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The Current Status of iPS Cells in Cardiac Research and Their Potential for Tissue Engineering and Regenerative Medicine

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Abstract

The recent availability of human cardiomyocytes derived from induced pluripotent stem (iPS) cells opens new opportunities to build in vitro models of cardiac disease, screening for new drugs, and patient-specific cardiac therapy. Notably, the use of iPS cells enables studies in the wide pool of genotypes and phenotypes. We describe progress in reprogramming of induced pluripotent stem (iPS) cells towards the cardiac lineage/differentiation. The focus is on challenges of cardiac disease modeling using iPS cells and their potential to produce safe, effective and affordable therapies/applications with the emphasis of cardiac tissue engineering. We also discuss implications of human iPS cells to biological research and some of the future needs.

Keywords

Reprogramming; Pluripotency; Induced pluripotent stem cells; Cardiac differentiation; Patient-specific stem cells; Cardiac disease models; Cardiac tissue engineering; Regenerative medicine

Introduction

Stem cells are undifferentiated cells that not only have the capability for self-renewal and proliferation but are able to generate progeny that can differentiate into multiple cell types. Stem cells can be classified as totipotent, pluripotent, multipotent and unipotent based on their differentiation potential [1–5]. *Totipotency* is the capacity to form all cell lineages of an organism including extraembryonic tissues, trophoctoderm (e.g. placenta). It is characteristic of the zygote (fertilized egg) and can give rise to an entire, viable organism as well as to three germ layers: ectoderm, endoderm, and mesoderm. Only the fertilized oocyte and first stages of cell division, about the 4-cell stage blastomer [6] are considered totipotent.

Pluripotent stem cells derived from blastocysts, such as embryonic stem (ES) cells, are defined by their capacity for unlimited growth and potential to differentiate/develop into all cell types derived from the three germ layers, but not to a functional organism. ES cells have ability to self-renew through repeated mitotic divisions and to generate differentiated cells that constitute multiple tissues. Somatic cells are multipotent and have capacity for self-renewal that enables these cells to regenerate damaged tissues [7]. These cells are found in bone marrow, brain, liver, skeletal muscle, and dermal tissue [7].

Progress in Reprogramming Methods for the Generation of iPS Cells

In 1998, Thomson and colleagues [2] generated the first human embryonic stem (ES) cells derived from in vitro fertilized blastocysts. ES cells can form teratomas (tumors composed of tissues from the three embryonic germ layers) and they need to be differentiated into stable phenotypes before implantation. Other limitations include ethical controversies as these cells originate from human embryos, and immunocompatibility as these cells are by their nature not patient-specific.

In 2006, Takahashi and Yamanaka [8] showed for the first time that fully differentiated somatic cells (e.g. fibroblasts) derived from tissues of adult and fetal mice could be reprogrammed to make cells similar to ES cells. Their method is based on the introduction of four genes (Oct3/4, Sox2, Klf4, and c-Myc) expressing transcription factors through retroviral transduction. The resulting cells are called induced pluripotent stem (iPS) cells, and they show many properties of ES cells such as: they form teratomas when grafted into immunocompromised mice and embryoid bodies in vitro (aggregates of embryonic stem cells than can spontaneously differentiate). Just a year later, Yamanaka [9] and Thomson [10] independently demonstrated the derivation of human iPS cells. Human fibroblasts were reprogrammed into cells similar to ES cells by introducing combinations of four transcription factors (i.e. Oct4, Sox2, Nanog, and Lin28) [10]. Human iPS cells exhibited the crucial characteristics of human ES cells in morphology, proliferation and teratoma formation when injected into immunodeficient mice [8]. Specifically, they were positive for alkaline phosphatase, expressed ES cell surface markers and genes, show telomerase activity, had normal karyotypes, and maintained potential to form teratomas containing derivatives of all three germ layers [9, 10]. The progress from mouse to human iPS cells has opened the possibility of autologous regenerative medicine in which patient-specific pluripotent stem cells could be generated from adult somatic cells.

The methods for generating iPS cells can basically be divided into integrating and non-integrating, excisable and DNA free approaches (Table 1). Retrovirus and lentivirus delivery can cause reactivation of the viral vector, after transplantation, resulting in tumors and other abnormalities [39]. To establish safe iPS cells, several methodologies have been studied to avoid transgene insertions into the host genome.

Non-viral or non-integrating methods involve transient expression of reprogramming factors without genomic integration. Adenovirus or plasmid-mediated transfections can avoid the potential problems associated with viral integration of transgenes [25–27]. Yamanaka group [27] reported the generation of mouse iPS cells without viral vectors. Repeated transfection of two expression plasmids, one containing the complementary DNAs (cDNAs) of Oct3/4, Sox2, and Klf4 and the other containing the c-Myc cDNA, into mouse embryonic fibroblasts resulted in iPS cells without plasmid integration [27]. Hochedlinger et al. [25] created murine iPS cells from fibroblasts and liver cells by using non-integrating adenoviruses; afterwards the same result was achieved in human cells [26]. Also, Kaji et al. [29] used virus-free transfection of a single multiprotein expression vector for establishing reprogrammed human cell lines from embryonic fibroblasts.

