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Harnessing cell pluripotency for cardiovascular regenerative medicine

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Abstract

Human pluripotent stem cells (hPSCs), in particular embryonic stem cells and induced pluripotent stem cells, have received enormous attention in cardiovascular regenerative medicine, owing to their ability to expand and differentiate into functional cardiomyocytes and other cardiovascular cell types. Despite the potential applications of hPSCs for tissue regeneration in patients suffering from cardiovascular disease, whether hPSC-based therapies can be safe and efficacious remains inconclusive, with strong evidence from clinical trials lacking. Critical factors limiting therapeutic efficacy are the degree of maturity and purity of the hPSC-derived differentiated progeny, and the tumourigenic risk associated with residual undifferentiated cells. In this Review, we discuss recent advances in cardiac-cell differentiation from hPSCs and in the direct reprogramming of non-myocyte cells for cardiovascular regenerative applications. We also discuss approaches for the delivery of cells to diseased tissue, and how such advances are contributing to progress in cardiac tissue engineering for tackling heart disease.

Pluripotency is defined as the capacity of cells to differentiate into multiple mature lineages, such as cardiomyocytes, hepatocytes, neurons or blood cells. When considering their sources, hPSCs — which have the ability to form all adult cell types — can be divided in two groups: human embryonic stem cells (hESCs), derived from the embryo's inner cell mass at the blastocyst stage after *in vitro* fertilization or somatic nuclear transfer; and human induced pluripotent stem cells (hiPSCs), generated via the ectopic expression of defined transcription factors[1,2]. Both hESCs and hiPSCs have unlimited self-renewal potential and therefore represent potentially unrestricted cell sources for the production of tissues for

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Author Contributions

H.C. and J.C.W. conceptualized the outline and contents of the article. H.C. and A.Z. participated in the researching and writing for the article and J.C.W. reviewed and edited the manuscript before submission.

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regenerative medicine[3]. However, their high capacity for self-renewal comes with safety concerns, owing to the possible formation of teratomas and to the instability of their genetic material[4]. A major challenge of stem cell therapy is to eliminate these risks in preclinical studies before the initiation of clinical trials.

The successful application of hPSCs as clinical therapy requires control over the maintenance of their pluripotent phenotype and their *bona fide* differentiation into terminal lineages. hPSCs can retain their pluripotency and self-renewal capacity when cultured in maintenance media containing self-renewal factors. Withdrawal of these factors then triggers the spontaneous differentiation of hPSCs into a mixture of cells representing all three germ layers. Directed differentiation can thus be evoked when pluripotency maintenance media are replaced with differentiating media supplemented with factors that activate or inhibit particular intracellular signalling pathways. During this process, cells lose their pluripotency and self-renewal capacity, and acquire cell-type-specific functions and markers. Different factors can be added into differentiation media to mimic the embryonic developmental signalling to obtain specific cell types[5].

The mammalian heart consists of many cell types, including atrial and ventricular cardiomyocytes, cardiac fibroblasts, endothelial cells, smooth muscle cells, and cells forming the conduction system such as pacemaker cells and Purkinje fibres. Given that cell death is a major aspect in tissue damage during injury and heart failure, cellular replacement using hPSC-derived cardiac cells is a promising strategy for stem-cell-based regenerative therapy. In this Review, we discuss the use of hPSCs and their progeny for the repair of the cardiovascular system, and summarize the in vitro cardiac-lineage differentiation protocols developed to date. We also discuss the direct reprogramming of non-myocytes into cardiomyocytes or cardiac progenitors (Fig. 1).

hPSC-derived cardiomyocytes for cardiac repair

One of the most studied cell types are hPSC-derived cardiomyocytes (hPSC-CMs), which have shown promising results in multiple studies for improving cardiac function in animal models[6–10]. Several key steps are involved in the preparation of hPSC-CMs for transplantation, including cardiomyocyte differentiation, purification and maturation. The efficiency of cardiomyocyte differentiation has improved dramatically over the past decade, rising from 1–10% efficiency using the embryoid-body formation protocol[11] to >90% using the monolayer protocol combined with metabolic purification to remove undifferentiated cells[12,13]. Most cardiomyocyte-differentiation protocols involve the modulation of signalling pathways involving Wnt, Activin A or bone morphogenetic protein-4 (BMP-4) in order to recall early cardiac development[5]. Although the differentiation and purification of hPSC-CMs has been progressively optimized, successful and efficient maturation has thus far eluded the field. hPSC-CMs show immature phenotypes, as measured by low sarcomere alignment, small action potentials in electrophysiology assessments, and fetal-like transcriptome profiles. Importantly, hPSC-CMs can exhibit heterogeneous electrophysiological phenotypes and spontaneous automaticity, carrying a risk of life-threatening arrhythmias when transplanted[8,14].

