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STATE-OF-THE-ART REVIEW

# The Promise and Challenge of Induced Pluripotent Stem Cells for Cardiovascular Applications

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## SUMMARY

The recent discovery of human-induced pluripotent stem cells (iPSC) has revolutionized the field of stem cells. iPSC have demonstrated that biological development is not an irreversible process and that mature adult somatic cells can be induced to become pluripotent. This breakthrough is projected to advance our current understanding of many disease processes and revolutionize the approach to effective therapeutics. Despite the great promise of iPSC, many translational challenges still remain. The authors review the basic concept of induction of pluripotency as a novel approach to understand cardiac regeneration, cardiovascular disease modeling, and drug discovery. They critically reflect on the current results of pre-clinical and clinical studies using iPSC for these applications with appropriate emphasis on the challenges facing clinical translation. (J Am Coll Cardiol Basic Trans Science 2016;1:510–23) © 2016 The Authors. Published by Elsevier on behalf of the American College of Cardiology Foundation. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

## ORIGIN OF THE INDUCED PLURIPOTENT STEM CELLS

The totipotency of a fertilized egg confers a unique ability to divide and differentiate into all tissue types to form an entire organism. To identify the exact role of a mammalian cell nucleus undergoing embryonic development, somatic cell nuclear transfer experiments were conducted in frogs in the 1950s (1). These experiments offered a proof of principle that pluripotency can be conferred to somatic cells by transferring their nuclear contents into oocytes (2). In fact, the nuclei of differentiated mammalian cells possess the ability to become pluripotent upon transfer of the nucleus into an oocyte or fusion with embryonic stem cells (ESC) (3,4). For many years, the major challenge was to reprogram the somatic cell nucleus without transferring its contents or using an oocyte (5). In 2006, Takahashi and Yamanaka (6) studied the effects of 24 transcription factors, which were known to confer pluripotency to early embryos and ESC. They succeeded in transforming the adult mouse fibroblasts into induced pluripotent stem cells (iPSC) using 4 select transcription factors: Oct4, Sox2, c-Myc, and Klf4 (6,7). These iPSC exhibited characteristics very similar to ESC. One year later, the same group

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generated human iPSC from human dermal fibroblasts using retroviral transduction with the same 4 transcription factors (8). These iPSC have morphology, cell surface markers, and genes characteristics similar to human ESC, exhibiting unlimited replication potential without telomere shortening or karyotype changes. The multilineage differentiation potential of iPSC has been confirmed both in vitro in embryoid bodies and in vivo based upon teratoma formation upon injection into severe combined immunodeficient (SCID) mice. Other groups reproduced the "stemness" or induced pluripotency of somatic cells using the same or slightly different transcription factors, demonstrating the robustness of this technique and revolutionizing the field of stem cell biology (9–11).

Since the ability to generate iPSC in culture from adult human skin fibroblasts has been established (8,9), this pluripotent state has been induced in a variety of human cells including keratinocytes (12), T-lymphocytes (13), peripheral mononuclear blood cells (14), cord blood (15), placenta (16), neural stem cells (17), adipose tissue (18), and renal epithelial cells present in urine samples (19). Ectopic expression of different combinations of reprogramming factors has been used, with Oct4 being the most consistent across protocols. Furthermore, several protocols have been developed for directed in vitro differentiation of iPSC into spontaneously contractile cardiomyocytes, smooth muscle cells, and vascular endothelial cells (20,21). The differentiation of iPSC into cardiomyocytes was confirmed by microscopic examination of beating colonies, immunostaining for cardiac proteins, electrophysiological testing, and real-time polymerase chain reaction analysis of cardiac markers (20,21) (**Figures 1A to 1C**). The iPSC overcame the ethical concerns and immunogenicity of human ESC, thus becoming an attractive alternative for autologous tissue repair and regeneration as well as a source for allogeneic transplantation. The therapeutic potential of iPSC-derived cells has been tested in many pre-clinical studies with some encouraging results in murine and porcine models of myocardial infarction (MI) (16,22–25). Currently, the first human trials of iPSC-derived cells, aimed at establishing the safety of these cells, are enrolling patients with age-related macular degeneration in Japan (26).

Beyond the potential for regenerative or transplantation therapies, iPSC offer an unprecedented opportunity to recapitulate both normal and pathologic human tissue formation in vitro, thereby providing novel cell-based biological models that enable better understanding of disease pathogenesis and drug discovery (27). From 2008 to 2015, more than 70 human iPSC-based disease models have been published in an exponential fashion (28). The ability to examine the direct effects and toxicity of new drugs on the patients' own cells could also represent an invaluable tool for drug development and discovery. For these reasons, both Sir John Gurdon, who performed nuclear transfer experiments, and Shinya Yamanaka were awarded the Nobel Prize in Physiology or Medicine in 2012. This review discusses the current status of the cardiovascular applications of iPSC for cardiac and vascular regeneration, disease modeling, and drug discoveries with a special emphasis on the current challenges for clinical translation (**Figure 1**).

**ABBREVIATIONS AND ACRONYMS**

**ESC** = embryonic stem cell(s)

**iCM** = induced pluripotent stem cell-derived cardiomyocyte(s)

**iEC** = induced pluripotent stem cell-derived endothelial cell(s)

**iPSC** = induced pluripotent stem cell(s)

**MI** = myocardial infarction

**SCID** = severe combined immunodeficient

## iPSC FOR CARDIOVASCULAR REGENERATION

With the growing epidemic of heart failure, cardiac regeneration represents a major priority of regenerative medicine. The ability of iPSC to differentiate into autologous tissue-specific cells, similar to ESC but without the need to destroy a human embryo, is an important breakthrough in human stem cell biology. A number of pre-clinical studies have explored the effects of intramyocardial injection of induced pluripotent stem cell-derived cardiomyocytes (iCM) into murine and porcine models of MI (a complete recent list of pre-clinical studies is provided in Lalit et al. [29]). Nelson et al. (30) were the first to perform intramyocardial injection of iPSC-derived cells into a murine model of acute MI. They found improved left ventricular ejection fraction, fractional shortening, and regional wall motion on echocardiography 4 weeks after permanent coronary artery ligation when compared with the fibroblast-injected animals. Interestingly, they reported teratoma formation upon subcutaneous and intramyocardial injections of iPSC in immunodeficient animals but none in immunocompetent mice (30).

Other studies have reported formation of intramural teratomas in both immunocompetent and SCID mice after intramyocardial injection of undifferentiated iPSC (31,32). Kim et al. (32) tracked the engraftment of iPSC at 4 weeks in a SCID murine model of MI. They demonstrated teratoma formation with no myocardial or endothelial cell differentiation despite the reported improvement in myocardial function and myocardial viability (32). This demonstrates a potential mechanistic role of cytokines released from the iPSC, which may restore cardiac function despite teratoma formation. Similar results were obtained with undifferentiated iPSC in a rat model of MI (33), which is consistent with the initial studies of