Another strategy is to replace the genetic method with chemically defined approaches, such as protein or RNA transfection, growth factor or small-molecule treatments [40]. Moreover, some chemical compounds can improve the reprogramming efficiency and reduce the required number of transcription factors to generate iPS cells [40–42]. Several studies described reprogramming without using oncogenic factors [13, 14, 43–45] and instead by using chemicals such as valproic acid (VPA), BIX01294 (BIX) and 5'-azacytidine (5'-azaC) as substitutes for these factors [13, 43]. VPA is a histone deacetylase inhibitor; BIX and (5'-azaC) are DNA methyltransferase inhibitors [13, 43]. It has been demonstrated that VPA, BIX and (5'-azaC) can enhance the efficiency of iPS colony formation [13, 43].

Some reprogramming factors, namely c-Myc and Klf4 have known oncogenic activity and are often overexpressed in several types of cancer [46, 47]. iPS cells without insertion of c-Myc have been established but with a low efficiency [44, 48, 49]. Melton and co-workers [13] made human iPS cells by replacing two of the four genes with chemicals. However, it was reported that c-Myc itself was not essential for reprogramming [44, 45]. Nakagawa et al. [50] reported that L-Myc, used instead of c-Myc, increased the reprogramming efficiency in human cells, but no tumor formation, in iPS cells-derived chimeric mice. Hypoxia or supplementation of vitamin C has also been described to increase the reprogramming efficiency [51, 52]. Kim et al. [53] in a later study presented another attractive method to generate neural stem cells from somatic cells, with only one transcript factor, Oct4.

Furthermore, iPS cells can also be established by using sendavirus (an RNA virus without DNA state, that cannot be integrated into the host genome) [38] and removable transposon systems [29–31]. Mouse and human iPS cells were established by Zhou et al. [33] and Kim et al. [32] by the direct delivery of recombinant proteins [32, 33]. These results provided strong evidence that insertional mutagenesis was not required for in vitro reprogramming. This was an important step in making the technology safer for human use. More recently, multiple groups have independently reported that suppression of the tumor-suppressor gene

p53 markedly enhances iPS cells generation [54–56]. Synthetic mRNA may represent another way to replace integrating vectors and has been reported to reprogram human somatic cells into iPS cells with great efficiency [34]. Furthermore, some microRNAs, such as miR-302 and miR-372, have also been described to improve the efficiency of iPS cells [35–37].

It is imperative for human applications to obtain non-integrating human iPS cells using methods that are robust, reproducible, fast, and efficient and involve easy harvesting procedures for donor cells. The non-integration methodologies can be very useful for generating iPS cells for clinical applications.

Sources of Somatic Cells

Most iPS cells derived from human [12, 15, 16, 35, 38, 44, 57, 58] or murine [8, 14, 27, 35, 44, 53, 58, 59] origin. It has been shown that iPS cells can be generated from a variety of cell types, which differ in terms of mutational burden, relicative potential in vitro, availability, accessibility, and reprogramming efficiency. Successful reprogramming of terminally differentiated cells such as liver and stomach cells [17], mesenchymal stem cells [60], neural stem cells [14, 53], adipose-derived cells [61], umbilical cord [62], B lymphocytes [63], hepatocytes, pancreatic beta cells [64], human mobilized CD34+ cells from peripheral blood [16], among others.

Cardiac Differentiation from iPS Cells

Cardiovascular disease is the first cause of death and disability worldwide [65]. Heart failure, chronic ischemic heart disease and acute myocardial infarction are the three most frequent causes of death. So far, there is no definitive therapy for end-stage heart failure, other than heart transplantation. However, several drawbacks such as the scarcity of donor hearts and immunological rejection are associated with this approach [66]. A clear need for new therapies for cardiovascular disorders, and recent advances in stem cell biology, indicate that regenerative medicine and tissue engineering could play a major role in the future of cardiac research.

A deeper understanding of the biological activity of stem cells, and their ability to repair, regenerate and remodeling of the injured heart is essential for developing tissue-engineered modalities for heart regeneration.

