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Stem Cells in Cardiovascular Medicine: the Road to Regenerative Therapies

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Abstract

Purpose of Review—The purpose of this review is to provide a broad overview of current trends in stem cell research and its applications in cardiovascular medicine. Researches on different stem cell sources, their inherent characteristics, and the limitations they have in medical applications are discussed. Additionally, uses of stem cells for both modeling and treating cardiovascular disease are discussed, taking note of the obstacles these engineered interventions must overcome to be clinically viable.

Recent Findings—Tissue engineering aims to replace dysfunctional tissues with engineered constructs. Stem cell technologies have been a great enabling factor in working toward this goal.

Summary—Many tissue-engineered products are in development that utilize stem cell technology. Although promising, some refinement must be made to these constructs with respect to safety and functionality. A deeper understanding of basic differentiation and tissue developmental mechanisms is required to allow these engineered tissues to be translated into the clinic.

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Compliance with Ethical Standards

Conflict of Interest Christopher W. Anderson, Nicole Boardman, Jiesi Luo, Jinkyu Park, and Yibing Qyang declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

Keywords

Stem cells; Cardiovascular; Regenerative medicine; Induced pluripotent stem cells; Embryonic stem cells; Tissue engineering

Introduction

Cardiovascular disease is globally the leading cause of mortality with an estimated 17 million annual deaths [1]. This is approximately 30% of reported annual deaths and is expected to have an increased incidence through the year 2030 [1, 2]. In the USA, there is an estimated total of 27 million individuals afflicted nationwide [1]. As people age, their natural ability to repair and regulate homeostasis of the cardiovascular system declines [3]. This natural decline in cardiovascular health is exacerbated by environmental factors, where clinical intervention will eventually be needed [3, 4]. One of the issues with current therapies for the treatment of cardiovascular disease is that different patients will respond in varying levels to the drugs administered. This discrepancy arises due to unique cellular and genetic conditions underlying these diseases that are specific to the individual. It is for this reason that the concept of personalized medicine has gained attraction in recent years by both physicians and researchers alike. The purposes of this review are to provide some insight into the advancements of stem cell technologies and examine their applications in cardiovascular medicine. In this review, we will discuss different stem cell sources and their current applications, while taking note of the limitations of each. This review takes the stance that induced pluripotent stem cell technology provides an exciting avenue for developing therapeutics with the aim of making personalized medicine through regenerative therapy a reality.

Embryonic Stem Cells

Brief History of Embryonic Stem Cells

Embryonic stem cells (ESCs) are cells derived from the inner cell mass of the preimplantation blastocyst that retain the ability to differentiate into all three germ layers [5]. Human ESCs have been derived from the human embryo and have displayed pluripotency [6]. Theoretically, ESCs are capable of being expanded in culture indefinitely, which is due to their active telomerase enzymes that prevent telomere shortening, senescence, and rapid apoptosis [7]. These are exciting features of ESCs because it not only allows for the generation of varied cell types for genetic modeling but also for the potential of generating a nearly unlimited supply of cells for use. This aspect makes it particularly attractive for use in cell-based therapies which require large cell numbers. Though human ESCs offered much promise in the years since they were first isolated by Thomson in 1998, public opinion heavily influenced how these cells ultimately could be used [8]. Because the establishment of an ESC line requires the destruction of the developing human embryo, the country was faced with a new ethical dilemma regarding the use of this cell source for research. This national dialogue resulted in a restricted progress in ESC research since there could be no new ESC lines generated with federal funds as declared by the Dickey-Wicker amendment in 1996, which is still in effect to this day. However, subsequent presidential administrations

have made suggestions on how to work within the guidelines of Dickey-Wicker while impeding scientific progress as little as possible [9]. Currently, the excess unused eggs from in vitro fertilization (IVF) are the main source of new ESC lines in the USA as they fall into a special category that is permissible under current law [10].