The challenges of controlling hPSC-CM maturation have been tackled with a variety of approaches, including targeting internal pathways, recreating the optimal environment, and providing proper physical stimulation (Box 1). To investigate differences between mature and immature hPSC-CMs, hESC-CMs have been cultured for over a year and have been found to have let-7 as the most highly upregulated microRNA. Consequently, overexpression of let-7 family members in hESC-CMs enhanced cell size, sarcomere length, the force of contraction, and respiratory capacity[15]. Additionally, for maturation of hPSC-CMs, metabolic approaches have been used to increase the use of fatty acids as the energy source. On the basis of metabolic differences between hPSC-CMs and primary cardiomyocytes, insulin, dexamethasone and 3-isobutyl-1-methylxanthine were found to drive metabolic maturation via the increase of mitochondrial oxidation, lipogenesis and lipolysis[16]. Nevertheless, these approaches only target single phenotypic features and cannot generate cells that are as mature as adult human cardiomyocytes.

hPSC-CM differentiation has also been achieved via specific environmental cues. For example, to replicate the environment of the naive myocardium, hPSC-CM maturity has been boosted by injecting cells into fetal animal hearts so as to provide the microenvironment for cardiomyocyte maturation[17,18]. Although increased sarcomere alignment was observed, the low throughput of the approach and the use of model animals as a cell source limit the future application of this approach.

Approaches relying on a scalable biopolymer-based microenvironment to enhance maturation have shown some potential. The ideal matrix stiffness for promoting cardiomyocyte maturation has been studied extensively, and is thought to be ~10 kPa. The matrix can incorporate patterns or elongated folds for optimal cell differentiation. For instance, hPSC-CMs have been cultured on 10 kPa polyacrylamide substrates patterned with Matrigel in 2,000 μm^2 rectangles, with aspect ratios between 5:1 and 7:1, using mechanical output and myofibril alignment as the measurement of maturity[19]; in this study, the mechanical output was highest in 7:1 hPSC-CMs. On the other hand, another study in which action potentials were used to evaluate cardiomyocyte maturity revealed that cardiomyocytes had the longest action potential when cultured on 9 kPa hydrogel[20].

In the quest for developing an optimal microenvironment for hPSC-CM differentiation, an on-going challenge is to determine the optimal permutation of parameters, such as type of material, its stiffness, matrix patterns and dimensions, and its co-culture with other cell types. To that end, physical cues have been used to stimulate maturation, for example with the use of gold nanowires to promote cardiomyocyte maturation, which increased electrical signal propagation across the cells and improved electrical synchronicity[21]. Others have found that transversal, shearing, or stretch stimuli can induce the proper maturation cues[22].

Despite these advances, the in vivo therapeutic use of hPSC-CMs requires a degree of maturity that is different from those encountered in in vitro disease modelling and drug screening. To date, the immaturity of hPSC-CMs is mainly defined by the comparison between adult cardiomyocytes and hPSC-CMs with respect to sarcomere organization, sarcoplasmic reticulum function, metabolism, energy handling, ion-channel density and conduction, calcium kinetics, and gene expression (reviewed in ref. [23]). Whereas immature

hPSC-CMs have been transplanted and engrafted in animal hearts, the survival of primary adult cardiomyocytes after transplantation is poor[24]. Mature hPSC-CMs may therefore be more sensitive to ischaemic stress, and have little or no capacity for cell regeneration, leading to poor survival. Additional studies are required to find the optimal balance between cell maturity and therapeutic efficacy (Fig. 2).

Cardiac repair with hPSC-derived non-myocyte cells

The survival of cardiomyocytes transplanted into heart tissue depends on other cell types that provide the necessary local support, including the supply of oxygen and extracellular matrix. In addition to hPSC-CMs, non-myocyte cells also have the therapeutic potential to repair a damaged heart.

Endothelial cells

Endothelial cells make up the interior surface of blood vessels, and are required for angiogenesis and vasculogenesis. As with cardiomyocyte differentiation, endothelial cells can be differentiated from hPSCs (hPSC-ECs) through the modulation of Activin/Nodal or Wnt pathways. The first hESC-EC generated from embryoid bodies involved spontaneous differentiation of hESCs in the presence of serum, followed by cell sorting with platelet endothelial cell-adhesion molecule-1 antibodies[25]. Improved protocols in the presence of different growth factors and small molecules[26–28] have been inspired by this approach. More recently, monolayer protocols in which hPSCs are grown and differentiated on surfaces coated with Matrigel followed by antibody purification with one or more endothelial markers (such as CD31 and CD144) have been reported[29–33].