Recent generation of iPS cells has created new expectations in the field of regenerative medicine. iPS cells hold great promise for cardiac research and therapeutic applications. Because the iPS cells are derived from the patient, there are no immunocompatibility problem, circumventing the important issue of tissue rejection associated with transplantation [67]. Also, these cells are derived from adult somatic cells (such as skin or blood) and thus do not raise ethical issues. At this time, iPS cells are likely the best candidates for heart regeneration therapies. iPS cells can be expanded in vitro, while maintaining their capacity to undergo differentiation towards endothelial cells, vascular smooth muscle cells and cardiomyocytes [68]. Testing these cells in cardiovascular injury models would help gain further understanding of their functional properties.

iPS colonies can be differentiated into functional cardiomyocytes using several methods, which are very similar to those used to generate cardiomyocytes from human ES cells. The three most frequently used methods to differentiate human pluripotent stem cells (ES cells and iPS cells) into cardiomyocytes include (please see review in [69]): (1) co-culture with mouse visceral endoderm-like (END-2) stromal cells [70–73], (2) spontaneous embryoid body (EB) differentiation in suspension [74–80], and (3) two-dimensional monolayer differentiation [81].

The first in vitro cardiac differentiation strategy uses signaling molecules secreted by END-2 stromal cells [70]. Usually, yield of cardiomyocytes using this strategy is low. However, co-culture with END-2 stromal cells has been used to successfully differentiate more than 10 human ES cells and human iPS cells into cardiac cells [70–72, 82, 83].

The second technique, EB differentiation in suspension, involves the culture of ES cells or iPS cells in the presence of differentiation signals (e.g. TGF- β , Wnts). Currently, this is the most common method of generating cardiomyocytes from iPS cells [76, 77, 84–87]. This technique results in the cells differentiating and forming 3-D aggregates named EBs. Using an EB formation assay, human ES/iPS cells can differentiate into beating cardiomyocytes in the presence of fetal bovine serum [88–90]. Under the serum-free conditions, with the supplementation of several cytokines, including Activin A and BMP4, the embryoid body can efficiently differentiate into cardiomyocytes [91]. Some protocols involve harvest of undifferentiated ES cells or iPS cells by manual dissociation or using collagenase [92, 93]. Other alternative involves the forced aggregation of ES cells or iPS cells dissociated to single cells [75]. With the “Spin EB” method, differentiation can be performed in a serum-free medium [94], allowing the cardiomyogenic effects of growth factors and small molecules to be systematically analyzed. The formation of human EBs with growth factor supplementation results in 23–60 % of EBs contracting [75, 91, 95] or suspension in END-2 conditioned medium results in ~12–70 % human EBs contracting [72, 95].

The third method, monolayer differentiation, allows the differentiation of pluripotent stem cells under fully defined conditions without involving the formation of EBs and derives specific mature cell types in large quantities. In a monolayer culture system of human ES cells and iPS cells, the directed differentiation into cardiomyocytes can be achieved by sequential treatment with activin A and BMP4 which yield >30 % cardiomyocytes [81].

To establish human-based cardiac cell therapy, efficient induction, purification, and transplantation methods for cardiomyocytes are required. High efficiencies of cardiomyocytes (approximately 30–80 %) have been described in the aforementioned studies. Current methods produce heterogeneous mixtures of cardiomyocytes and non-cardiomyocyte cells. A major limitation for cardiomyocyte purification is the lack of easy and specific cell marking techniques. However, several methods have been developed to derive highly purified populations of cardiomyocytes from human iPS cells [96, 97]. Hattori et al. [98] used a fluorescent dye, tetramethylrhodamine methyl ester perchlorate, to selectively mark human pluripotent stem cell-derived cardiomyocytes, and demonstrated that the cells could subsequently be enriched (>99 % purity) by fluorescence-activated cell sorting. Furthermore, Uosaki et al. demonstrated that purification of cardiomyocytes from

iPS cells by VCAM1 surface expression was almost no undifferentiated cell contamination after purification [96]. Also, reported an entirely novel system in which pluripotent stem cells-derived cardiomyocytes are purified by cardiomyocyte-specific molecular beacons [97].

In 2008, Mauritz et al. [99] demonstrated functional benefits of murine iPS-derived cardiomyocytes for cellular cardiomyoplasty and myocardial tissue engineering. The first human iPS-derived cardiomyocytes were generated by Zhang et al. [76]. These authors [76, 100] compared the cardiac differentiation potential of human iPS and ES cells, and found similar capacity for differentiation into cardiac cells with nodal-, atrial-, and ventricular-like phenotypes responsive to β -adrenergic stimulation. Later, iPS cells were differentiated into cardiovascular lineages [76, 101], and displayed properties similar to those of cardiomyocytes generated from ES cells [102, 103]. Also, Martinez-Fernandez et al. [48, 49] reported the robust cardiac differentiation potential of iPS cells without c-Myc.

iPS disease modeling requires high yields of phenotypically mature cardiomyocytes, through in vitro differentiation, into different subtypes (i.e. atrial, ventricular, or pacemaker cells). However, the efficiency of cardiac differentiation from iPS cells is still low for cardiac regenerative medicine applications. The cardiac differentiation efficiency from human pluripotent stem cells can be increased [58, 78], using screens for crucial signaling molecules in heart development. Signal proteins associated with cardiac development include canonical Wnt/ β -catenin [104–107], activin/nodal [108], FGF-2 [78] and BMP [78, 106, 109]. These signal proteins, together with small molecules (e.g. ascorbic acid [110], ITS [78] and cyclosporin-A [111]), have also been shown to increase the efficacy and quality of derived cardiomyocytes. A wide range of protocols is using these developmentally important signals for efficient and cardiac-specific differentiation of iPS cells [69].