The use of leftover IVF embryos alleviated some of the restrictions ESC research faced previously in that it allowed for a greater number of cell lines with a wide variety of genetic backgrounds to be tested for pathology in disease and necessary pathways in development. Healthy cell lines may also be generated for biobanking-defined human leukocyte antigen (HLA) lines for cell therapies [11]. However, storage protocols must be optimized as current methodologies have led to overall low viability rates for these embryos after thawing [12]. One reason an embryo may not be selected for IVF is that it is deemed to be of low “quality” as determined by the physical characteristics of the embryo that have been associated with low efficacy for implantation in utero [13–15]. Different quality blastocysts have been tested and, surprisingly, are able to give rise to high quality cells that display proper ESC morphology and expression patterns [16, 17]. However, there are some debates regarding the efficiency differences between cell lines in ESC derivation [18, 19]. These cells also show varying capacity to differentiate into cardiomyocytes expressing proper markers regardless of original blastocyst quality [20].

Differentiation of ESCs

The development of protocol for differentiation of ESCs into cardiomyocytes in vitro has been largely based on observations of cardiac development pathways in vivo. These pathways include activities of Wnt, activin/Nodal/TGF β , BMP, and FGF [21]. A typical protocol may include the following: ESC differentiation into cardiac mesoderm through addition of BMP4, Nodal/activin A, and Wnt/ β -catenin; subsequent inhibition of both Wnt/ β -catenin and Nodal/activin A to yield cardiac progenitor cells; continued inhibition of Wnt/ β -catenin and addition of FGF to induce differentiation into cardiomyocytes; and addition of Wnt/ β -catenin, IGF, NRG, FGF1, Notch1, and periostin to support further proliferation [21, 22, 23••, 24].

The generation of cardiomyocytes from ESCs may be a potential therapy for patients that have suffered from myocardial infarction, one occurrence of which can destroy as much as one billion cardiomyocytes [21]. Progress has been made in the development of functional cardiac tissue in vitro. Stevens et al. aimed to create 3D cardiac tissue consisting of ESC-derived cells alone, in a scalable method. To do so, ESCs were cultured and induced to differentiate into cardiomyocytes (through genetic pathways similar to those previously discussed), which were then incubated on a rotating orbital shaker. This yielded an aggregate of disc-shaped cardiac tissue in the center of each plate, which showed synchronous and spontaneous beating. The individual cells were shown to be electrochemically coupled to one another, resembling the functionality that occurs in normal cardiac tissue. This indicates that the experimentally developed cardiomyocytes could be able to functionally integrate with host cardiac tissue [25]. In fact, this functional integration was demonstrated to occur when cardiomyocytes derived from human ESCs were implanted into pig and guinea pig

hearts [26, 27]. Cardiomyocyte derived from ESCs has allowed for great advancements in the field of regenerative medicine but is currently limited in their potential for therapies.

Adult Stem Cells

Many tissues retain the ability to regenerate upon damage well into adulthood [28–30]. This regenerative capability is due to somatic stem cells, which retain the ability to differentiate into various cell types of their respective tissues. This is known as multipotency, and while these cells are thought to be more limited in their differentiation potential than ESCs, they do come with some added benefits. These cells are autologous which reduces the need for immunosuppression for potential therapies. In addition, these cells are derived from an adult individual and thus circumvent the ethical issues surrounding human ESC use.

For cardiovascular regenerative medicine, a candidate autologous stem cell type lies in the bone marrow. Bone marrow-derived stem cells (BMSCs) have been shown to aid in the regeneration of vasculature [31, 32], but their contribution to new cardiomyocyte generation remains controversial [33, 34]. These cells have even been shown to generate perfusable vascular networks for in vitro tissue models [35]. BMSCs have also been used to alleviate limb ischemia. In one study, rats received intravenous injections of bone marrow mesenchymal stem cells. It was found that the BMSCs significantly increased capillary density and improved renal function via an activation of the PI3K-Akt signaling pathway [36]. In another study, the effects of BMSCs in repairing acute ischemic-related renal damage were assessed by injecting Lin(–)Sca-1+ c-Kit+ BMSCs into an area of acute tubular necrosis in mice [37]. The cells differentiated into renal tubular epithelial cells, repairing the ischemic renal tubular injury. Both of these studies illustrated the potential of BMSC therapy in alleviating the effects of ischemia. However, bone marrow extraction is an invasive and painful procedure, and the availability of BMSCs varies from person to person and is further impaired in older or diseased patients. There is little known about the regulatory mechanisms for BMSCs. For these reasons, correcting ischemic damage through BMSC therapy has limited use in clinical applications.

