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APPLICATION OF STEM CELLS IN ISCHEMIC HEART DISEASE

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12.1 INTRODUCTION

Many diseases kill cells in the organs, claiming lives or impairing a person's ability to live a normal life. For example, about 5.8 million Americans have heart failure, and 700,000 are diagnosed with it each year (Centers for Disease Control). In heart failure, much of heart muscle itself dies, so the heart cannot sufficiently pump blood.

Similarly, about 24.6 million Americans and 5% of adult Indians have diabetes. About 5–10% of these people have type I diabetes in which the insulin-producing cells of the pancreas are dead. Finally, about 1 million Americans live with Parkinson's disease. In this disease, cells that make the neurotransmitter dopamine, which helps control movement, die. Patients with Parkinson's disease have tremors and uncontrollable movements. Could these patients be treated and live normal lives? That is the goal of stem cell research.

Heart disease is an endemic health problem of great magnitude in the world. Despite considerable clinical and research effort during the past decade and the development of new drugs and surgical modalities of therapy, the mortality and morbidity remain very high. Because the limited potential of the myocardium for self-repair and renewal, a significant proportion of cardiac muscle loses its ability to perform work; this loss may be the most important factor in the heart pump failure occurring in patients with coronary artery disease and dilated cardiomyopathy. Until

Micro and Nanotechnologies in Engineering Stem Cells and Tissues, First Edition. Edited by Murugan Ramalingam, Esmaiel Jabbari, Seeram Ramakrishna, and Ali Khademhosseini.

recently, reperfusion of the ischemic myocardium was the only intervention available to restore the various cellular functions affected by myocardial ischemia, including preventing cell death by necrosis or apoptosis. Unfortunately, reperfusion may result in extensive myocardial damage, including myocardial stunning, and the functional recovery of the heart may appear only after a period of cardiac contractile dysfunction that may last for several hours or days. It is evident that the limited capacity of regeneration and proliferation of human cardiomyocytes can prevent neither the scar formation that occurs after myocardial infarction (MI) nor the loss of heart function occurring in patients with cardiomyopathy and heart failure. Replacement and regeneration of functional cardiac muscle is an important goal that could be achieved either by stimulation of autologous resident cardiomyocytes or by the transplantation of allogenic cells (e.g., embryonic stem cells, bone marrow mesenchymal cells, or skeletal myoblast).[1–24]

The discovery of cardiogenesis in adult animals and human represent one of the most significant advances in cardiology in the past 25 years (Table 12.1). Previously, most cardiologists believed that the birth of new cardiomyocytes was only confined to the fetal and neonatal heart. This dogma recently collapsed when researchers discovered that the hearts of adult rats, mice, and humans undergo significant cardiac

TABLE 12.1 Major Milestones in Stem Cell Research

1981. First mouse embryonic stem cell (ESC) lines isolated and grown in culture
1981. First transgenic animals produced
1988. First cord blood transplant
1989. A clonal line of human embryonal carcinoma cells derived
1990. Britain passes the Human Fertilization and Embryology Act
1994. Human ES-like cells generated
1995. Evidence found for neural stem cells
1996. Dolly, the first cloned sheep, born in Scotland
1998. Scientists at University of Wisconsin-Madison and Johns Hopkins University isolated the first human stem cells
2000. Scientists in Singapore and Australia derive human ESCs from blastocysts
2001. Advanced Cell Technology creates the first cloned human embryos
2001. U.S. President George Bush blocks federal funding for creation of new stem Cell lines
2001. Human ESCs successfully developed into blood cells
2002. Neural stem cells successfully developed into functional neurons
2003. Institute of Stem Cell Research, Edinburgh, discovers key gene that keeps ESCs in a state of youthful immortality
2003. Dolly dies after developing progressive lung disease
2003. UK Stem Cell Bank established
2004. Web-based resource for international stem cell researchers launched
2004. Californians approve Proposition 71 to spend $3 billion over 10 years on stem cell research
2005. U.S. House of Representatives approves a bill to loosen restrictions on federal funding for stem cell research
2006. Judge rules in favor of proceeding with the financing of California's 10-year stem cell project (based on Proposition 71)

changes as a function of age. New cardiomyocytes are can be produced by, homing in to myocardial areas relevant to cardiac pathways; then they integrate structurally so that myocardial function can be restored and new tissue can be produced.[25–36]

12.1.1 Potential Uses of Human Stem Cells

New drugs have been tested for safety on differentiated cells generated from human pluripotent cell lines. Cancer cell lines, for example, are used to screen potential antitumor drugs. The generation of cells and tissues can be used for cell-based therapies; replacement cells; and tissues to treat diseases such as Parkinson's and Alzheimer's diseases, spinal cord injury, stroke, burns, heart disease, diabetes, osteoarthritis, and rheumatoid arthritis.

12.1.2 Various Sources of Stem Cells

Table 12.2 lists sources of stem cells.

Technical challenges with stem cells include the ability to obtain source material (ethical, concerns, abundance, vs. rarity), ability to direct differentiation and to select out and purify desired phenotypes, engraftment and integration versus migration, tolerance versus rejection, and tumor formation *in vivo*.

12.1.3 Unique Properties of Stem Cells

Stem cells have three general properties:

1. They are capable of dividing and renewing for longer periods.
2. They are unspecialized.
3. They can give rise to specialized cell types.

Stem cells are unspecialized. They do not have any tissue-specific structures that allow them to perform specialized functions. They cannot work with their neighbors

TABLE 12.2 Sources of Stem Cells

Adult tissue
Hematopoietic stem cells
Mesenchymal stem cells
Multipotent adult progenitor cells
Neural stem cells
Muscle-derived stem cells
Pancreatic stem cells
Hepatic stem cells
Epithelial stem cells
Cord blood and placenta
Fetal tissue
Embryos (embryonic stem cells)

to pump blood through the body (like a heart muscle cell), cannot carry molecules of oxygen through the bloodstream (like a red blood cell), and cannot fire electrochemical signals to other cells that allow the body to move or speak (like a nerve cell). However, unspecialized stem cells can give rise to specialized cells, including heart muscle cells, blood cells, or nerve cells.

12.1.4 Stem Cells Can Give Rise to Specialized Cells

Unspecialized stem cells become specialized cells; the process is called differentiation. The internal signals are controlled by a cell's genes. The external signals include chemicals secreted by other cells and physical contact with neighboring cells and certain molecules in the microenvironment.[11,37] It is unknown whether internal and external signals for cell differentiation are similar for all kinds of stem cells. It is also not known whether specific sets of signals promote differentiation into specific cell types.

Stem cells are essentially the building blocks of the human body. Stem cells are capable of dividing for long periods of time; they are unspecialized but can develop into specialized cells. The earliest stem cells in the human body are those found in embryos. The stem cells inside an embryo will eventually give rise to every cell, tissue, and organ in the fetus's body. Unlike a regular cell, which can only replicate to create more of its own kind of cell, a stem cell is pluripotent. When it divides, it can make any one of the 220 different cells in the human body. Stem cells also have the capability to self-renew; they can reproduce themselves many times over.[12] Commonly, stem cells originate from two main sources, and they are embryonic stem cells (ESCs) ESCs include those found within the embryo, the fetus or the umbilical cord blood. Depending on when they are harvested, ESCs can give rise to just about any cell in the human body.

12.1.4.1 Adult Stem Cells Adult stem cells can be found in infants, children, and adults. They reside in already developed tissues such as those of the heart, brain, and kidney. They usually give rise to cells within their resident organs. However, unspecialized stem cells can give rise to specialized cells, including heart muscle cells, blood cells, or nerve cells.

12.1.5 Embryonic Stem Cells

Embryonic stem cells are the most primitive of all stem cells. They develop as the inner cell mass in the human blastocyst derived from a 4- or 5-day-old human embryo that is in the blastocyst phase of development. The embryos are usually extras that have been created in *in vitro* fertilization (IVF) clinics, where several eggs are fertilized in a test tube but only one is implanted into a woman. Sexual reproduction begins when a male's sperm fertilizes a female's ovum (egg) to form a single cell called a zygote. The single zygote cell then begins a series of divisions, forming 2, 4, 8, 16 cells and so on. After 4–6 days before implantation in the uterus, this mass of cells is called a blastocyst. The blastocyst consists of an inner

cell mass (embryoblast) and an outer cell mass (trophoblast). The outer cell mass becomes part of the placenta, and the inner cell mass is the group of cells that will differentiate to become all the structures of an adult organism. This latter mass is the source of ESCs—totipotent cells.[4,16,22,37–40]

In a normal pregnancy, the blastocyst stage continues until implantation of the embryo in the uterus, at which point the embryo is referred to as a fetus. This usually occurs by the end of the 10th week of gestation after all major organs of the body have been created. However, when extracting ESCs, the blastocyst stage signals when to isolate stem cells by placing the "inner cell mass" of the blastocyst into a culture dish containing a nutrient-rich broth. Eventually, these undifferentiated cells can be stimulated to create specialized cells. In culture, they spontaneously form cystic structures known as embryoid bodies. Lacking the necessary stimulation to differentiate, they begin to divide and replicate while maintaining their ability to become any cell type in the human body.