Notwithstanding, some of the known barriers to efficient in vitro differentiation include incomplete reprogramming, the origin of somatic cell according to their epigenetic memory [112–114] and variability intrinsic to pluripotent cells [115]. Also, current protocols for generating cardiomyocytes from iPS cells tend to yield immature cells with fetal-like morphology [110], ion channel expression [116] and, electrophysiological function [117].

Although these results are promising, more studies are needed in both in vitro cell culture systems and translational animal models to gain insights into the potential and function of both human ES- and iPS-derived cardiovascular cells, and enable translation from experimental and pre-clinical studies to human clinical therapies.

Modeling Cardiac Disease Using Patient-Specific iPS Cells

A more immediate application of iPS cell technology that may have important impact on human health lies in the development of cellular models of disease that genetically match the patient [118]. This technology allows researchers to use human cells that reflect the genetic background of an individual in microassays that require minimal amounts of material. Pharmacological and toxicological testing using iPS cells could replace animal experiments, and help identify new therapeutic targets [119, 120]. Our understanding of human heart disease has been limited by lack of suitable models for studying human

cardiomyocyte behavior in vitro. Consequently, much of our knowledge of the cellular biology of heart disease has come from animal models that have inherent limitations and in many cases do not mimic human disease. Experimental mouse models (e.g. transgenic and/or knock out) are important for studying human disease but in many cases there are major differences in animal vs human physiology and gene function [121]. Mouse models do not always demonstrate the same phenotype as those observed in humans. For example, mice show a much higher rate of heart's beating (600 beats/min) and different action potentials in comparison to humans.

The use of iPS- derived cardiomyocytes is a unique method for studying human cellular mechanisms in vitro using cells from patients with cardiovascular disorders. iPS cells offer an exceptional opportunity and an unlimited cell source for creating disease-specific models, investigating underlying mechanisms, advanced studies on the pathophysiology of such diseases, and optimizing therapy. For regenerative medicine applications, using a person's own cells eliminates the risk of rejection and the need for immunosuppression drugs. iPS cells technology can generate cardiomyocytes that are genetically equivalent to the cells of transplant recipient. Patient-specific IPS cells would be a unique resource as therapeutic cell transplants if mature and functional cell derivatives were obtainable by in vitro differentiation.

In recent years, there is a significant increase in the number of publications about modeling cardiovascular disease in vitro using iPS cell technology [136–138] (Table 2). One of the first such reports described the generation of iPS cells from patients with several genetic diseases with complex inheritance patterns [139]. These disorders included adenosine deaminase deficiency-related severe combined immunodeficiency (ADA-SCID), Shwachman-Bodian-Diamond syndrome, Gaucher disease type III, Duchenne and Becker muscular dystrophy, Parkinson disease, Huntington disease, juvenile-onset, type 1 diabetes mellitus, Down syndrome/trisomy 21, and the carrier state of Lesch-Nyhan syndrome. Park et al. [139] proposed that disease-specific stem cells offer an unprecedented opportunity to recapitulate both normal and pathologic human tissue formation in vitro, allowing disease investigation and drug development.

One of the firsts studies reporting the use of iPS cell-derived cardiomyocytes from patients with hereditary cardiac disease presenting the phenotypic characteristics of the disease was published in 2010 by Carvajal-Vergara et al. [122]. The authors investigated patients with LEOPARD syndrome (Lentigines; electrocardiographic abnormalities; ocular hypertelorism; pulmonary valve stenosis; abnormal genitalia; retardation of growth; deafness) which is a rare multiple congenital anomalies condition, mainly characterized by skin, facial and cardiac anomalies [122, 140] with pleomorphic effects on several tissues and organ systems [122]. iPS cell-derived cardiomyocytes obtained from these patients showed increased surface area and a higher degree of sarcomeric organization as compared to cardiomyocytes derived from healthy individuals [122].

Inherited Cardiac Arrhythmias

Cardiac arrhythmias are a major cause of morbidity and mortality in developed countries. In younger patients most sudden cardiac deaths have an underlying Mendelian genetic cause.