The transplantation of hPSC-ECs can revascularize tissues and promote vascular regeneration[34,35]. In this context, hESC-ECs have been used to treat myocardial infarction in a rodent model, where the transplanted endothelial cells formed functional vessels and improved cardiac function[36]. Also, by incorporating hiPSC-ECs and hiPSC-CMs into engineered cardiac tissue sheets neovascularization and engraftment survival four weeks after transplantation could be increased[37]. Furthermore, in a porcine model of acute myocardial infarction, the engraftment of tri-lineage cardiac patches with hiPSC-CMs, hiPSC-ECs, and hiPSC-derived smooth muscle cells in combination with IGF-1–fibrin led to improvements in cardiac function without evidence of arrhythmias[38]. Similar to hPSC-CMs, hPSC-ECs purified by selecting for endothelial cell markers exhibited functional heterogeneity and displayed arterial, venous and lymphatic subtype markers[39]. Further refinements in protocols for subtype-specific hPSC-EC differentiation are expected.

Vascular smooth muscle cells

Vascular smooth muscle cells (VSMCs) are another major component of blood vessels, with both contractile and factor-release functions. The transplantation of smooth muscle cells into scar tissue can improve heart function[40]. To differentiate VSMCs, hPSCs are directed towards the mesoderm lineage by modulation of the Activin/Nodal pathway, followed by cell sorting to purify the cells of interest. The co-transplantation of hPSC-derived vascular smooth muscle cells with other cells can enhance the formation of blood vessels[41,42].

Cardiac progenitor cells

Cardiac progenitor cells (CPCs) are a group of resident cells in the heart that can proliferate and differentiate into mature cardiomyocytes following cardiac injury. CPCs are not yet well understood, and surface markers are often used to define them. Although terminally differentiated cell types such as cardiomyocytes and endothelial cells have been tested for their efficacy at heart repair in preclinical models, it is debatable whether progenitor cells that are tissue-committed yet still multipotent would be more suitable for regeneration, owing to their potential to expand and differentiate into multiple cardiac cell types after transplantation. The differentiation protocol for generating hPSC-derived cardiac progenitor cells relies on embryoid-body formation and fluorescence-activated cell sorting (FACS)-based selection of CPC surface markers such as Flk-1 (KDR in humans)[43,44], CD13/ROR2 (ref. [45]) and SSEA-1 (ref. [46]). Although hPSC-CPCs possess the ability to generate vascularized myocardium grafts after transplantation, their tumorigenic risk must be evaluated thoroughly, owing to the possibility of hPSC contamination and cell dedifferentiation.

Endothelial progenitor cells

Endothelial progenitor cells (EPCs) are circulating cells that participate in the formation of new blood vessels by adhering to endothelial cells at sites of hypoxic or ischaemic injury. As with CPCs, how EPCs should be defined remains unresolved. EPCs have been defined on the basis of the cell-surface markers CD31, CD34 and KDR (refs. [47,48]), whereas others have defined EPCs on the basis of their colony-forming capacity[47,49]. Protocols generating EPCs from hPSCs rely on embryoid-body formation[50,51] or on a combination of factors that include Activin A, BMP-4, FGF-2 and VEGF (ref. [52]).

Epicardial cells

The epicardium, the outer layer of the heart, has the ability to form epicardial-derived cells such as cardiac fibroblasts, cardiac muscle cells, coronary smooth muscle cells and vascular endothelial cells. Generation of hPSC-derived epicardial cells (EPIs) involves the temporal modulation of the BMP and WNT signalling pathways[53–55]. Treatment with TGF-β inhibitors enables the long-term culture of hPSC-EPIs without the spontaneous epithelial–mesenchymal transition[53] occurring. Epicardial cells have the ability to differentiate into smooth muscle cells, fibroblasts and endothelial cells[56], and can protect the heart following myocardial injury[57,58]. Interestingly, epicardial Hippo signalling plays a key role in the immunosuppressive response following myocardial infarction[59]‘; yet it remains unclear whether hPSC-EPIs retain these properties in a therapeutic setting.