Alternatively, adipose-derived mesenchymal stem cells (ADMSCs) have also been tested for use in cardiovascular regeneration [38–40]. These cells have the benefit of being easily extractable and in ample supply for most patients. Cardiac resident adipose tissue has also been implicated in normal heart repair and homeostasis [39, 41]. ADMSCs have been tested in donors of different health statuses to assess potential functional differences [42]. Because cardiovascular disease is often a consequence of the presence of risk factors such as hypertension and diabetes, ADMSCs must be tested in this context to paint a more accurate picture of their potential as a therapeutic. It was noted that even elderly ADMSCs that are healthy were able to produce functional vascular grafts. However, cells derived from diabetics caused thrombosis in the mouse model [42]. Additionally, the diabetic ADMSCs showed impaired remodeling of the implant due to a decreased capacity for fibrinolysis when compared to healthy ADMSC populations.

Induced Pluripotent Stem Cells

Although human ESCs have been crucial in understanding basic biology surrounding pluripotency and differentiation, current laws restricting their use hinder their efficacy for cell-based therapies. Autologously derived adult stem cells such as BMSCs seem to get around some of the ethical and compatibility issues faced by ESCs but ultimately are limited in that these cells are difficult to isolate or will be in scarce supply for elderly or diseased patients who would be prime candidates for regenerative therapy. An ideal cell source for regenerative medicine needs to be readily available, immunologically compatible, and not ethically dubious to obtain. In 2006, Shinya Yamanaka described successful reprogramming of human somatic cells into a pluripotent state that was similar to ESCs in both its phenotype and transcriptome [43••]. This was accomplished by using retroviral transduction of what have become known as the Yamanaka factors (Oct3/4, c-MYC, Klf4, Sox-2). Yamanaka also demonstrated the differentiation potential of these cells into various lineages via quantification of cell identifying lineage-specific marker expression. The advent of these induced pluripotent stem cells (iPSCs) generated a large interest in the stem cell and regenerative medicine communities because it opened many avenues for research and therapeutics.

Reprogramming of Somatic Cells

The introduction of the exogenous Yamanaka factors into dermal fibroblasts resulted in these cells taking on an ESC-like morphology in that they form rounded colonies. These transcription factors modulate pathways involved in development and metabolism in order to generate and maintain a pluripotent state [44]. Specifically, Oct4, Sox2, and Klf4 were found to regulate developmental pathways while c-Myc regulates metabolism. Subsequent studies show that while c-Myc is dispensable for reprogramming, it does increase the speed at which this process takes place [45]. The exact mechanism of reprogramming is still being actively investigated, although some reports utilizing histone deacetylase inhibitors, or other small molecules that influence chromatin remodeling proteins, have increased the efficiency of reprogramming using only Oct4 and Sox2 overexpression [46, 47]. These findings suggest that chromatin modification is a key component of this process. Elucidating the mechanistic effects of cell reprogramming could help in increasing the efficiency of the overall process. While the genetic mechanisms are being worked out, researchers are adapting methodologies of cell culture and Yamanaka factor introduction to optimize reprogramming protocols.

The original protocol for generating iPSCs relied upon using an integrating viral vector and mouse embryonic fibroblasts (MEF) as feeder cells to aid in reprogramming. Both of these cause significant concerns for translation of these cells into regenerative therapies in human. The presence of xenogenic feeder cells complicates the isolation of human cells, and any residual MEF cells in an implanted tissue could cause unwanted immune reactions and thereby harm the engrafted construct. In addition, the integrated DNA that supplied the Yamanaka factors could have off-target effects, depending on where it was inserted. To address this, protocols using Matrigel® have been developed for feeder-free culture of iPSCs so as to assure that the final differentiated cells are purely human derived [48]. A few alternatives to integrating viral vector for introducing Yamanaka factors have been studied.

Among these are small molecules, ready-made proteins and CRISPR systems for knocking in and out constitutively expressed Yamanaka genes [49–51]. The important theme in these different reprogramming protocols is that they produce iPSCs without integrating vectors. Although these protocols are innovative and may be advantageous over existing protocols, the most efficient way to accomplish this goal currently is by using a nonintegrating Sendai virus as the vector for the reprogramming factors [52, 53]. Using Sendai virus for reprogramming is straightforward, has a high transduction efficiency, and is nonintegrating. These qualities make it ideal for clinically relevant uses such as modeling and treating cardiovascular diseases.