Further studies were focused on congenital Long QT syndrome (LQT) [126, 141–143] a familial arrhythmogenic syndrome characterized by delayed repolarization, a prolonged QT interval in the electrocardiogram and a life-threatening polymorphic ventricular tachycardia known as “torsade de pointes” (TdP) and sudden cardiac death [84, 124]. Most of the arrhythmic events are precipitated by stress, emotion, or physical activity. Patients are usually treated with antiadrenergic therapy (β -blockers). Human iPS cell models have been described for the cardiac arrhythmia syndromes: LQT1, LQT2, LQT3/Brugada syndrome, LQT8/Timothy syndrome and cat-echolaminergic polymorphic ventricular tachycardia (CPVT).

Moretti et al. [84] were the first to report on human iPS-derived cardiomyocytes model for electrical disease, long-QT syndrome type 1 (LQT1). LQT1 is a repolarization disorder characterized by a prolongation of the QT interval on ECG due to mutations in the KCNQ1 gene. The authors obtained fibroblasts from two asymptomatic patients with the KCNQ1-G569A mutation and two healthy patients [84]. They have shown that patient-derived cells maintained the disease genotype and generated functional cardiomyocytes, and also recapitulated the electrophysiological characteristics of the disorder [84].

Itzhaki et al. [124] reported the development of a patient/disease-specific human iPS cell line from a patient with a diagnosis of LQT2 due to KCNH2-A614V mutation. The obtained iPS cells were differentiated into the cardiac lineage. Results revealed significant prolongation of the action-potential duration in LQTS human iPS cells-derived cardiomyocytes (the LQTS phenotype) when compared to healthy control cells. These cells were then used to evaluate the potency of existing and new therapeutic agents to prevent arrhythmias [124]. Later, Matsa et al. [125] demonstrated that cardiomyocytes derived from patient/disease-specific human iPS-derived cardiomyocytes of a symptomatic and asymptomatic patients with KVN2-G1681A mutation responded appropriately to clinically relevant pharmacology. Lahti et al. [73] described the cardiomyocytes differentiation from two iPS cell lines derived from an individual with LQT2 carrying the R176W mutation in the KCNH2 gene. The authors concluded that iPS cell-derived cardiomyocytes from an individual with LQT2 displayed the disease cardiac phenotype in cell culture conditions. Also, this model provided the means to explore the differences between clinical patients and mutation carriers and to scan the cardiac effects of different drugs sensitivity in LQT2 [73].

LQT3 and Brugada syndrome have been linked to mutations in SCN5A gene encoding the sodium (Na^+) channel. In the case of LQT3, these mutations cause an increased persistent Na^+ current which acts to prolong cardiomyocytes repolarization and increase of action potential (AP) duration [144]. For Brugada Syndrome, SCN5A mutations are associated with loss of channel function [144]. Davis et al. [83] generated iPS cell-derived cardiomyocyte model of a patient carrying the SCN5A-1795insD mutation. They concluded that these cells could serve as a model of the cardiac Na^+ channel overlap syndrome that evokes multiple cardiac rhythm disturbances. They also showed that human iPS cells can model loss-of-function Na^+ channel mutations [83].

LQT8/Timothy syndrome (TS) is a calcium channelopathy caused by a mutation in the gene encoding the L-type calcium $\text{Ca}_v1.2$ channel (CACNA1C) [145], a malignant multisystem

genetic disorder characterized by lethal arrhythmia, congenital heart defects, QT prolongation, ventricular tachycardia, syndactyly, and autism [128, 145]. Yazawa et al. [127] studied human iPS cell-derived cardiomyocytes of two patients with LQT8/TS. Electrophysiological recording and calcium imaging studies of these cells revealed irregular contraction, excess calcium influx, prolonged action potentials, irregular electrical activity and abnormal calcium transients in ventricular-like cells. The authors found that roscovitine, a compound that increases the voltage-dependent inactivation of $\text{Ca}_v1.2$, restored the electrical and calcium signaling properties of cardiomyocytes from LQT8/TS patients [127]. The same group published this year another study modeling LQT8/TS iPS cell-derived cardiomyocytes generated from patients [128]. The TS ventricular-like cardiomyocytes exhibited deficits in contraction, electrical signaling, and calcium handling, as demonstrated by live cell imaging and electrophysiological studies. The authors tested candidate drugs in TS cardiomyocytes and found that roscovitine could successfully rescue the cellular phenotype. Also, compared to iPS cell models of other LQTSs [84, 124], ventricular like cardiomyocytes derived from TS iPS cells showed a prolonged action potential (AP) and irregular APs that are similar to delayed after depolarizations (DADs). The results obtained in this study suggested that the duration of APs in iPS cell models of LQTS are consistent with the QT interval in patients, as TS patients demonstrated a longer QT interval than other LQTS patients [128].