Benefits and barriers of combinatorial stem cell therapy

The idea that cardiac stem cell therapy may benefit from using multiple cell types is based on the following hypotheses[60]: (i) no single cell type can regenerate all cardiac cells *in vivo*; (ii) both cardiomyocytes and non-cardiomyocyte cells are required to repair the damaged heart; and (iii) synergistic effects from the interaction of multiple cell types are known to improve implant survival (Fig. 3). These hypotheses, especially the third one, need to be tested in preclinical studies.

In particular, a side-by-side comparison is needed to determine the optimal combination of cell types. The comparison of different cell types for cardiac repair is difficult because of the different models and procedures required for their generation. A comparison among hPSC-CMs, hPSC-CPCs and bone marrow mononuclear cells[61] showed that injecting a total of 10×10^6 hPSC-CMs and hPSC-CPCs into rat hearts improved cardiac contractility, whereas bone-marrow-derived mononuclear cells enhanced host vascularization without improving contractility. Additional experiments will thus be needed to test whether the delivery of cell types with complementary roles may lead to improved effects.

Moreover, the optimal stoichiometry and delivery approach of implanted cells still need experimental validation. When using three cell types (hPSC-CMs, hPSC-ECs and hPSC-SMCs) in a 1:1:1 ratio for transplantation in a fibrin patch loaded with insulin growth factors[38], 27.1% ± 5% of surviving transplanted cells were hiPSC-CMs, 34.2% ± 10% were hiPSC-ECs, and 40.5% ± 1% were hiPSC-SMCs at 4 weeks after injury, resulting in a ratio of 0.67:0.84:1. When the same three types of cells were used in a ratio of 2:1:1 (ref. [62]), the ratio became 0.74:0.52:1 (CMs:ECs:SMCs) at week 1 post transplantation, and 0.78:1.16:1 at week 4. This ratiometric change is likely to be due to different cell-replication rates and to tolerance to ischaemic stress; in both cases, hPSC-CMs suffered the most from cell loss. More work is needed to evaluate the effect of cell ratio on therapy efficacy.

Cardiac repair with direct reprogramming

hPSC-derived cells may have a high risk of tumorigenicity, owing to possible contamination of the transplanted suspension with residual undifferentiated cells. Therefore, the possibility of directly reprogramming non-myocyte cells to differentiate into cardiomyocytes is being explored. Unlike cardiac differentiation from hPSCs, reprogramming of non-myocyte cells into cardiomyocytes or CPCs could regenerate injured heart tissue by either *in vitro* reprogramming followed by transplantation, or by directly injecting the reprogramming factors into infarcted sites for re-muscularization. This has been reviewed elsewhere[63,64].

Induced cardiomyocytes

Fibroblasts can be directly reprogrammed into cardiomyocytes via transfection of cardiac transcription factors[65,66]. Inspired by the pioneering reprogramming of somatic cells into hPSCs by ectopic expression of Yamanaka factors, fibroblasts can be induced to undergo transdifferentiation into cardiomyocytes via overexpression of cardiomyocyte master transcription factors. Known as GMT factors, the overexpression of Gata4, Mef2c, and Tbx5 using retrovirus vectors[67] or Sendai virus[68] in mouse fibroblasts resulted in cardiac lineage transdifferentiation. Unlike hPSC-based cell therapy, induced cardiomyocytes can be generated *in vivo* through gene therapy to reprogram cardiac fibroblasts into functional cardiomyocytes[69].

Despite these achievements in cardiomyocyte transdifferentiation, clinical trials of *in vivo* direct reprogramming should be approached with caution. First, the efficiency of direct reprogramming to induced cardiomyocytes is low[70]. Because of the lack of consensus on cardiomyocyte markers (such as Troponin T, αMHC, or Nkx2.5) and phenotypes (such as spontaneous beating), the efficiency of transdifferentiation can range from 0% (ref. [70])

to 10% (ref. [68]). Second, results from rodent models cannot be reliably translated into human trials, owing to molecular and physiological differences between the two species. For example, GMT alone is insufficient in generating induced cardiomyocytes from human fibroblasts, and additional factors (such as MESP1, HAND2, or specific microRNAs) are needed to enhance reprogramming[65,66]. Similarly to hPSC-CMs, induced cardiomyocytes have an immature phenotype, as indicated by myofilament disarray, electrophysiology, and global gene expression. Additional head-to-head comparisons between hPSC-CMs and induced cardiomyocytes are necessary in order to evaluate the therapeutic potential of these two cell types.