The beating embryoid bodies contain a mixed population of newly differentiated cell types, including cardiomyocytes, based on the expression of cardiac-specific genes such as cardiac-myosin heavy chain; cardiac troponin I and T; atrial natriuretic factor; and cardiac transcription factors GATA-4, Nkx2.5, and MEF-2; cellular ultrastructure; and extracellular electrical activity. These cardiomyocytes can be of the pacemaker atrium- and ventricle-like type, and they are distinguishable by their specific patterns of action potential. Although the precise cellular and molecular events comprising the pathway of ESC cardiomyocytes specific differentiation remain largely undetermined, significant progress has been made in identifying the regulatory factors that can enhance or inhibit the process. Differentiation into a particular cell type depends on these factors. For instance, inhibition of bone morphogenetic protein (BMP) signaling by its antagonist Noggin induces cardiomyocytes differentiation from mouse ESCs, and retinoic acid specifically induces the formation of ventricular-specific cardiomyocytes. Nitric oxide (NO), generated either by NO synthase activity or exogenous NO exposure, has also been implicated in the promotion of cardiomyocyte-specific differentiation from mouse ESCs. Cardiomyocyte differentiation of human ESCs could be enhanced by treatment with 5-aza-2′-deoxycytidine. Also, insulin-like growth factor 1 promotes cardiomyocyte differentiation phenotype and the expression of the cardiomyocyte phenotype in ESCs *in vivo*. Interestingly, increased levels of oxidative stress appear to reduce the cardiotypic development of embryoid bodies.[12,41–43] ESC-derived cardiomyocytes show macromolecular sarcomeric organization, which gives rise to calcium sparks and ionic currents and leads to functional and anatomical integration with surrounding cardiomyocytes, which leads to propagation of electrical activity as well as pacemaker activity. ESCs spontaneously differentiate into fully functionally active, fetal-like cardiomyocytes *in vitro*. Studies have shown a dose-dependent incidence of tumor formation, particularly teratocarcinoma formation[44] (Table 12.3).

Various chemicals and molecules have been used to enhance cardiomyogenic differentiation of ESCs, including retinoic acid,[4] ascorbic acid, transforming growth factor (TGF), and BMPs.[45–52] More recently, human ESC-derived cardiomyocytes have been shown to successfully engraft and electromechanically integrate when

TABLE 12.3 Markers of Stem Cell–Derived Cardiomyocyte Differentiation

Cell Type	Differentiation Agent	Markers of Differentiated Cardiomyocyte
ES cells		
ESCs	IGF-1, TGF-β	α-Sarcomeric actin, connexin 43, MHC I, sarcomeric myosin
P19 embryonal carcinoma line	5′-Azacytidine	BMP-2, BMP-4, Bmpr la, Smadl, GATA-4, Nkx2.5, cardiac troponin I, desmin
BMC		
Bone marrow (MSCs)	Insulin, ascorbic acid, dexamethasone	α-Skeletal actin, β-MHC, MLC-2v, CaV1.2, cardiac troponin I, sarcomeric tropomyosin, cardiac titin
Cardiac stem cells		
CKIT + Lin–	NA	c-kit+
isll+	NA	Csx/Nkx-2.5, GATA4
Sca-1+ cKit–	5′-Azacytidine oxytocin	High telomerase activity, Sca-1+ Csx/Nkx-2.5, GATA4, MEF-2C, α + β–MHC, MLC-2, cardiac-α actin
Cardiosphere	cKit+	Cardiac troponin I, myosin heavy chain, atrial natriuretic peptide
SP cells	NA	ATP-binding cassette transporter (ABCG2)

NA, not available.
Source: Adapted from Ref. [17].

injected into uninjured hearts. In these studies, the injected cells behaved as a biological pacemaker and electrically excited the rest of the ventricle.[7,53] Problems are large-scale generation of ESC–derived cardiomyocytes, immunologic rejection, differentiation into undesirable lineages, and ethical concerns.

12.1.6 Recommendations

The therapeutic use of ESC transplantation in cardiac diseases primarily needs a rigorous demonstration that it can work in a stable fashion and with limited adverse effects.

Despite the limitations on federally funded research presently imposed, new sources of ESCs and cell lines for ESC transplantation studies need to be developed and likely will be, given the strong worldwide corporate- and state-funded interest in this technology and its purported benefits. Investigation into novel ways to isolate and culture autologous ESCs should also prove to be of significance.

Our overall understanding of the factors that may elicit the homing of ESCs to the heart and stimulate or direct the differentiation of ESCs to functional cardiomyocytes is presently rudimentary (a critique also applicable to adult stem cells). Identification of these factors and their mechanism of action will likely optimize both the homing

and the differentiation processes and will also to contribute to defining the best-case scenarios in which ESC transplantation will be beneficial.

12.1.7 Limitations and Concerns with Embryonic Stem Cell Transplantation

Considerable ethical and legal concerns about ESCs remain, and these concerns have significantly hampered further research efforts, which could provide needed cell lines as well as answers to many of the questions regarding the efficacy, long-term stability, function, and even the extent of the negative effects of ESC transplantation in cardiovascular disease. A concern often raised regarding the use of ESCs relates to their source (i.e., whether they originate from a cell line or directly from embryo), primarily heterologous versus autologous, posing the potential problem of generating an allogenic response or immunorejection upon transplantation. In addition, pluripotent ESCs that have unlimited growth potential can have tumorigenic side effects, making the screening for teratoma formation well advised. Moreover, evidence shows that differentiation of a heterogeneous ESC population is rather inefficient, although several agents (e.g., retinoic acid) appear to be effective in activating a greater extent of ESC-mediated cardiomyocyte-specific differentiation. The long-term stability of ESC-differentiated phenotype has also received mixed reviews because several studies have shown a loss of ESC=differentiated cardiomyocytes over time[5,28,29,54] (Table 12.4).

Transplanted ESC progeny may not always have a normal function because ESCs may promote arrhythmias in the transplanted hearts. On the other hand, the application of ESCs in repairing damaged, aging hearts may also be limited. This limitation has been proposed, but currently there is not solid data to support it. Nevertheless, cell transplants (either ESCs or adult stem cells) in the hearts of older individuals have frequently proved to be less effective. The inability of the damaged myocardium to provide the appropriate molecular signals for stem cells engraftment seems to limit their capacity for recruitment and integration into the aging myocardium (Coburn et al., 2005;[56–58]).

12.2 ADULT SKELETAL MYOBLAST CELLS

Transplanted satellite stem cells (myoblasts) from skeletal muscle can successfully home and engraft within a damaged myocardium, preventing progressive ventricular dilatation and improving cardiac function.[59] These myoblasts can be delivered into the myocardium by either intramural implantation or arterial delivery, and recently, effective deployment of a less invasive catheter approach has been reported. Skeletal muscle satellite cells can proliferate abundantly in culture and can be easily grown from the patients themselves (self-derived or autologous), thereby avoiding a potential immune response. Myoblasts are relatively ischemia resistant (compared with cardiomyocytes, which become injured within 20 min) because they can withstand several hours of severe ischemia without becoming irreversibly injured. The functional benefits of intramyocardially transplanted skeletal myoblasts in

TABLE 12.4 Different Types of Stem Cells for Cardiovascular Diseases

Cell Types	Advantages	Disadvantages
Embryonic stem cells	• Pluripotent and unlimited supply • Patient-specific cells for autologous transplantation possible via therapeutic donning	• Social and ethical concerns • Risk of rejection and required immunosuppression for allogenic transplant • Limited supply of human oocytes • Risk of tumor formation • Proarrhythmic risk because of immature phenotype of derived cardiomyocyte
Induced pluripotent stem cells	• Pluripotent and unlimited supply • Patient-specific cells for autologous transplantation possible	• Risk of tumor formation • Risk of viral vector • Proarrhythmic risk due to immature phenotype of derived cardiomyocyte
Skeletal myoblast	• Autologous transplantation without the need for immunosuppression or risk of rejection • Can be expanded *in vitro* with high yield, resistant to ischemia and fatigue	• Cannot differentiate into cardiomyocyte phenotype • Lack of integration with host cardiomyocyte with arrhythmogenic potential
Bone marrow stem cells	• Autologous transplantation without the need for immunosuppression or risk of rejection • Can induce angiogenesis; possibly pluripotent	• Limited ability to differentiate into cardiomyocyte • Limited supply and need for *in vitro* expansion • Difficult to isolate and propagate in culture
Mesenchymal stem cells	• Autologous transplantation without the need for immunosuppression or risk of rejection • Can induce angiogenesis and possible pluripotent • Lower risk of rejection and possibility for allogenic transplantation	• Limited ability to differentiate into cardiomyocyte • Limited supply and need for *in vitro* expansion • Difficult to isolate and propagate in culture
Adult cardiac stem cells	• Cardiomyocyte phenotype with no need for differentiation • Can integrate with host cardiomyocyte • Autologous transplantation without the need for immunosuppression or risk of rejection	• Very limited supply • Difficult to isolate and propagate in culture • Proarrhythmic risk because of immature phenotype of derived cardiomyocyte

Source: Adapted from Ref. [55].

improving the damaged myocardium secondary to ischemia have been well documented.