Differentiating into Cardiovascular Lineages

Different stimuli can be employed to differentiate iPSCs into the desired lineage. Included within these parameters will be both biochemical and physical cues, as well as general media components, and each of these components must be optimized for efficient differentiation. Specifically, cardiovascular cell differentiation relies upon temporally controlled activation of the Wnt signaling pathway in order to generate multipotent, brachyury⁺ mesodermal progenitor cells that can be further differentiated into vascular endothelial cells, vascular smooth muscle cells, or cardiomyocytes [22, 54, 55]. Provision of a small molecule agonist of the Wnt pathway in a temporal-specific manner can markedly improve the generation of various cells of the cardiovascular lineage.

Cardiomyocyte differentiation relies on both up- and downregulation of Wnt signaling. Inhibition of Wnt signaling must be done after they reach the brachyury⁺ progenitor phase in order to generate high cardiomyocyte yields [22]. This can be accomplished via shRNA knockdown of WNT or small molecule inhibitors [23••, 24]. Utilizing small molecule inhibitors would be better suited for regenerative medicine applications as it does not alter the genome of these cells in any way. Reports of using this method of controlling the Wnt pathway, cultured in insulin and growth factor free medium, have boasted ~90% purity in their differentiated cell populations [23••]. This is a marked increase from the original embryoid body differentiation protocols that would see relatively low cardiomyocyte yields from those populations [56, 57]. Smooth muscle and endothelial cells can be derived from these brachyury⁺ progenitors without subsequent inhibition of Wnt signaling. These progenitor cells can be cultured on Matrigel® and placed in either commercially available vascular smooth muscle or endothelial cell media, respectively, in order to derive these lineages. Endothelial cells can be further matured into venous or arterial phenotypes using biomimetic bioreactors to provide physical cues in the form of fluid shear stress, which has been shown to have profound effects on vascular endothelial cell behavior [58•, 59, 60]. Differentiated endothelial cells exposed to the fluid shear stress were found to have upregulated vasoprotective markers KLF2 and 4 in addition to upregulating NOTCH signaling that is associated with mature arterial endothelial cells. This type of maturation event will be very important for certain applications of these differentiated cells which will be discussed in the next section.

Applications of iPSCs for Cardiovascular Regenerative Medicine

Modeling Cardiovascular Disease with iPSCs

An attractive feature of iPSCs is that they retain all genetic traits of the cell donor, and this can be leveraged to model diseases in vitro. This method is advantageous, compared to using somatic myocardium, because iPSCs have a larger replicative capacity and are much more readily available than donor heart tissue. iPSC technology has been used to study hypertrophic cardiomyopathy (HCM) where cellular phenotype was shown to be conserved in iPSC differentiated cardiomyocytes [61]. This model was the first to show that RAS signaling was perturbed in human cardiomyocytes with HCM causing mutation in *PTPN11*. Other genetic backgrounds of HCM have been assessed using iPSC to gain insights into the diseases' electrical alterations [62, 63••]. These studies showed that high calcium concentration during diastole causes a progression of HCM phenotype in cardiomyocytes with a mutated MYH7. Human iPSC-based disease modeling provided a unique perspective over mouse models since the structure of sarcomeric proteins between mouse and human is quite different [64]. These differences cause alternate cellular phenotypes in mouse and human like sarcomeric backgrounds with the same mutation [65].

Vascular diseases such as supravalvular aortic stenosis (SVAS) can also be modeled with hiPSC technology [66]. SVAS is characterized by haploinsufficiency of elastin and eventually the reduction in luminal diameter due to proliferating vascular smooth muscle cells in the supravalvular aortic region. This phenotype was recapitulated using iPSC-derived smooth muscle cells in that they were noted to have an increased proliferation rate as compared to wild-type cells. The iPSC modeling system also revealed a disorganization of contractile bundles in SVAS smooth muscle cells, indicating that the cellular phenotype is particularly robust in terms of modeling SVAS. iPSC modeling systems offer a dual capacity for investigators in that they cannot only offer a platform for gaining valuable insights into the mechanisms of disease progression but can also be used as a tool for drug screening to treat the disease [67]. Drug screens on human cells will give a better picture of how human cells will react to treatment than using mouse models. This concept may also be applied in the clinic as a means of personalized medicine to determine what drugs are the most effective for an individual's cells [68, 69, 70••].