Catecholaminergic bidirectional ventricular tachycardia (CPVT) is an uncommon form of arrhythmogenic disease occurring in children and adolescents with a structurally intact heart, manifesting ventricular premature beats and bidirectional or polymorphic ventricular tachycardia in response to emotional or physical stress that can lead to sudden cardiac death [146, 147]. It is a clinically and genetically heterogeneous disease caused by mutations in the cardiac ryanodine receptor type 2 gene (RYR2). Fatima et al. [129] established the first in vitro human iPS cell-based model of CPVT1 and demonstrated that iPS cell-derived cardiomyocytes with RYR2 mutation reflected the Ca^{2+} signalling phenotype of CPVT1 patients.

Friedreich's ataxia (FRDA) is an autosomal recessive disorder characterized by neurodegeneration and cardiomyopathy, generally late in childhood. Liu et al. [131] reported the generation of iPS cell lines derived from skin fibroblasts from two FRDA patients. Each of the patient-derived iPS (FA-iPS) cell lines maintained the gene expression characteristic of the patient. The authors [131] have shown that following in vitro differentiation the FA-iPS cells give rise to the two cell types primarily affected in FRDA, peripheral neurons and cardiomyocytes.

Arrhythmogenic right ventricular cardiomyopathy (ARVC) or arrhythmogenic right ventricular dysplasia (ARVD) is a rare inherited heart-muscle disease causing sudden death in young people and athletes [148]. This disorder is characterized by fibrous-fatty replacement of the right ventricle and the sub-epicardial region of the left ventricle, that could be detected on cardiac magnetic resonance imaging [87]. Clinical diagnosis can be established by demonstrating function and structure changes of the right ventricle, electrocardiogram depolarization and depolarization abnormalities, ventricular arrhythmias, and fibrofatty replacement through endomyocardial biopsy [148]. Significant differences

between the functional and electrofunctional properties of animal and human hearts limit the interpretation and applicability of comparing studies. Ma and colleagues [87] studied in vitro cellular model of ARVC using patient-specific cardiomyocytes, which could recapitulate key features of the disease phenotype. Cardiomyocytes derived from iPS cells of patients with ARVC displayed some key features of the disorder, namely cell surface localization of desmosomal proteins and a more adipogenic phenotype [87].

Glycogen Storage Diseases (GSDs)

Most lysosomal storage diseases (LSDs) or glycogen storage diseases (GSDs) are life threatening genetic diseases [149], and include the Pompe, Fabry, Danon and von Gierke (GSD type 1a) diseases. Most GSDs are caused by deficiency of a single lysosomal enzyme or protein, which leads to abnormal accumulation of substrate in cells and subsequent cellular dysfunction [149]. The paucity of mechanistic studies of GSDs is due largely to the absence of faithful in vitro disease model systems.

Pompe disease is an autosomal recessive disorder caused by mutations in the gene encoding the lysosomal glycogen-degrading enzyme, acid alpha-glucosidase [133]. Huang et al. [133] successfully generated Pompe disease-specific human iPS cells and showed that these cells possess characteristics similar to human ES cells and pluripotent developmental propensity. Furthermore, they demonstrated that iPS cells were able to give rise to cardiomyocytes that exhibit multiple pathophysiological phenotypes of Pompe disease in vitro.

Fabry disease is an X-linked lysosomal hydrolase α -galactosidase A (GLA) deficiency. It is associated with severe multisystem abnormalities including cardiac hypertrophy and stroke. Meng et al. [59] investigated the generation and characterization of iPS cells from mouse models of Fabry disease.

Danon disease (GSD type IIb) is an X-linked cardiomyopathy caused by mutations in the lysosomal associated membrane protein 2 (LAMP2). It is an inherited metabolic disorder of autophagy. In 2011, investigators successfully created the first in vitro human model of Danon disease. Furthermore, they confirmed the critical role of LAMP2 in the regulation of autophagy. This study highlights the utility of this in vitro system for elucidating the pathophysiology of inherited cardiovascular disease [135]. Other cardiac diseases, such as short-QT [10, 12] and Brugada syndrome [2, 46], might be studied in a similar fashion using patient-specific iPS cells.

Application of iPS Cells in Regenerative Medicine and Cardiac Tissue Engineering

Human cardiac disease models generated so far to test various drugs using cardiomyocytes derived from patient-specific iPS cells recapitulated at least some features of the disease phenotype [84, 124, 125]. The cardiomyocytes derived from patient-specific iPS cells enable “personalized medicine” approaches, through the use of the cells from a specific patient for determining the effects of a particular drug on the individual from which the cells were obtained. Human cardiac cells containing the genetic information of patients with cardiac disorders allowed studies of the cellular and molecular mechanisms of diseases in the cells

affected by the disease. These studies of patient-specific iPS cells show a remarkable potential for understanding cardiac pathologies and how can they be treated. However, to accurately model cardiac disease using IPS cells technology, it might be necessary to use tissue-engineering techniques.