Initial clinical trials have shown the efficacy of autologous skeletal myoblast transplantation in patients with left ventricular (LV) dysfunction. The use of skeletal myoblasts delivered by multiple intramyocardial injections was effective in restoring LV function in a genetically determined Syrian hamster model of dilated cardiomyopathy, demonstrating that the functional benefits of transplanted skeletal myoblast can be extended to nonischemic cardiomyopathy.[59,60]

12.2.1 Advantages to Myoblast Transplantation

Because myoblasts can be of autologous origin and can be robustly expanded in culture, a large number of cells can be obtained from only a small skeletal muscle biopsy sample in a relatively short period of time. Compared with transplanted cardiomyocytes, myoblast cells appear to be more resistant to ongoing apoptotic damage, which tends to be prevalent at ischemic sites. Skeletal myoblasts were the first cells to be tried for cell-based cardiac therapy. They do not form tumors as with ESCs. Moreover, they can be easily handled and expanded *in vitro* (millions of myoblasts can be grown from a single muscle biopsy within a relatively short time). Myoblasts after injection into the infarcted heart have been shown to exhibit long-term engraftment;[61,62].

12.2.2 Disadvantages with Skeletal Myoblasts

Skeletal myoblasts do not adopt a cardiomyogenic differentiation .Moreover, they lack gap junctions, have not been shown to integrate electromechanically with the surrounding myocardium. They do not beat in synchrony and are isolated from the rest of the myocardium. Clinical trials of myoblast therapy have shown improvements in ejection fraction that persist 10 months following injection.[63,64]

12.2.3 Further Recommendations

Although preclinical studies with stem cell and myoblast transplantation have shown similar levels of efficacy, there is a need for a detailed evaluation on the relative benefits, adverse effects, and efficiency of skeletal myoblast and stem cell transplants in the clinical setting (e.g., heart failure) vis a vis the restoration of myocardial function. New methods to better assess and optimize posttransplanted myoblast recruitment and survival, particularly in the long term, need to be developed, and the repertoire of effective, less invasive cell delivery technologies needs to be expanded.

12.3 ADULT BONE MARROW–DERIVED STEM CELLS

The bone marrow contains a varied assortment of progenitor cells, including hematopoietic stem cells (HSCs), mesenchymal stem cells (MSCs), multipotent

adult progenitor cells, and side population (SP) cells. Interest in bone marrow–derived stem cells has been mainly motivated by their neovascularization and angiogenesis properties, and these effects are enhanced by the presence of specific growth factors and cytokines (e.g., granulocyte colony-stimulating factor). Orlic and coworkers reported that after injection of bone marrow hematopoietic progenitors into the infarcted heart, the infarcted heart regenerated 68% of the myocardium and improved echocardiographic and hemodynamic indices of LV function.[65,66] Bone marrow progenitor cells evidently improve cardiac performance after myocardial injury.

It is important to point out that bone marrow contains several stem cell populations with overlapping phenotypes, including HSCs, endothelial stem/precursor cells (EPCs), mesenchymal stem cells (MSCs), and multipotent adult progenitor cells (MAPss). When endothelial progenitor cells originating from a common hemangioblast precursor in bone marrow, are delivered to the myocardial target area, they may implant, differentiate *in situ*, and promote new vessel growth, an approach that has been applied to several animal models of myocardial ischemia. These bone marrow–derived stem/precursor cells also can prevent the progression of cardiomyocytes, apoptosis, and stem cardiac remodeling.[65–69]

12.3.1 Advantages of Adult Bone Marrow Cell Transplantation

There is evidence that treatment with bone marrow cells (BMCs) can ameliorate both myocardial and vascular damage with increasing angiogenesis.[70] The effect of transplanted BMCs (which can include endothelial precursor cells) on vascular growth may significantly impact the recovery of the damaged heart (i.e., by improving oxygen availability), although this may depend on the myocardial setting, whether acute myocardial infarction or established heart failure. Moreover, autologous-derived cells for transplantation are an attractive alternative because bone marrow mesenchymal cells can be readily isolated in most cases. In addition, the expansion of BMC number by *in vitro* growth can be readily achieved by vigorous growth of mesenchymal cells in culture. It is significant that this method bypasses much of the ethical and legal concerns associated with the use of ESCs. Currently, it seems that MSCs likely exert their beneficial effects through the mechanisms pertaining to myocardial protection, ventricular remodeling, angiogenesis, and possibly myocyte regeneration.[71] The advantages are the immunotolerant properties stem cells (i.e., easy handling, their ability to home to injured myocardium after systemic delivery) have made them attractive tools for cardiac regenerative therapy (Beeres et al., 2003).

12.3.2 Limitations and Concerns with Adult Bone Marrow Cell Transplant

The mechanism of BMC-mediated augmentation of cardiomyocyte number and function remains controversial. Some studies have suggested that the effects of adult stem cell transplantation on the recipient heart are not a consequence of transdifferentiation but likely arise as a result of cell fusion with preexisting

cardiomyocytes or occur as a function of paracrine effects of transfected cells. Others maintain that there is evidence for a transdifferentiation event. Cell fusion has been demonstrated between cardiomyocytes and noncardiomyocytes *in vivo* and *in vitro*, and the data in support of transdifferentiation (particularly with HSCs) have not always been replicable. Further research is needed to clarify these issues and reconcile the contradictory claims as well as provide additional information about the extent of cell fusion and when it occurs. A limitation of the majority of the clinical studies with adult noncardiac stem cell transplantation relates to the potential stability of the differentiated phenotype because these studies have primarily examined the short-term benefits.[72]

12.3.3 Resident Cardiac Progenitor Cells

Cardiac progenitor cell are capable of multilineage differentiation. Beltrami and coworkers[73] were the first to describe the existence of a resident cardiac progenitor cell (CPC) capable of differentiating into myocytes as well as endothelial and smooth muscle cells. It has been shown that when these resident cardiac progenitor cells are injected into the postinfarct heart, there is some improvement of cardiac function.[74]

12.3.4 Adult Stem Cells

Adult or somatic stem cells exist throughout the body after embryonic development and are found inside of different types of tissue. These stem cells have been found in tissues such as the brain, bone marrow, blood, blood vessels, skeletal muscles, skin, and liver. They remain in a quiescent or nondividing state for years until activated by disease or tissue injury. They are multi(pluri)potent.[73,75]

Adult stem cells are unique cells that can divide or self-renew indefinitely, enabling them to generate a range of cell types from the originating organ or even regenerate the entire original organ. They have the ability to differentiate through a committed lineage. It is generally thought that adult stem cells are limited in their ability to differentiate based on their tissue of origin, but some evidence suggests that they can differentiate to become other cell types. These adult stem cells comprise at least three different groups: bone marrow–derived stem cells, the circulating pool of stem or progenitor cells, and tissue resident stem cells Adult stem cells typically generate the cell types of the tissue in which they reside (e.g., a blood-forming adult stem cell in the bone marrow), and they normally gives rise to the many types of blood cells such as red blood cells, white blood cells, and platelets. Stem cells from one type of tissue may be able to give rise to cell types of a completely different tissue; that is, they exhibit plasticity (e.g., blood cells becoming neurons and heart muscle, liver cells that can be made to produce insulin).

Stem cells, depending on their lineage commitment, possess the ability to differentiate into cells of various tissues; this property of stem cell is called differentiation. Embryonic cells are pluripotent and can generate tissues belonging to all three germ layers. Adult stem cells are thought to be more committed and

possess a limited ability to differentiate along a specific lineage. In contrast to ESCs, differentiation of adult bone marrow stem cells into functional cardiomyocytes has been more difficult to demonstrate. *Transdifferentiation* is a term used to define a committed stem cell crossing lineage boundaries and differentiating into cells belonging to another lineage. An HSC giving rise to cardiomyocytes is an example of transdifferentiation. *Fusion* refers to the phenomenon in which stem cells fuse with somatic cells; the resultant hybrid cells usually assume the more undifferentiated phenotype but possess some characteristics of both cell types. For example, bone marrow cells, when grown in culture with ESCs, could fuse with ESCs and adopt the recipient phenotype. Stem cells can regulate tissue regeneration and repair. Apart from angiogenesis and wound healing, stem cells could exert a host of other paracrine effects on myocardial protection, cardiac contractility, myogenic differentiation of resident cardiac progenitors, and scar formation.[19,76]

12.3.5 Advantages of Adult Stem Cells

Although the implantation of skeletal myoblasts and adult BMC transplantation appears promising, adult stem cell transplantation might be more effective than adult BMC transplantation because cardiac stem cells may be better programmed. The further identification, purification, and characterization of the adult stem cells as well as a detailed knowledge of their interactions with the cardiac milieu or niche are essential if we are to achieve the major goal of regenerating or transplanting the tissue to treat myocardial infarction.

12.3.6 Limitations of Adult Stem Cells

Until recently, data on the presence of adult stem cells have been scarce. This subset of stem cells appears to be extremely limited in number and difficult to identify and expand in culture, thereby limiting their characterization and utilization and likely contributing to difficulties in reproducing experiments concerning their isolation and transplantation. In addition, there is presently no consensus regarding the definition of selective markers specific for adult stem cell type.

12.3.7 Culturing Embryonic Stem Cells in the Laboratory

Human ESCs are isolated by transferring the inner cell mass to a plastic laboratory culture dish that contains a nutrient broth known as culture medium. The cells divide and spread over the surface of the dish. The inner surface of the culture dish is typically coated with mouse embryonic skin cells that have been treated so they will not divide. This coating layer of cells is called a *feeder layer*. This layer provides a sticky surface for the inner cell mass to which they can attach. Also, the feeder cells release nutrients into the culture medium. Culture media without the feeder cell layer is also available, so there are no chances of transmission of viral disease. ESCs that have proliferated in cell culture for 6 or more months without differentiating, are

pluripotent, and appear genetically normal are referred to as an ESC line.[77] After cell lines are established, or even before that stage, batches of them can be frozen and shipped to other laboratories for further culture and experimentation. The process of growing large numbers of ESCs has been easier than growing large numbers of adult stem cells, but progress is being made for both cell types.