Induced CPCs (iCPCs)

Two studies[71,72] have reported different strategies for the direct reprogramming of adult mouse fibroblasts into cardiovascular progenitor cells. Whereas one study[71] started with an extensive search for CPC-inducing transcription factors and revealed five (MTGNB: Mesp1, Tbx5, Gata4, Nkx2.5, and Baf60c) factors that were sufficient to induce CPCs, the other study[72] transiently overexpressed the four Yamanaka factors in combination with a JAK inhibitor to induce mesodermal specification, followed by a treatment of various combinations of modulators to induce expandable CPCs. Unlike what was shown previously for induced cardiomyocytes, both these studies also showed that iCPCs are expandable, with the ability to propagate for >18 passages in vitro. They also showed that iCPCs can generate different cardiac lineages *in vivo*, and improve cardiac function and survival following transplantation into a myocardial infarction animal model.

Reprogrammed endothelial cells (rECs)

Direct lineage conversion with cell-type-specific transcription factors has been attempted to generate rECs directly from somatic cells. The ETS (E26 transformation-specific or E-twenty-six) family of transcription factors, especially ETV2 (ETS variant 2; also known as ER71), drive endothelial-cell development, and their overexpression leads to conversion of somatic cells into rECs (refs. [73]). In lieu of the overexpression of endothelial-cell transcription factors, a cocktail of growth factors combined with an immunostimulant was also sufficient to induce rEC transdifferentiation[74]. Although not tested in a cardiac injury model, rEC transplantation has been shown to increase neovascularization and to enhance blood perfusion in a rodent limb ischaemia model[73,74].

Delivery and transplantation strategies

A central determinant in the survival and efficacy of transplanted hPSC-CMs is the delivery method. Thus far, two primary delivery methods have been explored: direct injection, and transplantation via a gel patch. Each method has its own advantages and disadvantages, with injection being the most used method of delivery in preclinical trials to date. However, progress in the development of gel patches shows them as an increasingly attractive alternative, and has enabled their integration into cardiac tissue to stabilize transplanted hPSC-CMs, as evidenced by progressive vascularization of the patches and by their stable engraftment into the host after transplantation[22,75–77].

The transplantation of hPSC-CMs via gel patches offers a host of advantages. The engineered tissue offers contractile, paracrine and passive mechanical support. In designing a patch for cell transplantation, two key factors are the selection of patch material and the design of the patch architecture. It is essential to select a biopolymer that is scalable and capable of integrating into the cardiac tissue while faithfully relaying the signals and contractions of the seeded hPSC-CMs. Additional functionalities, such as an advantageous degradation rate, are also important aspects when selecting polymers for cell delivery. Although many biopolymers have been proposed, thus far collagen, fibrin, alginate and Matrigel have been the most applied[78]. For example, collagen type-I hydrogels have been used extensively, owing to their integral role in the native heart's extracellular matrix, ability to advance the maturation of hPSC-CMs, and well-characterized properties for widespread use in multiple clinical scenarios[22,79]. Alginate, an algae-derived polysaccharide, is also an attractive material, owing to its low cost, biocompatibility and highly tunable properties. Alginate has been investigated for patients with myocardial infarction in an attempt to reduce the demands on the injured heart, and as a patch for the transplantation of hPSC-CMs. Cardiac-cell-seeded microporous alginate scaffolds have been successfully implanted into infarcted rat hearts[80,81].

Although also popular, fibrin has been less extensively studied than the aforementioned materials, perhaps because as a standalone material it cannot be optimized for cardiomyocyte support[78]; however, it has been studied for its resemblance to the main protein constituent of engineered heart tissue. Using hESC-CMs, a 2D cell monolayer and a 3D fibrin-based cardiac patch were found to increase cardiac maturity with higher conduction velocities, longer sarcomeres, and increased expression of contractility genes. Not only could cardiomyocytes be seeded onto fibrin patches; the constructs also promoted maturity[82]. In a fibrin patch containing hESC-derived cardiac progenitors, the left ventricular ejection fraction (LVEF) was found to improve after 2 months in rats with the cardiac patch, without evidence of arising teratomas, thus validating the efficacy and safety of a fibrin-based patch[46]. Subsequently, a clinical trial of hESC-derived CPCs in patients with severe ischemic left ventricular dysfunction[83] reported no tumour formation or arrhythmias in all patients during a 1-year follow-up. Interestingly, although this trial was not powered to assess efficacy, a slight improvement in wall motion and an overall increase in ejection fraction was observed.