Tissue Engineering and Regenerative Therapies

Stem cells also offer a great platform for developing clinical interventions to treat cardiovascular disease. A few examples currently in the clinical trial stage are listed in Table 1. The goal of regenerative medicine is to replace or restore nonfunctional tissues. A very exciting, albeit ambitious, approach is to generate entire hearts for transplant patients [71, 72]. This methodology aims to address two major issues transplant recipients face: the lack of availability of organs and complications due to immune rejection. The perfusion decellularization method seeks to remove all endogenous cells within a mature adult heart while leaving behind the extracellular matrix that keeps the organ geometry and vascular structure intact. This allows investigators to utilize xenogenic organs of similar size to help fill the clinical demand. Different methodologies for recellularizing the extracellular matrix (ECM) scaffold have been explored where it was noted that re-endothelialization prior to

parenchymal recellularization increased cell infiltration and ventricular functionality [72]. Human iPSC-derived cardiac progenitors have been used to repopulate mouse heart ECM scaffolds. This strategy resulted in proper endothelial and myocardial differentiation and localization [73]. However, arrhythmic beating and poor contractile force were noted in the construct. This implies that, although the ECM can direct proper differentiation of progenitors, the tissue may not be maturing properly. This could be due to missing mechanical or electrical cues that are necessary for functional tissue maturation and myocyte coupling. Additionally, other cell types may be necessary for maturation of functional heart tissue such as cardiac fibroblasts [74, 75]. More basic biology of its constituent components must be understood in terms of how to generate physiologically relevant vasculature, myocardium, and pacemakers before the dream of generating a fully functional heart can be realized.

Each of these components of healthy heart tissue can be generated individually in order to provide insights into the mechanisms that facilitate proper development and to potentially provide therapeutic interventions at a smaller scale. Tissue-engineered vascular grafts (TEVG) aim to provide fully functional vasculature for patients in need of a bypass surgery [76•]. Methods have been attempted to use artificial scaffolds of different porosity to generate vessels in situ [77–79]. These grafts showed infiltration of native endothelial and vascular smooth muscle cells. Although there was some remodeling and ECM deposition, the artificial polylactic acid scaffolds remained largely intact after 12 months. It was noted that these acellular scaffolds showed macrophage infiltration, which has positive implications for vascular remodeling by influencing vascular cell behavior and ECM deposition [80–82]. In addition, these scaffolds also need to be preseeded with autologous bone marrow-derived endothelial cells. Ideally, a TEVG would utilize a cell source that is less invasive to collect. Some labs are utilizing iPSC technology to develop engineered blood vessels. Two-millimeter-thick polyglycolic acid (PGA) scaffolds have been utilized to develop TEVG by seeding them with vascular smooth muscle cells (VSMC) [76•, 83]. Static culture for approximately 8 weeks on this scaffold yielded an implantable vessel with inherent ECM composition and is perfusable in vivo. These vessels do not harbor sufficient strength to withstand burst pressure which may be due to lack of sufficient collagen deposition and/or suboptimal remodeling efficiency. These vessels also must be coated with endothelial cells to prevent thrombogenicity and be able to interact with circulating leukocytes. Endothelial specification must be taken into account as arterial endothelium behaves much differently than venous [84, 85]. Endothelial cells contain robust mechanical sensing mechanisms that influence gene expression and remodeling, and these abilities have been leveraged to specify differentiation into an arterial-like phenotype via biomimetic luminal flow [58•, 86].

Cardiac patches offer a great therapeutic to aid repair after myocardial infarction by reducing scar tissue formation and global remodeling [87–89]. Similar to what is seen in whole organ engineering attempts, poor electrical coupling of cells is seen within cardiac patches [90]. The lack of electrical coupling of these cells is a recurring issue that researchers are attempting to circumvent via biomaterial and cell maturational interventions. Nanostructural modifications of the scaffolds used in generating these tissues have provided improvements in tissue morphology and electrical coupling of mature cardiomyocytes [90–

92]. Additionally, biomaterial modifications aimed at mimicking natural ECM protein and polysaccharide composition have been developed to aid progenitor cell differentiation into cardiomyocytes [93]. The modified hyaluronic acid patches showed an increased expression of cardiac markers, including the gap junction protein connexin43, as compared to other synthetic scaffolds. However, testing of the signal propagation characteristics of these differentiated cardiac cells is needed to confirm if this expression is sufficient to allow synchronous contraction with native myocardium. As an alternative approach, cardiac patches may also be used as a mode of delivery for cardiac progenitor cells (CPC) [94]. In this way, the functionality of the patch tissue itself is not a factor because the progenitor cells will translocate from the pericardium into the infarct site where they will mediate repair.