12.3.8 Stem Cell Lines

After stem cells have been allowed to divide and propagate in a controlled culture, the collection of healthy, dividing, and undifferentiated cells is called a stem cell line. These stem cell lines are subsequently managed and shared among researchers. When under control, the stem cells can be stimulated to specialize as directed by a researcher, a process known as directed differentiation. ESCs are able to differentiate into more cell types than adult stem cells.[78]

12.3.9 Tests Used to Identify Embryonic Stem Cells

During the process of generating embryonic stem cell lines to see whether they exhibit the fundamental properties that makes them embryonic stem cells, various tests are carried out, this process is called *characterization*. The process includes growing and subculturing the stem cells for many months. This ensures that the cells are capable of long-term self-renewal. Microscopy used to see that the cells look healthy and remain undifferentiated. Surface markers are found only on undifferentiated stem cells.[77]

12.3.10 Tests Used in Identifying Adult Stem Cells

Labeling the cells in a living tissue with molecular markers and then determining the specialized cell types they generate, removing the cells from a living animal, labeling them in cell culture, and transplanting them back into another animal to determine whether the cells repopulate their tissue of origin. Isolating the cells, growing them in cell culture, and manipulating them, often by adding growth factors or introducing new genes, to determine what differentiated cells types they can become.

12.4 TYPE OF STEM CELLS USED TO TREAT CARDIAC DISEASES

A brief comparison of the advantages and limitations of the cell types presently used in cardiac transplantation is shown in Table 12.5. Although no clear-cut choice has yet emerged as to which cell type is best to transplant in myocardial repair, there are reasons to believe that the development of a multiplicity of approaches in the application of cell engineering will be required to develop novel therapies for different cardiac disorders.

The approach to treat heart failure may require the transplantation of cell types (e.g., skeletal myoblasts) that are different than those used in the targeted treatment of cardiac arrhythmias, conduction disorders, and congenital defects. It is also

TABLE 12.5 Advantages and Disadvantages of Potential Clinical Strategies for Stem Cell Delivery

Strategies	Potential Clinical Applications	Clinical Experience	Advantages	Disadvantages
Intravenous	Acute MI	+/−	• Easy available and avoids the risk of any invasive procedure	• Low efficacy of cell delivery • Not applicable to patients with an occluded • Risk of systemic administration unclear
Intracoronary	Acute MI Chronic MI	+++ +	• Possible wide use in catheterization laboratory • Limited risk of systemic administration • Clinical trials ongoing	• Efficacy of cell delivery to the myocardium uncertain • Not applicable to patients with a occluded artery • Not applicable for stem cell with large cells
Catheter-based direct intramyocardial injection	Chronic myocardial ischemia Acute MI	+++ +	• Avoid the risk of open heart surgery • Higher efficacy of cell delivery • Short-term safety proven • Clinical trials ongoing	• Need for specialized catheters and imaging technology to guide the procedure
Open heart direct epicardial injection	Chronic myocardial ischemia Chronic MI	++ ++	• Applicable to patients who need open heart surgery • Allows direct visualization of the site of injection	• Risk of mortality and morbidity of open heart surgery
Direct epicardial patch	Chronic MI	–	• Applicable to patients who need open heart surgery • Allows uneven distribution of cell in the myocardium • Allows direct delivery of a large amount of cell	• Needs tissue engineering to create cellular patch • Risk of mortality and morbidity of open heart surgery

Source: Adapted from Ref. [80].

possible that the long-term repair of a fully functioning myocardium may require more than a single cell type (e.g., cardiomyocytes, fibroblasts, and endothelial cells) in the generation and integration of a stable and responsive cardiac graft.[13,30,31,84–87]

12.4.1 Potency

Stem cells are categorized by their potential to differentiate into other types of cells. ESCs are the most potent because they must become every type of cell in the body. The full classification includes totipotent, pluripotent, multipotent, oligopotent, and unipotent. *Totipotent* is the ability to differentiate into all possible cell types. Examples are the zygote formed at egg fertilization and the first few cells that result from the division of the zygote. *Pluripotent* is the ability to differentiate into almost all cell types. Examples include ESCs and cells that are derived from the mesoderm, endoderm, and ectoderm germ layers that are formed in the beginning stages of ESC differentiation. *Multipotent* is the ability to differentiate into a closely related family of cells. Examples include hematopoietic (adult) stem cells that can become red and white blood cells or platelets. *Oligopotent* is the ability to differentiate into a few cells. Examples include (adult) lymphoid or myeloid stem cells. *Unipotent* is the ability to only produce cells of their own type but having the property of self-renewal required to be labeled a stem cell. Examples include (adult) muscle stem cells. ESCs are considered pluripotent instead of totipotent because they do not have the ability to become part of the extraembryonic membranes or the placenta.

12.4.2 Identification of Stem Cells

Although there is not complete agreement among scientists of how to identify stem cells, most tests are based on making sure that stem cells are undifferentiated and capable of self-renewal. Tests are often conducted in the laboratory to check for these properties. The standard procedure for testing bone marrow or HSCs is by transplanting one cell to save an individual without HSCs. If the stem cell produces new blood and immune cells, it demonstrates its potency. Clonogenic assays (a laboratory procedure) can also be used *in vitro* to test whether single cells can differentiate and self-renew. Researchers may also inspect cells under a microscope to see if they are healthy and undifferentiated, or they may examine chromosomes. To test whether human ESCs are pluripotent, scientists allow the cells to differentiate spontaneously in cell culture, manipulate the cells so they will differentiate to form specific cell types, or inject the cells into an immunosuppressed mouse to test for the formation of a teratoma (a benign tumor containing a mixture of differentiated cells).

12.4.3 Mechanisms of Action of Stem Cells

A co-culture technique was developed whereby stem or progenitor cells are cultured together with rat neonatal ventricular cardiomyocytes to simulate a cardiac like environment *in vitro*. The neurohormone oxytocin or cytokines of the Wnt or

platelet-derived growth factor (PDGF) family have been used to induce or enhance differentiation of adult stem cells. The identification of subsets of adult stem cells with a higher capacity to differentiate into cardiac myocytes is currently under investigation. Progenitor cells may improve neovascularization and thereby augment nutrients and oxygen supply. Neovascularization can be mediated by the physical incorporation of progenitor cells into new capillaries or by perivascular accumulation of cells. Incorporated progenitor cells of most, if not all, types may release growth factors that promote angiogenesis by acting on mature endothelial cells. Paracrine factors may also beneficially influence cardiac repair by protecting cardiomyocytes from apoptotic stimuli or activate cardiac resident stem cells to enhance the endogenous repair capacity. The release of various cytokines affects the cardiac remodeling processes by altering the development of fibrosis development during scar formation or by modulating inflammatory processes .The extent to which progenitor cells contribute to vasculogenesis depends on the environment to which the cells are exposed. Thus stem cells mechanisms to be considered include transdifferentiation of stem cells, enhanced neovascularization, alterations in scar formation, and cytoprotection.

12.4.4 Immunomodulatory Effect of Stem Cells

Numerous studies have demonstrated that human mesenchymal stem cells (hMSCs) avoid allorecognition, interfere with dendritic cell and T-cell function, and generate a local immunosuppressive microenvironment by secreting cytokines. It has also been shown that the immunomodulatory function of human MSCs is enhanced when the cells are exposed to an inflammatory environment characterized by the presence of elevated local interferon-γ (INF-γ) levels. Other studies contradict some of these findings, reflecting both the highly heterogeneous nature of MSC isolates and the considerable differences between isolates generated by the many different methods under development.

Mesenchymal stem cells are multipotential nonhematopoietic progenitor cells capable of differentiating into multiple mesenchymal tissues. hMSCs are characterized by a low expression of major histocompatibility complex (MHC) class I and the absence of co-stimulatory molecules such as CD80, CD86, or CD40. Moreover, hMSCs fail to induce proliferation of allogeneic or xenogeneic lymphocytes.

These characteristics support the possibility of exploiting universal donor MSC for therapeutic applications. MSCs constitutively express low levels of MHC-I molecules, but, as a general rule, they do not constitutively express MHC class II molecules However, recent evidence indicates that MSC can function as antigen-presenting cells and activate immune responses under appropriate conditions. Although one study reported constitutive MHC class II expression on MSC, several groups reported that both MHC class I and class II molecules are upregulated after IFN-γ treatment, thus inducing a T-cell response to recall antigens.

Mesenchymal stem cells have an immunomodulatory effect, which is currently being exploited in the clinical setting for the treatment of coronary artery diseases.

12.4.4.1 Drugs That Have Immunomodulatory Effects on T Cells and Dendritic Cells A new group of tyrosine kinase inhibitors, exemplified by the Bcr-Abl inhibitor imatinib, avoids the side effects of systemic chemotherapies and the high morbidity and mortality risks associated with HSC transplantation. Concurrently, however, increasing evidence has emerged to indicate that these drugs exert profound immunomodulatory effects on T cells and antigen-presenting cells, such as dendritic cells, that play major roles in immune tumor surveillance and the outcome of HSC transplantation. Targeted tyrosine kinase inhibitor therapy may thus control cancer cell growth both directly and indirectly by changing the immunologic microenvironment. Furthermore, such molecules might help to unravel the complexities of the human infectious processes.