Whereas the major consideration in evaluating a cell-delivery approach is to maximize viable cell retention in the target tissue, other variables such as cell differentiation and maturation can also affect the choice of delivery strategies. To support the survival of fully differentiated cardiomyocytes, the appropriate extracellular matrix, as well as growth factors, can be co-injected to provide a favourable microenvironment[84]. When endothelial cells or vascular progenitor cells are delivered, pro-angiogenic factors (such as VEGF) can be encapsulated in the hydrogels or in other biomaterials to support blood-vessel formation. Recent developments in 3D bioprinting have created additional opportunities to generate multicellular engineered-tissue constructs with pre-defined biomaterials and microstructure[85]. By using a 3D bioprinter, cardiac-muscle patches can be created with multiple cell types embedded for transplantation[62,86]. Even for hPSCs, which are not suitable for direct transplantation owing to the risk of teratomas formation, acellular

approaches, such as those involving extracellular vesicles isolated from culture supernatants, can induce cardiac repair[87].

Outlook

The heart has a very limited endogenous regenerative capacity; yet, lessons learned from developmental biology have brought us closer to the goal of generating appropriate types of cells to repair damaged hearts. Despite notable progress in the development of cell-differentiation protocols, hurdles in the way of clinical translation, such as achieving appropriate cell maturity, cell purity, and the ability to generate enough cells for therapeutic efficacy, remain mostly unsurpassed. Preclinical studies are necessary to identify the best combinations of cell types for myocardial regeneration.

Other applications of hPSCs in cardiovascular medicine include disease modelling and drug discovery. Using primary patient-derived cells, hiPSCs have been used for studying cardiovascular disorders caused by genetic mutations, and the emergence of genome-editing tools has further expanded the application of hiPSCs in precision medicine. Advances in hiPSC technology also facilitate the screening for personalized pharmacological drugs (thoroughly reviewed elsewhere[88]).

There are further obstacles to the translation of cardiac stem cell therapy. Primarily, a lack of knowledge of the mechanisms of regeneration[89]; in this context, incorporating molecular imaging in animal experiments to track cell fate after transplantation may help elucidate the mechanisms[90]. And great caution is advised when evaluating differences between species before translating animal results into human trials[91]. Naturally, economic, regulatory and ethical issues persist[4]. Because cardiac stem cell therapy requires large investments in preclinical research and in clinical trials, closer collaboration between government agencies, non-profit foundations, private and strategic investors, universities, and research institutes is necessary in order for cell therapies to hopefully revolutionize the understanding of heart regeneration and ameliorate heart disease.

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Box 1.

Four main strategies used to replicate the environment of cardiomyocytes to promote maturation of cardiomyocytes derived from pluripotent stem cells.

Cell Biology Approaches • Extended Culture • Co-Culture ○ Endothelial cells ○ Fibroblasts • Cell Fusion ○ To form larger and multinucleated cells	Genetic Approaches • Overexpression of cardiac genes ○ Ion channels or calcium handling proteins (e.g., Kir 2.1, calsequestrin) • Gene expression network perturbation ○ MicroRNA (e.g., let 7, miR-1)
Biophysical approaches • Tissue engineering • Electric pacing • Mechanical loading • Conductive substrate • Substrate stiffness • Micropatterning	(Bio)chemical approaches • Growth Factors • Hormones (e.g., thyroid/glucocorticoid hormones) • Adrenergic agonists • ROS removal