Stem cells may also be used to generate biological pacemakers, which is a crucial development for whole organs to be viable for translation. The sinoatrial node (SAN) is thought to be the initiator of the electrical signal that controls beating [95]. Current strategies for generating biological pacemakers aim to functionally mimic nodal cells of the SAN. Human mesenchymal stem cells have been transduced to express the pacemaker-specific HCN2 ion channel and subsequently expressed cardiac gap junction proteins [96, 97]. It is important to note that these cells are not pacemakers within themselves but rather provide a mechanism of depolarization for the resident cardiomyocytes coupled to the HCN2⁺ hMSC via the I_f current generated by HCN2. This multicell setup initiated subsequent action potential firing when resident cardiomyocytes became hyperpolarized between beats in a canine model [98], although it is unclear if pacing was due entirely to implanted cells. Researchers have attempted to isolate nodal-like cells from the heterogeneous mixture of hESC-derived cardiomyocytes but ultimately have been unable to show their ability to regulate sustained contraction in vivo [99, 100, 101••]. Transcription factors Shox2 and Tbx18 have each been implicated in the maintenance of cardiac pacing in nodal cells and have been used to generate autonomous pacemakers from mouse stem cells [102, 103]. Although these cells were found to have pacing capabilities, they still must be further refined to fully recapitulate physiologic pacing and be shown to maintain pacing functionality long term.

Conclusion

The ability to generate cells of the cardiovascular system allows for more relevant in vitro disease modeling systems and designing of tissue-engineered therapeutics. Various cell sources have been assessed for generating cardiac, endothelial, and smooth muscle cells with robust phenotypes. Studies with embryonic stem cells offered great insights into the methodologies that can reproducibly differentiate into these lineages although their use was limited due to political and ethical concerns of the public. Autologous adult stem cell sources are very attractive, from a therapeutics perspective, due to their inherent immune compatibility with the host. However, they are not always abundant or the most accessible. In cases where the cells can be extracted, there are still issues with the maintenance of these cells due to the lack of knowledge of the basic biology for BMSCs. iPSCs represent the most versatile stem cells in that they have the combined attributes of being autologous, easily accessible, and not ethically dubious. These qualities make them a prime candidate for both

experimental studies in different genetic backgrounds and engineering therapies as outlined in Fig. 1. Reprogramming and differentiation to a specific cell lineage requires time, so interventions using this cell source would be limited to nonurgent treatments as patients would need to wait for weeks for the tissue to mature. Still, stem cells show great promise in recapitulating the physiology and architecture of complex tissues, though more fine-tuning of the differentiation protocol is necessary to become more efficient in cardiomyocyte generation. In order to accomplish this goal, more of the basic biology of stem cells must be pursued so that these therapeutics approaches can become more robust. An interdisciplinary approach is necessary to accomplish the goal of personalized regenerative medicine. Collaborations between stem cell biologists, bioengineers, and material scientists will offer the most efficient path to translation for these projects. The expertise from these fields will allow researchers to discern and recapitulate the proper cell environments to allow for the maturation of these tissues that will yield in physiologically relevant implants.