12.4.4.2 Immunomodulatory Properties of Mesenchymal Stem Cells Derived from Dental Pulp and Dental Follicle are Susceptible to Activation by Toll-Like Receptor Agonists Adult MSCs have recently become a potent tool in regenerative medicine. Because of certain shortcomings of obtaining bone marrow MSCs, alternate sources of MSCs have been sought. MSCs from dental pulp (DP-MSCs) and dental follicle (DF-MSCs), isolated from the same tooth or donor, to define differences in their phenotypic properties, differentiation potential, and immunomodulatory activities. Both cell types showed colony-forming ability and expressed typical MSCs markers but differed in the levels of their expression. DF-MSCs proliferated faster, contained cells larger in diameter, and exhibited a higher potential to form adipocytes and a lower potential to form chondrocytes and osteoblasts compared with DP-MSCs.

In contrast to DF-MSCs, DP-MSCs produced TGF-β and suppressed proliferation of peripheral blood mononuclear cells (PBMCs), which could be neutralized with anti–TGF-β antibody. The treatment with Toll-like receptor 3 (TLR3) agonist augmented the suppressive potential of both cell types and potentiated TGF-β and interleukin-6 secretions by these cells. TLR4 agonist augmented the suppressive potential of DF-MSCs and increased TGF-β production but abrogated the immunosuppressive activity of DP-MSCs by inhibiting TGF-β production and the expression of indolamine-2,3-dioxygenase-1. Some of these effects correlated with the higher expression of TLR3 and TLR4 by DP-MSCs compared with DF-MSCs. Dental MSCs are functionally different, and each of these functions should be further explored *in vivo* before their specific biomedical applications are used.

12.5 APPLICATION

12.5.1 Routes of Application

Progenitor cells for cardiac repair can be delivered in different ways via the intracoronary route or by direct injection into the myocardium using a percutaneous catheter-based or surgical epicardial approach. Intracoronary infusion using standard balloon catheters has been used in all clinical trials treating patients with acute

myocardial infarction. The advantage is that cells can travel directly only into myocardial regions in which nutrient blood flow and oxygen supply are preserved, thereby ensuring a favorable environment for cell survival, a prerequisite for stable engraftment.

Trials using the intracoronary approach have administered the cells in the culprit artery from 5 to 14 days after MI. Direct intracoronary injection after revascularization has the obvious advantage of the cells reaching previously underperfused regions of the myocardium. Potentially, the perfused myocardium also creates a more suitable environment for engraftment of the progenitor cells. In the heart, less perfused regions of the myocardium receive fewer cells; thus, this route may be inefficient for successful targeting of underperfused myocardium, an important consideration for patients with extensive microvascular disease. The type of stem cell administered is another important consideration for selecting the route of administration.[88–95]

Skeletal myoblasts are larger cells and may even obstruct the microcirculation and lead to greater injury. A decrease in distal blood flow and embolic risk may be clinically significant, especially if the cells are administered at the time of primary revascularization after MI. Direct injection of stem cells into the heart may obviate the problems of decreased uptake of cells in less perfused regions of the myocardium but runs risks of cardiac perforation. Moreover, the necrotic, hypoxic, and inflamed myocardium, into which the cells are directly injected, may not provide the cells with the best microenvironment for effective tissue repair. Intraarterially, homing of intraarterially applied progenitor cells requires migration out of the vasculature into the surrounding tissue, which may mean that unperfused regions of myocardium will be targeted far less efficiently, if at all. Bone marrow–derived and blood-derived progenitor cells are known to extravasate and migrate to ischemic areas. Other cell types may not, and they may even obstruct the microcirculation, which leads to embolic myocardial damage. Clinical trials of cardiac cell therapy suggest that injection of stem cells at least 4 days after myocardial injury leads to improvement in ejection fraction compared with earlier injection. Focal injection of stem cells may not be the optimal route in diseases that affect the myocardium more globally such as nonischemic dilated cardiomyopathy.

12.5.2 Complications

The risk for ventricular perforation is high. Most cells, if injected directly, simply die. In diffuse disease such as dilated nonischemic cardiomyopathy because focal deposits of directly injected cells might be poorly matched to the underlying anatomy and physiology, so additional strategies to augment cell homing and to promote homogeneous integration of cells may be required.

12.5.3 Using Stem Cells in Clinical Application and to Treat Disease

Currently, a variety of autologous adult progenitor cells are undergoing clinical evaluation. The first clinically relevant cells proposed for cardiac myocytes were

skeletal muscle myoblasts, undifferentiated proliferation competent cells that serve as precursors to skeletal muscle. For clinical use, autologous human myoblasts are isolated from skeletal muscle biopsies, propagated and expanded *ex vivo* for a few days or weeks, and then injected directly into the ventricular wall. Bone marrow is, at present, the most frequent source of stem cells used clinically for cardiac repair. Bone marrow is aspirated under local anesthesia in most of the studies; the entire mononuclear cell fraction is obtained. Isolated bone marrow–derived cells have been injected into the heart without further *ex vivo* expansion. These circulating "EPCs" are the basis for virtually all clinical studies that have used bone marrow or its circulating derivatives for the treatment of ischemic myocardium.[8,9,11,14,15,30,31,43,55,57,96–98]

The first step in using stem cells for disease treatment is to establish stem cell lines, which researchers have accomplished. Next, scientists must be able to turn on specific genes within the stem cells so that the stem cells will differentiate into any cell they wish. But scientists have not learned how to do this yet, so studying stem cell differentiation is an active area of research. When scientists are able to create differentiated cells from stem cells, then there are many possibilities for their use, such as drug testing and cell-based therapies. For example, let us say you want to test new drugs to treat heart diseases. Currently, new drugs must be tested on animals. The data from animal research must be interpreted and then extrapolated to humans before human clinical trials. But suppose you could test them directly on human heart cells. To do this, human stem cell lines could be treated to differentiate into human heart cells in a dish. The potential drugs could be tested on those cells, and the data would be directly applicable to humans. This use could save vast amounts of time and money in bringing new drugs to market.

Stem cell–based therapies are not new. The first stem cell–based therapy was a bone marrow transplant used to treat leukemia. In this procedure, the patient's existing bone marrow is destroyed by radiation, chemotherapy, or both. Donor bone marrow is injected into the patient, and the bone marrow stem cells establish themselves in the patient's bones. The donor bone marrow cells differentiate into blood cells that the patient needs. Often, the patient must take drugs to prevent his or her immune system from rejecting the new bone marrow. But this procedure uses existing hemopoietic stem cells. How would you use stem cell lines? Let us look at how stem cells might be used to treat heart failure.

Ideally, to treat a failing heart, scientists could stimulate stem cells to differentiate into heart cells and inject them into the patient's damaged heart. There, the new heart cells could grow and repair the damaged tissue. Although scientists cannot yet direct stem cells to differentiate into heart cells, they have tested this idea in mice. They have injected stem cells (adult, embryonic) into mice with damaged hearts. The cells grew in the damaged heart cells, and the mice showed improved heart function and blood flow. In these experiments, exactly how the stem cells improved heart function remains controversial. They may have directly regenerated new muscle cells. Alternatively, they may have stimulated the formation of new blood vessels into the damaged areas. And the new blood flow may have stimulated existing heart stem cells to differentiate into new heart muscle cells. These experiments are currently being evaluated.

One major obstacle in stem cell use is the problem of rejection. If a patient is injected with stem cells taken from a donated embryo, his or her immune system may see the cells as foreign invaders and launch an attack against them. Using adult stem cells or induced pluripotent stem cells (IPSCs) could overcome this problem somewhat because stem cells taken from the patient would not be rejected by his or her immune system; however, the adult stem cells are less flexible than ESCs and are harder to manipulate in the laboratory.[99] IPSC technology is too new for transplantation work.[100]

Finally, by studying how stem cells differentiate into specialized cells, the information gained can be used to understand how birth defects occur and possibly how to treat them. So, if there is so much potential in stem cell research, why all the controversy? Let us investigate the current ethical and political issues.[5,27,28,29,38,56,71,101–111]

12.5.4 Results of Clinical Trials

In patients with acute myocardial infarction, progenitor cell transplantation aims to prevent or ameliorate postinfarction LV remodeling, thereby reducing postinfarction HF. Such an effect might be achieved by enhanced neovascularization and reduced cardiomyocyte apoptosis, irrespective of long-term engraftment and transdifferentiation. In patients with chronic HF, cardiomyogenesis in its pure sense would be desirable.[58,85,91,96,112–115]

12.5.5 Cell Therapy in Acute Myocardial Infarction

Infusion of autologous bone marrow mononuclear cells (BMCs) is safe and feasible in patients with acute myocardial infarction, which is supported by the TOPCARE-AMI, the BOOST trial, which showed there is improvement in global LV ejection fraction by 7% to 9%, and there is significant reduction in LV end-systolic volume, which has improved perfusion in the infarcted area in 4–6 months after cell transplantation. Whereas the randomized, controlled trial by Janssen did not reveal a significant effect on global ejection fraction, it did show an increase in regional ejection fraction and a reduction of the infarct size in the BMC group. Another trial, ASTAMI (Autologous Stem Cell Transplantation in Acute Myocardial Infarction), did not show any benefit.

