced capacity for in situ tissue repair following myocardial infarction or other adverse cardiac events—which frequently mandates heart transplants in advanced cases of disease. Patients can wait over 6 months for a transplant[17], with some succumbing to heart disease before finding an appropriate donor match. iPSC-CMs have garnered great interest in clinical studies and the development of novel regenerative treatments as they possess the potential for in vitro expansion and, in early transplantation studies, appear to improve contractile function ([18]). Moreover, using the patients' cells to produce iPSCs and subsequent cardiomyocytes reduces the risk of immune rejection as the patient receives cardiomyocytes produced from his or her original cells. Once iPSCs have gone through the cardiomyocyte differentiation protocol, cells must be sorted to exclude under-differentiated cells to minimize the risk of forming a teratoma, or benign tumor, after transplantation. For this, immunomagnetic and fluorescent-activated cell sorting approaches may be used to enrich and select for cells that exhibit surface markers associated with heart tissue progenitor identity[19]. Early studies attempting both direct intramyocardial iPSC-CM injection and pre-seeded structure (e.g., biocompatible fibrin patches loaded with cardiomyocyte progenitors) transplants (Fig. 3) have found structural approaches to be more advantageous in terms of contractile function recovery following myocardial infarction[19].

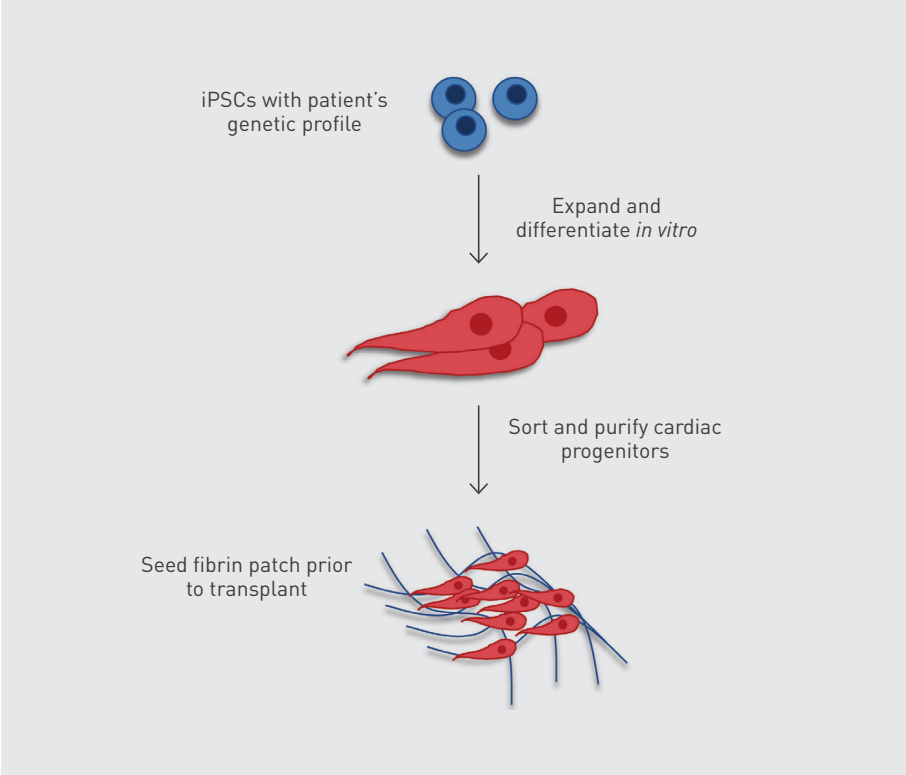

Figure 3: Cardiomyocytes generated from patient-derived induced pluripotent stem cells have been proposed as a possible treatment method for heart damage related to myocardial infarction. Pre-seeded biocompatible fibrin patches have demonstrated restoration of cardiac function following transplant.

Pre-clinically, iPSC-CMs have been identified for their usefulness in early drug discovery and efficacy trials[21]. iPSC-CMs can be used to screen potential treatments for cardiac diseases as well as for drug cardiotoxicity (Fig. 4). While iPSC-CMs may be generated "in-house", pre-seeded, pre-differentiated cardiomyocytes are commercially available to expedite early screen throughput[21].

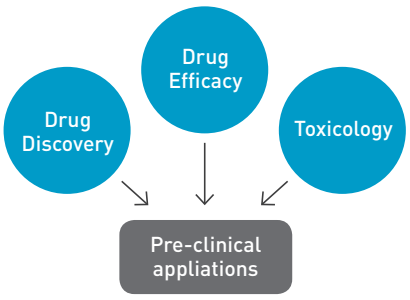

Figure 4: Induced pluripotent stem cell-derived cardiomyocytes have proposed uses in pre-clinical applications, including drug discover, drug efficacy, and toxicological evaluations.

Summary

Current treatments for cardiac disease are limited, and many treatments are only able to slow disease progression. iPSC-CMs offer the opportunity for in vitro cardiac disease modeling and development of clinical methods aimed to better understand disease onset and progression as well as to help develop novel treatment methods. Disease model evaluations rely on a combination of assays that evaluate cardiac function, molecular changes, and structural aberrations. Clinical applications capitalize on iPSC scalability and differentiation capacity to generate healthy cells for transplant. Differentiation outcomes depend on initial iPSC quality in both disease modeling and clinical applications. For best results, iPSCs are maintained in a reliable pluripotency maintenance medium like NutriStem® hPSC XF.

Citations

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