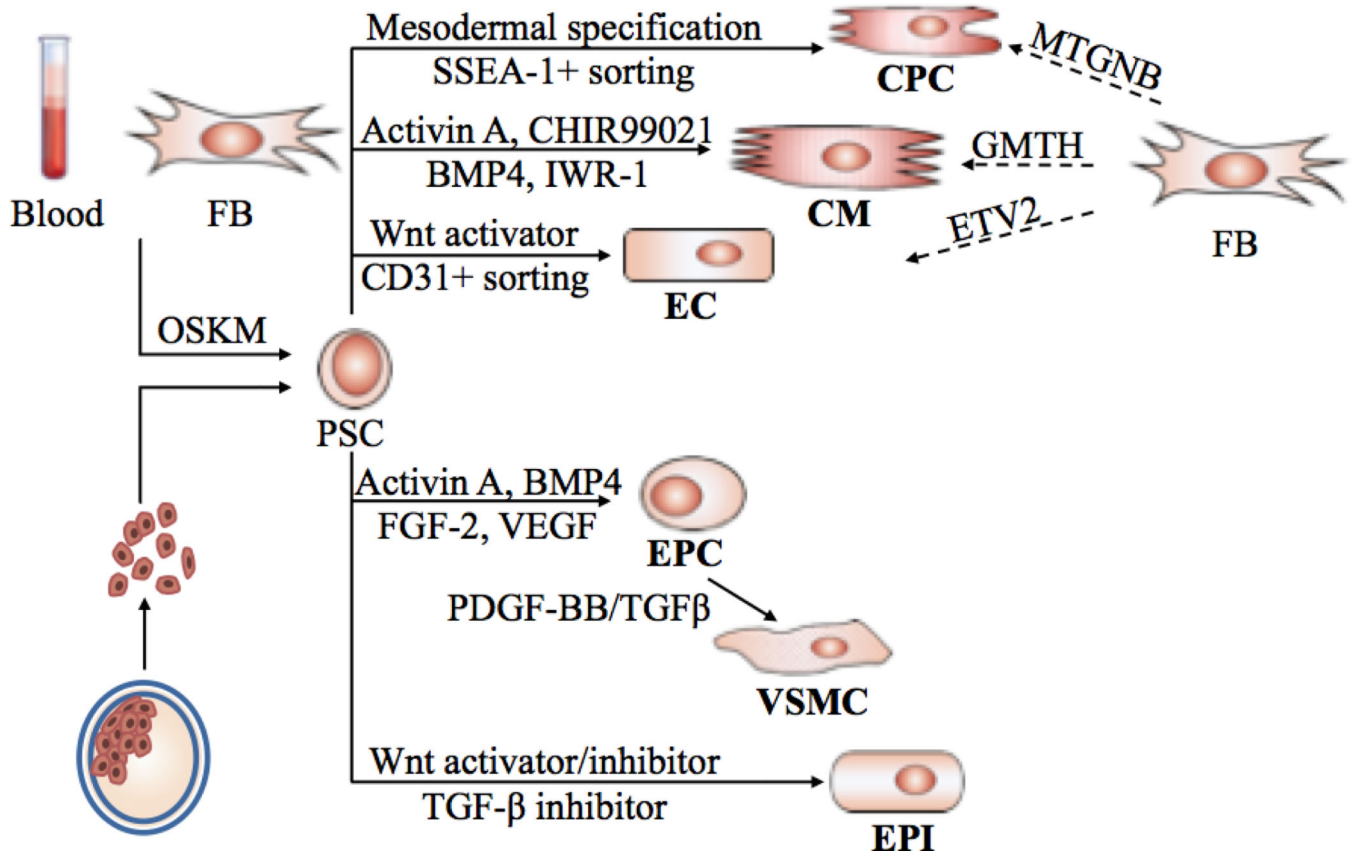


Fig. 1. Pluripotent stem cell therapy for cardiovascular regeneration.

Patient-specific hiPSCs reprogrammed from somatic cells or hESCs derived from fertilized embryos are differentiated into cells with different cardiac lineages: cardiomyocytes (CMs), endothelial cells (ECs), cardiac progenitor cells (CPCs), endothelial progenitor cells (EPCs), epicardial cells (EPIs) and vascular smooth muscle cells (VSMCs). Fibroblasts (FBs) are reprogrammed directly into CMs, CPCs or ECs via transfection of transcription factors. OSKM: Oct3/4, Sox2, Klf4, and c-Myc. MTGNB: Mesp1, Tbx5, Gata4, Nkx2.5, and Baf60c. GMTH: Gata4, Mef2c, Tbx5, and Hand2. ETV2, ETS variant 2.