Overall, the clinical data available indicate that cell therapy with bone marrow–derived cells is feasible and safe at least for the follow-up presently available (up to 5 years in the pioneering studies). None of the studies so far reported an increased incidence of arrhythmias (as has been seen in myoblast trials). Moreover, restenosis, which was considered as a potential side effect by progenitor cell–mediated plaque angiogenesis or plaque inflammation, was increased only in one study using $CD133^+$ cells, Because $CD133^+$ cells were isolated by using a mouse antibody, one may speculate that the remaining antibody might have elicited a local proinflammatory reaction. All other studies did not observe an augmented risk for restenosis; if anything, there was a significantly decreased necessity for revascularization

procedures in the REPAIR-AMI trial. Careful evaluation of the 18-month follow-up data of the BOOST trial indicates that the ejection fraction of the cell therapy group is maintained from 6 to 18 months of follow-up. The long-term 2 year follow-up MRI-derived data of the TOPCARE-AMI trial showed that the ejection fraction is maintained and even further augmented in the treated patients, in parallel with a sustained reduction in NT pro-BNP serum levels, suggesting a beneficial effect of long-term LV remodeling. Taken together, these data may provide the rationale to assess the effects of intracoronary BMC infusion on clinical outcome in a large patient cohort with severe acute myocardial infarction.[14,111,116–126]

12.5.6 Research with Stem Cells

Scientists and researchers are interested in stem cells for several reasons. Although stem cells do not serve any one function, many have the capacity to serve any function after they are instructed to specialize. Every cell in the body, for example, is derived from first few stem cells formed in the early stages of embryologic development. Therefore, stem cells extracted from embryos can be induced to become any desired cell type. This property makes stem cells powerful enough to regenerate damaged tissue under the right conditions.

12.5.7 Organ and Tissue Regeneration

Tissue regeneration is probably the most important possible application of stem cell research. Currently, organs must be donated and transplanted, but the demand for organs far exceeds supply. Stem cells could potentially be used to grow a particular type of tissue or organ if directed to differentiate in a certain way. Stem cells that lie just beneath the skin, for example, have been used to engineer new skin tissue that can be grafted on to burn victims.[127,128]

12.5.8 Brain Disease Treatment

Additionally, replacement cells and tissues may be used to treat brain disease such as Parkinson's and Alzheimer's diseases by replenishing damaged tissue, bringing back the specialized brain cells that keep unneeded muscles from moving. ESCs have recently been directed to differentiate into these types of cells, so treatments are promising.[129–131]

12.5.9 Cell Deficiency Therapy

Healthy heart cells developed in a laboratory may one day be transplanted into patients with heart disease, repopulating the heart with healthy tissue. Similarly, people with type I diabetes may receive pancreatic cells to replace the insulin-producing cells that have been lost or destroyed by the patient's own immune system. The only current therapy is a pancreatic transplant, and it is unlikely to occur because of a small supply of pancreases available for transplant.[130,132–136]

12.5.10 Blood Disease Treatments

Adult HSCs found in blood and bone marrow have been used for years to treat diseases such as leukemia, sickle cell anemia, and other immunodeficiencies. These cells are capable of producing all blood cell types, such as red blood cells that carry oxygen to white blood cells that fight disease. Difficulties arise in the extraction of these cells through the use of invasive bone marrow transplants. However, HSCs have also been found in umbilical cords and placentas. This has led some scientists to call for an umbilical cord blood bank to make these powerful cells more easily obtainable and to decrease the chances of a body's rejecting therapy.[137,138]

12.5.11 General Scientific Discovery

Stem cell research is also useful for learning about human development. Undifferentiated stem cells eventually differentiate partly because a particular gene is turned on or off. Stem cell researchers may help to clarify the role that genes play in determining what genetic traits or mutations we receive. Cancer and other birth defects are also affected by abnormal cell division and differentiation. New therapies for diseases may be developed if we better understand how these agents attack the human body. Another reason why stem cell research is being pursued is to develop new drugs. Scientists could measure a drug's effect on healthy, normal tissue by testing the drug on tissue grown from stem cells rather than testing the drug on human volunteers.[139,140]

12.5.12 Transplantation and Left Ventricular Devices

Left ventricular devices to an earlier stage of heart failure when candidates with the highest risk can be avoided. By ratcheting back on the severity of illness, it is believed that perioperative mortality rate and complications will be reduced to a minimum, and the treatment will be a test of the pump's reliability and biocompatibility versus the best available medical therapy. A number of micropumps are under development to target less ill patients. They are designed for implantation either by a minimally invasive surgical procedure or, remarkably, by interventional catheter-based techniques. Their small size places the optimal rates of flow in the range of 2–3 l/min. These devices, unlike larger pumps that replace LV function, are designed to assist and more correctly are defined as ventricular assist devices. One can only conjecture as to whether these types of devices will reduce progression of heart failure or even reverse remodel early-stage disease.

12.6 OTHER DEVELOPING TECHNOLOGIES IN CELL ENGINEERING

12.6.1 Hybrid Embryos

British scientists plan to create the world's first human stem cells from embryos that are part human and part animal.

Human skin cells will fuse with empty pig eggs to create embryos that contain 99.9% human DNA and 0.1% pig DNA. Stem cells extracted from the embryos will then be treated with chemicals to destroy the pig DNA before they are grown into human heart cells. The animal DNA is destroyed to make the cells behave more like human cells.

This will represent a landmark in stem cell science and give researchers a way to make almost unlimited stocks of human ESCs, which in principle can grow into any tissue in the body. Scientists have so far been unable to create stem cells using human eggs, which are in short supply.

Although the stem cells will not contain any animal DNA, they will not be suitable for treating humans directly. Instead, the scientists will use the cells to learn how genetic mutations cause heart cells to malfunction and ultimately cause life-threatening cardiomyopathy. Ultimately, they will help us understand where some of the problems associated with these diseases arise, and they could also provide models for the pharmaceutical industry to test new drugs.

12.6.2 Upcoming Techniques in Guidance to Homing of Stem Cell

Adipose tissue is another rich source of distinct subsets of stem and progenitor cells that are potentially useful for cardiac repair and neovascularization improvement. Both mesenchymal stem cells and endothelial progenitor cells have been isolated after enzymatic digestion of adipose tissue and showed beneficial effects in experimental studies. Very recently, pluripotent spermatogonial stem cells from adult mouse testis that possess the capacity to differentiate to fully active cardiac myocytes *in vitro* have been identified.

In diffuse disease such as dilated nonischemic cardiomyopathy, focal deposits of directly injected cells might be poorly matched to the underlying anatomy and physiology. Thus, it is likely that the nature of the patient's cardiomyopathy will ultimately influence, if not dictate, the source and route chosen among potential progenitor cell therapies. Intravenous administration of cells may be hampered by trapping of the cells in the pulmonary circulation. Indeed, in clinical trials with labeled bone marrow–derived cells, no homing to the heart in acute myocardial infarction was observed after intravenous cell administration. However, intravenous application of allogeneic mesenchymal stem cells was used safely and is currently being tested in a clinical phase II study.

Randomized controlled trials currently assessing the effects of intracoronary administration of BMCs in patients with acute myocardial infarction: are the REGENT (Myocardial Regeneration by Intracoronary Infusion of Selected Population of Stem Cells in Acute Myocardial Infarction), FINCELL (Effects of Intracoronary Injection of Mononuclear Bone Marrow Cells on LV Function, Arrhythmia Risk Profile, and Restenosis After Thrombolytic Therapy of Acute Myocardial Infarction), and ASTAMI (Autologous Stem Cell Transplantation in Acute Myocardial Infarction) studies (Tables 12.6–12.8).

Three of the trials were placebo controlled. The primary end point common to all these trials was change in LVEF at 4 to 6 months. In four of the trials, recovery of global LVEF was significantly greater in the BMC-treated patient group compared

TABLE 12.6 Randomized Controlled Trials in Acute Myocardial Infarction

	No. of Patients	Cell Types and Numbers ($\times 10^6$)	Time to Therapy (Days)	Follow-up Duration (Months)	Primary End Point	LVEF (%)	Infarct Size	Side Effects
BOOST-2 trial	33 versus 30 controls	2460 BMC with gelatine–polysuccinate density gradient (9.5 $CD34^+$)	4.8	18	LVEF (MRI), safety	6 months: +6.7% versus +0.7% (↑ 6%) 18 months: +5.9% versus +3.1% (NS)	NS	Nil
Jannsens et al.	33 versus 34 controls	304 BMC with Ficoll (2.8 $CD34^+$)	1	4	LVEF (MRI)	+3.4% versus +2.2% (NS)	↓	Nil
ASTAMI	50 versus 50 controls	68 BMC with Lymphoprep Ficoll (0.7 $CD34^+$)	6	6	LVEF infarct size (MRI, SPECT, ECHO)	+1.2% versus +4.3% (NS)	NS	Nil
MAGIC CELL-3-DES	25 versus 25 controls	1500 G-CSF mobilized PBC (7×10^6 $CD34^+$)	4	6	LVEF (MRI)	+5.5% versus 0% (↑ 5.5%)	↓	Nil
REPAIR-AMI	95 versus 92 controls	236 BMC with Ficoll (3.6 $CD34^+$)	4.4	12	LVEF (LV angiography)	5.5% versus + 3.0% (↑ 2.5%)	NS	Nil
Meluzin et al.	44 (high and low) versus 22 controls	High: 100 BMC Low: 10 BMC	7	3	LVEF (SPECT)	High: +5%; low: +3% Controls: 2% (↑2.0%)	NS	Nil
REGENT	117 (selected and unselected) versus 20 controls	Unselected: 178 BMC Selected: 1.9 $CD34/CXCR4^+$	7	6	LVEF (MRI)	Unselected +3% Selected: +3% Control: 0% (NS)	NA	Nil
FINCELL	40 versus 40 controls	402 BMC	2–6	12	LVEF (ECHO)	+7.1% versus +1.2%	NA	Nil

BMC, bone marrow cells; g-CSF, granulocyte colony simulating factor; PBC, peripheral blood cells; LVEF, left ventricular ejection fraction; MRI, magnetic resonance imaging; NA, not available; NS, non-significant.
Source: Adapted from Ref. [80].