The fields of stem cells, tissue engineering, and regenerative medicine are starting to realize how important the entire context of the cell environment is. Expectedly, tissue engineering is becoming increasingly oriented toward biologically inspired environments for directing stem cell differentiation for tissue regeneration [150]. Regenerative medicine relies today both on tissue engineering (fabrication of tissues using cells in conjunction with scaffold materials and bioreactors) and cell therapy (the transplantation or manipulation of cells in diseased tissue in vivo, [151], with an increasing focus on individualized tissue repair.

When myocardial infarction occurs, a significant loss of cardiomyocytes leads to a permanent reduction in contractile function, and can lead to heart failure. The heart cannot repair itself to sufficient extent by native processes. Instead, scar tissue develops over damaged myocardium, and the scar keeps the organ intact but with impaired contractile function. Clinical intervention should ideally avoid scar formation, or replace the scar with functional cardiac muscle, following a paradigm of “regenerative cardiology” [152, 153]. Several studies described cell injections into the beating myocardium that have lead to low retention rate (<10 %) in experimental animals [154–156] and intracoronary infusion in patients [157].

One current challenge is to derive phenotypically stable cardiac and vascular cell populations from human iPS cells in numbers sufficient for tissue engineering [158]. The purpose of tissue engineering is to create a viable environment through the use of biological 3D structures that form a functional interface with the host myocardium and mimic its structure and function, including normal cardiac conduction, vascularization, adequate mechanical properties, and porosity [159]. An “ideal” biomaterial should have high elasticity, high extensibility, high robustness and low Young’s modulus [160]. Various biopolymers, such as alginate [161], chitosan [162], gelatin [161, 163], collagen [162, 164], silk [165, 166], fibrin [167], poly(glycerol sebacate) (PGS) [168] have been employed on cardiac tissue engineering, also in conjunction with iPS cells [169, 170]. The group of Yamanaka [171] argued for the autologous approach to cardiac tissue engineering. In their opinion, autologous stem cells, which differentiate into cardiomyocytes, need to be used to enhance the contractile ability of the myocardium in situations where large numbers of cardiomyocytes were damaged [171].

Nelson *et al.* [172] reported that intramyocardial delivery of iPS cells into the infarcted hearts of immunocompetent mice significantly recovered cardiac function and that transplanted cells were successfully differentiated into cardiomyocytes, smooth muscle cells, and endothelial cells in the heart. Mauritz and colleagues [173] demonstrated that injection of fetal liver kinase-1 (Flk-1) into the ischemic myocardium in mice improved cardiac function following myocardial infarction, and that Flk-1 positive cells differentiated into cardiovascular lineages, in vitro and in vivo [173].

The use of an engineered tissue patch instead of simply injecting stem cells or their derivatives into the heart has been investigated for a long time, to alleviate low retention and survival of the cells injected into the myocardium or delivered by infusion [156]. One of the most advanced tissue engineering approaches has been established using cell sheet technique [174]. This method involves thermosensitive surfaces for cell culture that allow the detachment of cell monolayers [171, 175–177]. Recently, Okano's group described the first implantations of cell sheets into a patient with severe dilated cardiomyopathy [178]. Hibino et al. [179] evaluated the use of a sheet created from iPS cell-derived vascular cells as a potential source for the construction of a living tissue-engineered vascular graft (TEVG) for cardiovascular surgery. The differentiated iPS cell sheet was made using temperature-responsive dishes and then seeded onto a biodegradable scaffold composed of polyglycolic acid–poly-L-lactide and poly(L-lactide-co-ε-caprolactone) with a diameter of 0.8 mm. The scaffolds were implanted in the inferior vena cava for 1, 4 and 10 weeks. All mice survived without thrombosis, aneurysm formation, graft rupture, or calcification. Differentiated iPS cells provided an alternative cell source for constructing TEVGs using the sheet engineering technique. Tulloch et al. [180] also generated engineered heart tissues from human ES and iPS cells by using collagen [181, 182] and a commercially available FlexCell system.

A “heart in a dish” is still a dream, but repairing injured hearts with engineered myocardial tissue patches is a viable and increasingly realistic perspective in regenerative cardiology [183]. iPS cells represent a new breakthrough that may overcome some of the disadvantages of ES cells. However, the low efficiency and time-consuming protocols for iPS cells remains a huge obstacle for their application [184]. Cardiac tissue engineering is emerging as an enabling technology that can help utilize the enormous biological potential of iPS cells for cardiac regeneration and repair.