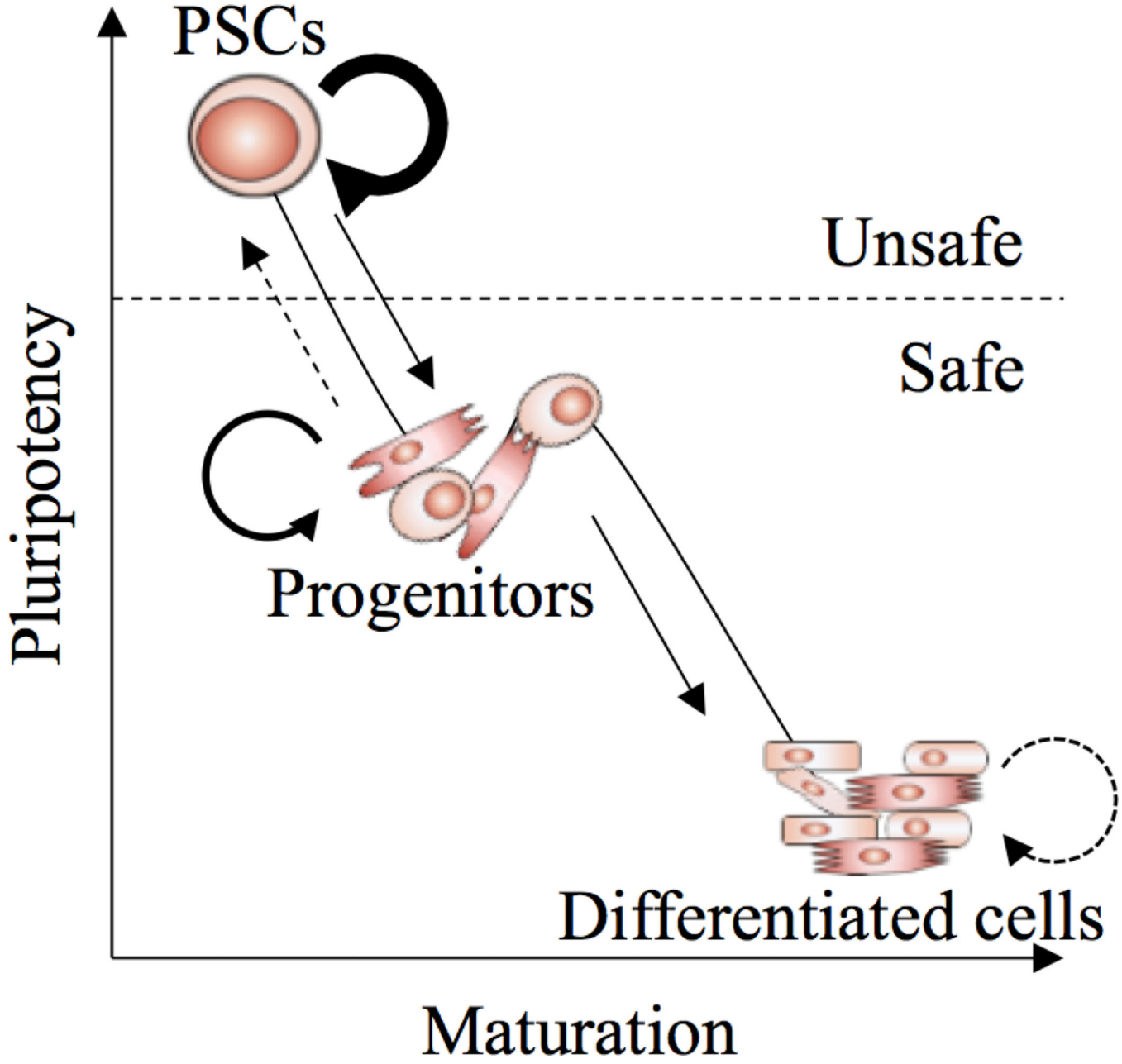


Fig. 2. The trajectory from pluripotent stem cells to fully differentiated cells through progenitor cells.

Both progenitors and fully differentiated cells can be candidates for transplantation. Pluripotent stem cells are however not suitable because of their high self-renewal capacity and potential tumorigenicity.

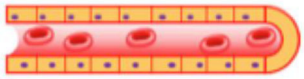
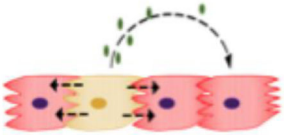
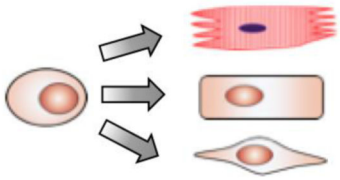
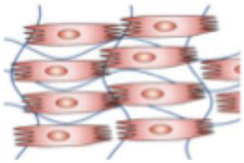
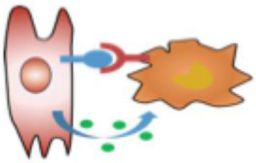
Mechanism	Remuscularization	Revascularization	Material transfer
Cell types	CM, CPC, EPI	EC, EPC, VSMC, EPI	CM, EC, VSMC
			
Mechanism	In vivo differentiation	Provision of extracellular matrix	Immunomodulation
Cell types	CPC, EPC, EPI	Tissue patch	MSC
			

Fig. 3. Mechanisms of cell-based therapy for cardiovascular regeneration.

Depending on cell types and delivery routes, one or more mechanisms may be used. When a combination of cell types is introduced, synergistic effects may occur to promote therapeutic efficacy. Progenitor cells including CPCs and EPCs, as well as epicardial cells, can further differentiate into other functional cells and may regenerate multiple cell types after transplantation. Extracellular matrix may be supplied through tissue patches to support stem cell survival and reverse heart remodelling.