TABLE 12.7 Randomized Controlled Trials in Chronic Myocardial Ischemia

	No. of Patients	Cell Types and Numbers ($\times 10^6$)	Follow-up Duration (Months)	Primary End Points	Secondary End Points	Side Effects
Losordo et al.	18 versus 6 controls	0 05–0.5 per kg G-CSF mobilized PBC	6	Anginal frequency: −12 6 versus −4.5 (↓8.1 NS) Safety: NS	SPECT perfusion score −1.5 versus −2.2 (NS) Exercise time: +0.5 versus +0.3 min (NS)	Nil
PROTECT-CAD	19 versus 9 controls	42 BMC with Ficoll (1.38 $CD34^+$)	6	Exercise time: ↑53%	LVEF (MRI): +3.7% versus −0.4% (↑5.4%) SPECT perfusion score: −0.5 versus +2.4 (↓2.7, NS)	Nil
Van Ramshorst et al.	25 versus 25 controls	98 BMC with Ficoll	6	SPECT perfusion score: −3.4 versus −1.1 (↓2.44)	LVEF (MRI): +3% versus −1% (↑3%) Exercise capacity: +9W versus +2W (↑7W)	Nil

Source: Adapted from Ref. [80].

TABLE 12.8 Randomized Controlled Trials in Congestive Heart Failure

	No. of Patients	Cell Types and Numbers ($\times 10^6$)	Follow-up Duration (Months)	Primary End Point	LVEF (%)	LV Dimension	Side Effects
TOPCARE-CHD	24 (PBC), 24 (BMC) versus 23 (controls)	Intracoronary injection: 22 PBC or 205 BMC with Ficoll	3	LVEF (LV angiogram)	PBC: −0.4%; BMC: +2 9%; control: −1.2%	LV EDV-PBC: −3%; BMC: 0%; control: −3% (NS) LVESV-PBC: −2%; BMC: +2%; control: −1% (NS)	Nil
MAGIC	63 high and low dose versus 34 controls	Epicardial injection: 400 skeletal myoblast (low dose); 800 skeletal myoblast (high dose)	6	LVEF and changes in number of akinetic segments (ECHO)	High dose: +5.2% Low dose: +3.4% Control: +4.4% (NS)	High: −12.6 Low: −3.9 Control: +5.9 (↓ 12.8) LV EDV (ml/m)- High: −8.3 Low: −6.5 Control: −2.1 (↓ 8.1)	NS higher number of arrhythmic events in myoblast group

LV, left ventricular; ESV, end-systolic volume; EDV, end-diastolic volume.
Source: Adapted from Ref. [80].

with the placebo or control group; one trial demonstrated only regional contractile improvement in the infarcted segments, and one trial did not show any differences between the treatment and the control groups. The Leuvin-AMI trial, which showed only regional contractile improvement in the infarcted segment, differed importantly from the other trials with respect to the timing of BMC administration, which was performed within 24 hours after reperfusion therapy for acute myocardial infarction. Although not yet published in manuscript form, the results of the HEBE trial (Bone Marrow Cell Therapy After Acute Myocardial Infarction) have been presented recently in abstract form.

In the HEBE trial, 200 patients with acute myocardial infarction were randomly assigned either to receive an infusion of mononucleated BMCs or mononucleated cells isolated from PBMCs or to primary percutaneous angioplasty alone (1:1:1 ratio). Despite promising results in the pilot trial, intracoronary infusion of mononucleated BMCs or PBMCs did not improve regional LV systolic function (primary end point) or global LV function and LV remodeling (secondary end points) at 4 months, assessed by MRI. The reasons that the ASTAMI and HEBE trials failed to show a benefit of cell therapy are unclear. However, preclinical work suggested that the processing techniques used to isolate the cells may have affected the outcome of the ASTAMI trial. The reasons for these negative findings of the HEBE trial are unclear and will remain speculative until publication of the full trial.

Stem cell researchers use the signaling molecules that selectively adhere to the receptors on the surface of the cell as a tool that allows them to identify stem cells. Many years ago, a technique was developed to attach to the signaling molecule another molecule (or the tag) that has the ability to fluoresce or emit light energy when activated by an energy source such as an ultraviolet light or laser beam.

There are two approaches of how researchers use the combination of the chemical properties of fluorescence and unique receptor patterns on cell surfaces to identify specific populations of stem cells. One approach for using markers as a research tool is with a technique known as fluorescence-activated cell sorting (FACS).

A second method uses stem cell markers and their fluorescent tags to visually assess cells as they exist in tissues. Often researchers want to assess how stem cells appear in tissues, and in doing so, they use a microscope to evaluate them rather than the FACS instrument.

Recently, researchers have applied a genetic engineering approach that uses fluorescence but is not dependent on cell surface markers. The importance of this new technique is that it allows the tracking of stem cells as they differentiate or become specialized. Scientists have inserted into a stem cell a "reporter gene" called green fluorescent protein. The gene is only activated or "reports" when cells are undifferentiated and is turned off when they become specialized. After activation, the gene directs the stem cells to produce a protein that fluoresces in a brilliant green color.

12.6.3 Future Perspectives in Myocardial Repair and Regeneration

The refinement of nuclear transfer, cybrid and cell fusion techniques may allow further engineering of stem cells to provide cardio protection or stimulate antioxidant

or antiapoptotic responses in the myocardium. These cell engineering techniques might also allow the specific targeting of mitochondrial-based cytopathies. To identify aspects of the cardiac milieu that may contribute to the growth and development of transplanted myoblasts *in vivo*, 3-dimensional matrices have been designed to serve as a novel *in vitro* system that mimic some aspects of the electrical and biochemical environment of the native myocardium. These structures may allow a finer resolution of electrical and biochemical signals that may be involved in myoblast proliferation and plasticity. Myoblasts have been grown on 3D polyglycolic acid mesh scaffolds under controlled conditions in the presence of cardiac-like electrical current fluxes and in the presence of culture medium that had been conditioned by mature cardiomyocytes. Such scaffolds containing either fetal or neonatal aggregates of contracting cardiac cells have been used to generate artificial cardiac grafts transplanted into injured myocardium with recuperation of ventricular function and formation of functional gap junctions between the grafted cells and the myocardium (Table 12.9).

The combination of gene therapy and stem cell engineering is an attractive approach for treating cardiac disorders. Overexpression (and in some cases, inhibition of expression) of specific proteins can result in striking changes in cardiomyocytes and in cardiac phenotype. Specific cardiomyocyte functions, including ion channel, cardiac conduction, contractility, and myocyte proliferation, have been shown to be effected by the gene transfer and expression of specific proteins. Cell-based therapies for injured or dysfunctional hearts can be enhanced by the use of *ex vivo* genetically modified stem cells to deliver genes and proteins. For instance, transplanted MSCs have been shown to be effective devices to deliver channel proteins involved in pacemaking activity (e.g., channel protein HCN2), resulting in the modification of cardiac rhythm *in vivo*.[35,126,141–143]

Several open questions are likely to be answered in the future:

1. What is the optimal time of delivery after acute myocardial infarction?
2. Is there a dose–response relationship?
3. How do different cell types compare?
4. The mechanism by which stem and progenitor cells achieve a functional improvement, which is difficult to test in the clinical scenario. In chronic ischemic HF, a superimposed question is whether identifying hibernating myocardium to direct cell therapy is essential to an effective outcome. The treatment for nonischemic heart disease is not yet addressed.