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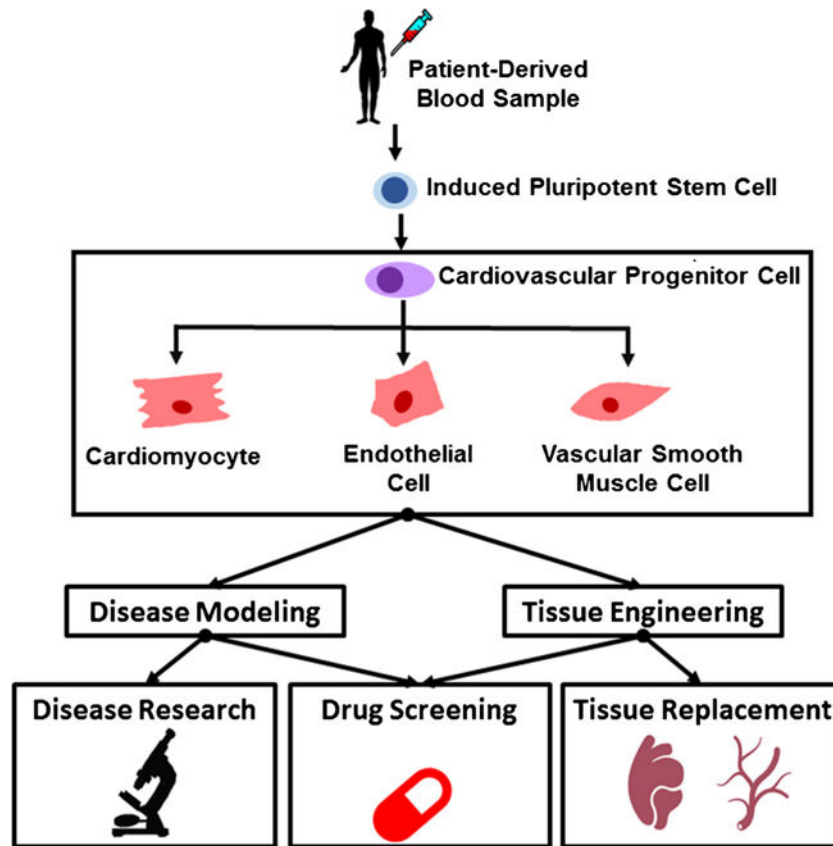


Fig. 1. Schematic of stem cell applications in cardiovascular medicine. Schematic depiction of iPSCs derived from peripheral blood that are differentiated into a cardiovascular lineage. These cells can be utilized for either tissue engineering constructs to replace dysfunctional vasculature or myocardium for clinical interventions or drug screening in complex, 3D tissues. 2D cell culture also allows for modeling of disease with these cells and high-throughput drug screening

Table 1

Cardiovascular stem cell therapies in the USA

Disease	Type	Status	Time frame	Cell source	Phase	Delivery mechanism	Clinical trial ID
Ischemic cardiomyopathy	Interventional	Completed	September 12, 2005–June 05, 2015	Autologous BMSC	1	Intramyocardial injection	NCT00203203
Chronic ischemic left ventricular dysfunction	Interventional	Ongoing	December 02, 2013–	Allogenic hMSC	2	Transendocardial injection	NCT02013674
General aging	Interventional	Ongoing	July 18, 2016–	Allogenic hMSC	2	Intravenous injection	NCT02065245
Coronary artery disease	Interventional	Ongoing	April 10, 2006–	Autologous BMSC-derived aldehyde dehydrogenase bright cells	1	Intramyocardial injection	NCT00314366
Ischemic cardiomyopathy	Interventional	Completed	May 15, 2007–October 15, 2014	Autologous c-kit+ cardiac stem cells	1	Intracoronary injection	NCT00474461
Peripheral arterial disease	Interventional	Recruiting	April 25, 2016–	Autologous ADSC	1	Intravenous injection or intramuscular injection	NCT02756884
Congenital heart disease	Interventional	Ongoing	January 29, 2008–	BMSC	2	Cardiomyoplasty	NCT01034007
Myocardial infarction	Interventional	Completed	July 2014–April 2016	Allogenic mesenchymal bone marrow cells	3	Intravenous injection	NCT02672267
Acute myocardial infarction	Interventional	Ongoing	June 2014–	Allogenic cardiac stem cells	2	Intracoronary infusion	NCT02439398
Cardiomyopathy	Observational	Recruiting	June 2014–	hiPSC	–	–	NCT02417311
Chronic myocardial ischemia	Interventional	Recruiting	January 2016–	Autologous MSC	2	Intramyocardial injection	NCT02462330
Cell type			No. of trials	% Observational		% Interventional	
hiPSC			5	100		0	
ADSC/BMSC			41	4.9		95.1	

Examples of recently completed or ongoing stem cell-based therapy trials. Total trials listed on clinicaltrials.gov for cardiovascular diseases are given for each cell type. Currently, there are no clinical trials using embryonic stem cells in the USA, so this category was omitted from this table