Conclusions and Future Directions

Two main trends have arisen in recent years, after the advent of iPS cells that invigorated research in the field: the opportunity of personalized stem cell therapies using human iPS cells, and the creation of in vitro models of human disease. The generation of iPS cells from human somatic cells results in patient-specific stem cell lines carrying the genotype of the patients, and providing opportunities to develop new therapeutic modalities [7, 185].

Patient/disease-specific human iPS cells represent a promising paradigm to study disease mechanisms, optimize patient care (personalized medicine), and aid in the development of new therapies. Combining tissue engineering with protocols for generating cardiomyocytes from iPS cells holds promise for entering into a new era of cardiac regenerative medicine. Still, many challenges remain before we can effectively translate the promising experimental results into curative treatment modalities for cardiac disease.

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Table 1

Reprogramming strategies to generate iPS cells [adapted from [11]]

Vector type	Reprogramming method	Source of somatic cells	Efficiency (%)	Advantages	Limitations	References
Integrating	Retroviral transduction	Fibroblasts, Neural stem cells, keratinocytes, Blood cells, Hepatocytes, Stomach cells, Adipose cells	~0.001–1	Reasonable efficiency	Genomic integration Slow kinetics Incomplete proviral silencing	[8, 9, 12–18]
Non-integrating	Lentivirus transduction	Fibroblasts, pancreatic beta-cells, and keratinocytes	~0.1–1.1	Reasonable efficiency	Genomic integration	[19–24]
	Adenoviral transfection	Fibroblasts Liver cells	~0.001	Reasonable efficiency No genomic integration	Low efficiency	[25, 26]
Excisable	Plasmid transfection	Hepatocytes Fibroblasts	~0.001	Minimize the potential for insertion mutagenesis	Diminished reprogramming efficiencies	[27, 28]
	Transposons	Fibroblasts	~0.1	Reasonable efficiency No genomic integration	Low efficiency	[29–31]
DNA free	Proteins-based methods	Fibroblasts	~0.001	No genomic integration Direct delivery of transcription factors No DNA-related complications	Extremely low reprogramming efficiencies Short half-life Need of large quantities of pure proteins and multiple applications of them	[32, 33]
	Synthetic mRNAs	Fibroblasts Keratinocytes	~2	No genomic integration High efficiency	Low efficiency	[34]
	MicroRNAs	Adipose stromal cells, Dermal fibroblasts	~1–4.4	No genomic integration High efficiency Faster reprogramming kinetics compared with lentiviral or retroviral vectors	Requires multiple rounds of transfection	[35–37]
	Sendai virus (SeV)	Human fibroblasts	~0.1	No risk of altering host genome	Sequence-sensitive RNA replicase, and difficulty in purging cells of replicating virus	[38]

Table 2

Cardiac disease modeling

Disease	Clinical phenotype	Source of somatic cell	Reprogramming method	Reference
LEOPARD syndrome	Electrocardiographic conduction Abnormalities pulmonary stenosis Cardiac hypertrophy Hypertrophic cardiomyopathy	Fibroblasts	Retrovirus	[122, 123]
LQT1, LQT2, LQT3 and Brugada Syndrome	Prolongation of the QT interval Ventricular arrhythmia Ventricular fibrillation Abnormal ion channel function	Fibroblasts	Retrovirus Lentivirus	[73, 79, 83, 84, 124–126]
LQT8/Tyothy Syndrome	Congenital heart defects QT prolongation Lethal arrhythmia Ventricular tachycardia Autism	Skin fibroblasts	Retrovirus	[127, 128]
Catecholaminergic polymorphic ventricular tachycardia (CPVT)	Ventricular arrhythmia Bidirectional or polymorphic ventricular tachycardia	Dermal fibroblasts	Retrovirus	[129, 130]
Supravalvular aortic stenosis syndrome	Abnormal proliferation of vascular smooth muscle cells Narrowing or blockage of the ascending aorta and other arterial vessels	Human foreskin fibroblasts	Polycistronic lentiviral vector	[57]
Dilated cardiomyopathy	Ventricular dilatation Systolic dysfunction Progressive heart failure	Skin fibroblasts	Lentivirus	[86]
Friedreich ataxia (FRDA).	Ataxia, loss of coordination, abnormal speech, heart disease, muscle weakness, diabetes, scoliosis, hearing and vision loss	Skin fibroblasts	Retrovirus	[131, 132]
Arrhythmogenic right ventricular cardiomyopathy	Ventricular arrhythmias	Dermal fibroblasts	Retrovirus	[87]
Pompe disease	Hypertrophic cardiomyopathy Arrhythmia Muscle weakness	Dermal fibroblasts	Retrovirus	[133, 134]
Fabry disease	Cardiac hypertrophy and stroke Arrhythmia, neuropathy	Mouse tail-tip fibroblasts	Retrovirus	[59]
Danon disease	Cardiomyopathy Muscle weakness	Dermal fibroblasts	Retrovirus	[135]