12.6.4 New Method Helps Stem Cells Find Damaged Tissue Better

Because the ability of stem cells migrating to damaged areas is well known, stem cells also have the ability to detect proteins that are secreted from the damaged tissue. Stem cells are chemotactic to detect movement (as amoeba, white blood cells attracted to chemicals and the movement around it). Research teams compared stem

TABLE 12.9 Myocardial Transplants: Advantages and Limitations Associated with Cell Type

Cell Type	Source	Advantages	Limitations
Cardiac stem cells	Allogenic fetal, neonatal, or adult heart	1. Recognition of myocardial growth factors and recruitment to myocardium are likely faster and more efficient than with other cell types 2. *In vivo* electrical coupling of transplanted cells to exiting myocardium has been demonstrated	1. Poor cell growth *in vitro* 2. Transplanted cells are very sensitive to ischemic insult and apoptotic cell death 3. Availability from fetal, neonatal, or adult sources is low at present; likely immune rejection; fetal and neonatal cells pose ethical difficulties
Skeletal myoblast	Autologous skeletal muscle biopsy	1. Cells proliferate *in vitro* (allowing for autologous transplant) 2. Ischemia resistant 3. Transplanted myoblasts can differentiate into slow-twitch myocytes (similar to cardiomyocytes), enabling cellular cardiomyoplasty 4. Reduces progressive ventricular dilatation and improves cardiac function 5. Can use adult cells	1. Likely do not develop new cardiomyocytes *in vivo* 2. Electrical coupling to surrounding myocardial cells is unclear (may cause arrhythmias) 3. Long-term stability of differentiated phenotype unknown
Adult bone marrow stem cells	Autologous bone marrow stromal cells (mesenchymal); bone marrow (endothelial progenitor cells)	1. Pluripotent stem cells can develop into cardiomyocytes 2. Stem cells are easy to isolate and grow well in culture 3. Neovascularization can occur at sue of myocardial scar reducing ischemia 4. Transdifferentiation of cells into cardiomyocyte *in vivo* has been shown 5. Can be derived from autologous source; no	1. New program of cell differentiation is required 2. Efficiency of the differentiation into adult cardiomyocytes appears limited 3. Signaling, stability and regulation of differentiation unknown

TABLE 12.9 (*Continued*)

Cell Type	Source	Advantages	Limitations
		immune-suppression treatment 6. Can improve myocardial contractile function	
Embryonic stem cells	Allogenic blastocyst (inner mass)	1. Easy propagation and well-defined cardiomyocyte differentiation process 2. *In vivo* electrical coupling of transplanted cells to existing myocardial cells 3. Pluripotent cells	1. Potential for tumor formation and immune rejection (allogenic) 2. Incomplete response to physiological stimuli 3. Legal and ethical issues 4. Donor availability

Source: Adapted from Ref. [17].

cell activity in the environment of chemokines and growth factors. Both factors induce the migration of stem cells; however, growth hormone appeared to be more effective. In particular, PDGF-AB, TGF-β1, TNF-α were exposed to growth hormones to observe the active migration of stem cells.

Interestingly, the factors that enhance the migration of stem cells exhibited improvement when used to stimulate stem cells. Among these factors, TNF-α showed the best response from stem cells. It was confirmed that stem cells' homing effect improved up to 4.4 times when stimulated by chemotactic chemokines and growth hormones.

12.6.5 Shortcomings in Stem Cell Applications

Use of ESCs for research involves the destruction of blastocysts formed from laboratory-fertilized human eggs. For those who believe that life begins at conception, the blastocyst is a human life, and to destroy it is unacceptable and immoral.

The range of different types of cells that stem cells can change into may be limited to a set of cells that may not be useful for certain diseases. The complexity of individual diseases will govern whether stem cell therapy is applicable.

Another possible restriction is the problem of tissue rejection when stem cells from other sources are used to treat a person who is tissue incompatible. This is a situation in which adult stem cells from the patient could have great use because they would not be recognized as foreign by their body.

Use of stem cell lines from alternative nonembryonic sources has received more attention in recent years and has already been demonstrated as a successful option for treatment of certain diseases. For example, adult stem cells can be used to replace blood cell–forming cells killed during chemotherapy in bone marrow transplant patients.

Biotech companies are researching techniques for cellular reprogramming of adult cells, use of amnionic fluid, or stem cell extraction techniques that do not damage the embryo and that provide alternatives for obtaining viable stem cell lines. ESCs can be isolated in greater numbers and are less limited in the number of cell types they can generate, which makes them attractive for study, but their current use has funding and ethical constraints. So the potential application of stem cell therapy to disease treatment is broad but not without its limits.

12.6.5.1 What Does the Future Hold? The potential for stem cell therapy is immense, and much of this potential is applicable to the use of adult stem cells, which makes things less complicated from the ethics point of view. However, with efforts from individual organizations and states underway to fund human ESC research, this field will also move forward rapidly. We think in the next 15 years that amazing things will be achieved in the field as research moves along at a quickening pace. Thus the future is bright for the field of stem cell research, its application to the treatment of a variety of diseases, and the resulting enhancement to the quality of life for many.

On March 9, 2009, President Barack Obama removed certain restrictions on federal funding for research involving new lines of human embryonic stem cells. Federal funding originating from current appropriations to the Department of Health and Human Services (including the National Institutes of Health) under the Omnibus Appropriations Act of 2009, remains prohibited under the Dickey Amendment for (1) the creation of a human embryo for research purposes; or (2) research in which a human embryo or embryos are destroyed, discarded, or knowingly subjected to risk of injury or death greater than that allowed for research on fetuses in utero.

12.6.6 Stem Cell Research Controversy

Stem cell research is governed by country-specific guidelines and involves ethical concerns and legal and religious issues.

12.6.7 Problems with Embryonic Stem Cell Research

12.6.7.1 The Issue of Who or What Cloning technology destroys the scientific and legal basis of distinguishing a preembryo from an embryo, the popular distinction made at 14 days after conception. This is significant because this distinction determines the handling and treatment of human life less than 14 days old, which is so basic to all ESC research. There is no real preembryo–embryo distinction, and that all human life begins at conception. Therefore, as a nation, we should rightly adjust the moral and legal treatment and status of all embryos to people, not property, from the point of conception.

12.6.7.2 Embryonic Stem Cell Research is Related to Human Cloning Understanding how ESC research and human cloning relate requires delineation between the two forms of human cloning: reproductive and therapeutic. Reproductive cloning creates a later-born twin from a single cell of another person by transplanting the DNA

of the adult cell into a human egg whose nucleus has been removed. This process is somatic cell nuclear transfer. In this procedure, the resulting embryo is implanted in a woman and carried to birth. Therapeutic cloning begins with the same procedure as reproductive cloning. Whereas the goal of reproductive cloning is to produce a baby, the goal of therapeutic cloning is to produce ESCs for research or treatment.

12.6.7.3 There is Law that Could Apply to ESCR Originally attached to the 1995 Health and Human Services (HHS) appropriations bill, the "Dickey Amendment" has prohibited federal funding of "any research in which a human embryo or embryos are destroyed, discarded or knowingly subjected to risk of injury or death." Unfortunately, there are no laws to protect preembryos (embryos younger than 14 days old) or that prohibit private individuals, research firms, or pharmaceutical companies from forming, manipulating, or destroying stem cells, human clones, or embryos.

12.6.7.4 ESCR Currently has Major Disadvantages One minor complication is that use of human ESCs requires lifelong use of drugs to prevent rejection of the tissue. Another more serious disadvantage is that using ESCs can produce tumors from rapid growth when injected into adult patients. A third disadvantage reported in the March 8, 2001, *New England Journal of Medicine* was of tragic side effects from an experiment involving the insertion of fetal brain cells into the brains of patients with Parkinson's disease. Results included uncontrollable movements, including writhing, twisting, head jerking, arm flailing, and constant chewing. Fourth, a recent report in the *Journal Science* reported that mice cloned from ESCs were genetically defective. Finally, the research may be hampered because many of the existing stem cell lines were grown with the necessary help of mouse cells. If any of this research is to turn into treatments, it will need approval from the FDA, which requires special safeguards to prevent transmission of animal diseases to people. It is unclear how many of these cell lines were developed with the safeguards in place. This leads to a host of problems related to transgenic issues.

12.6.8 Challenges Remain for Stem Cell Therapies

There are many challenges to making stem cell therapies, such as regenerative medicine, actually work in a therapeutic setting. We might be able to harvest stem cells, from either blastocysts or by creating pluripotent cells from already differentiated tissues, but that is really only the beginning of a medically viable process. After a cell line is cultured in a maintainable way, the following questions remain:

- How to direct differentiation into the desired tissue type and optimizing growth conditions and the physical environment for cell cultures or for growing organs for transplantation
- How to inject and transport stem cells to the target location in the body
- Finding ways to generate induced pluripotent stem cells without inducing tumor formation in future recipients of stem cell therapies.

Reprogramming of human somatic cells uses readily accessible tissue, such as skin or blood, to generate embryonic-like iPSCs. This procedure has been applied to somatic cells from patients who are classified into a disease group, thus creating "disease-specific" iPSCs. Here, the challenges and assumptions are in creating a disease model from a single cell of the patient. Both the kinetics of disease onset and progression as well as the spatial localization of disease in the patient's body are challenges to disease modeling. New tools in genetic modification, reprogramming, biomaterials, and animal models can be used for addressing these challenges.

Despite these many hurdles and the newness of the technology, there are already some glimmers of hope for clinical applications of hiPSCs. Diseases of the retina may offer an early test bed for hiPSC-derived cells in the form of retinal pigmented epithelium, given the relative isolation of the tissue and the small number of cells required. A second intriguing possibility would be the use of hiPSCs to produce functional cells for use in extracorporeal applications, such as mature hepatocytes for use in bioartificial livers. If such early applications prove successful, it may help to allay concerns over safety and increase public and regulatory acceptance of the clinical use of hiPSCs, enabling them to establish a solid footing before attempts are made at treating more complex and deeply rooted disorders. At the same time, it will be important for cell therapy pioneers to investigate alternative routes for funding their work, so as to ensure the standards of safety and efficacy expected of small molecules are also met by cellular applications.

ACKNOWLEDGMENTS

We thank Narayana Medical College and Hospital for providing necessary resources. We also thank the publishers of the handbook of *Micro- and Nanotechnologies in Engineering Stem Cells and Tissues*, Wiley—IEEE Press, for allowing figures and tables for this chapter.

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