STEM CELL AND GENE THERAPY FOR CARDIOVASCULAR DISEASE

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Preface

The use of stem cells and genes has become an area of increasing research in both basic science and clinical trials. This book provides a blend of these two areas and is intended to provide the reader with the most up-to-date status of not only stem cell and genes, but also progress in the area of tissue engineering to enhance retention. These new therapeutic options are intended for patients with all forms and stages of cardiovascular disease, with the goal of reducing the limitations of their condition and the risk to their lives and well being.

The book is dedicated to all of the patients now afflicted and all those who are at risk, or will develop cardiovascular disease in the future, and the growing number of students, trainees, research scientists, and clinicians involved in the care of these patients with the goal of finding new therapeutic options to reduce the limitations of their condition.

Regenerative Medicine and the Cardiovascular System: A Good Start*

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All humans are developmental products of stem cells. The union of our father's sperm and our mother's egg generated billions of stem cells that through genetic signaling became every organ in our body and all about us, including our appearance, our soul, and our intellect. Each of us also has a "rescue" system of stem cells circulating in the blood and residing in our heart (and other organs) that is intended to repair injuries. However, early after birth, the heart's ability to regenerate itself is inhibited [1]. Improving our understanding of stem cells and building on the capabilities of the rescue stem cell system may ultimately enable researchers to use cell therapy not only to repair injuries locally, but also to regenerate the whole heart and other organs, when necessary. Although preclinical studies provide valuable information, proof of the safety and efficacy of stem cell therapy has and will continue to come from studies in humans with cardiovascular diseases—the best model for this work. Thus, we should carry on our human research of stem cell therapies as Shakespeare urged, "till truth makes all things plain."†

Multiple small-scale clinical studies of cell therapy for cardiovascular diseases have shown that these treatments are safe and may provide clinical benefits [2–10]. Studies have examined a variety of cell types, including bone marrow-derived mononuclear cells, bone marrow-derived aldehyde dehydrogenase-bright stem cells, adipose tissue-derived mesenchymal cells, and cardiac stem cells. For example, Perin and colleagues [6] found transendocardial injections of autologous bone marrow-derived mononuclear cells to be safe in patients with end-stage ischemic heart disease, and their findings suggested that the treatment may have had positive effects on myocardial perfusion and contractility. Likewise, transendocardial injections of autologous bone marrow-derived aldehyde dehydrogenase-bright stem cells in patients with ischemic heart failure were found to be safe and to potentially contribute to improvements in perfusion and left ventricular (LV) function [9]. In a study examining the use of autologous adipose tissue-derived mesenchymal cells in patients with ischemic cardiomyopathy, the cells were found to be safe when injected transendocardially, and the treated patients showed preserved LV function and possible benefits in coronary blood flow, scar size, and LV contractility [7]. Furthermore, a study by Bolli and colleagues [Cardiac Stem Cell Infusion in Patients with Ischemic CardiOmyopathy (SCIPIO) trial] [2] showed that intracoronary infusion of autologous c-kit$^+$ cardiac stem cells after coronary artery bypass surgery was safe in patients with post-infarction LV dysfunction, and that this treatment led to a significant increase in LV ejection fraction (LVEF), a reduction in infarct scar size, and improvements in the New York Heart Association (NYHA) functional class and quality of life. In the CADUCEUS (CArdiosphere-Derived aUtologous stem CElls to reverse ventricULar dySfunction) trial [4,5], investigators showed that intracoronary infusion of cardiosphere-derived cells (a mixture of resident cardiac stem cells, including mesenchymal cells, CD105$^+$ cells, and c-kit$^+$ cells [11]) obtained from endomyocardial biopsy specimens in patients with recent large myocardial infarcts resulted in reduced infarct mass and improved regional LV function in patients with LVEFs of 25% to 45%.

Larger clinical studies have also shown the safety and potential efficacy of stem cell therapy in heart disease [12–15]. In the REPAIR-AMI (Reinfusion of Enriched Progenitor cells And Infarct Remodeling in

*Modified from a manuscript published in *Circulation Research* 2014;115(12);271–78.

†A Midsummer Night's Dream, Act V, sc. 1, line 128.

Acute Myocardial Infarction) study [13], for example, the intracoronary administration of unfractionated mononuclear cells taken from patients' bone marrows resulted in a small, but significant, increase in LVEF in patients with acute ST-segment elevation myocardial infarction (MI) when the patients' pretreatment LVEFs were less than 48%. In treated patients from the same REPAIR-AMI trial, the risk of death, recurrent MI, and rehospitalization also decreased [12]. In a trial from the Cardiovascular Cell Therapy Research Network, transendocardial injections of autologous bone marrow-derived mononuclear cells in patients with ischemic cardiomyopathies and no other option for revascularization resulted in a small but significant increase in LVEF [15]. This increase correlated with the percentage of $CD34^+$ and $CD133^+$ cells in the bone marrow samples. Specifically, every 3% increase in $CD34^+$ or $CD133^+$ cells was associated with an absolute unit increase in LVEF of 3% or 5.9%, respectively, in a multivariable model that included age and treatment as predictor variables ($P = 0.04$ for both). The therapeutic potential of $CD34^+$ cells has also been suggested by positive findings by Losordo et al. in a trial of patients with refractory angina where patients who received intramyocardial injections of the low-dose treatment (1×10^5 autologous $CD34^+$ cells/kg body weight) showed a subsequent decrease in angina frequency and improvement in exercise tolerance [14]. Although these examples suggest that stem cell therapy may be beneficial for some patients, the measurable effects have generally, even in larger trials, been modest. Thus, a better understanding of the factors that contribute to the effectiveness of stem cell therapy is needed.

Adult stem cells have also been used to treat patients with nonischemic cardiomyopathies. Vntovec et al. administered CD 34^+ cells to 28 patients by the intracoronary route and had 27 control patients [16]. In the cell-treated patients, CD 34^+ cells were mobilized by granulocyte-colony stimulating factor and collected by aphaeresis. After a one-year follow-up, patients treated with cells had an increase in LVEF from 25.5% to 30% ($P = 0.03$) and a decrease in NT-pro BMP from 2069 $^+$ 1996 pg/ml to 1037 I 950 pg/ml ($P = 0.01$). A secondary end point of one-year mortality or heart transplantation was lower in patients receiving stem cell therapy (2/28, 7%) than in controls (8/27, 30%) ($P = 0.03$), and the stem cell therapy was the only predictor of outcome by multivariate analysis ($P = 0.04$). The beneficial effect was sustained during a 5-year follow-up [17] and was greater when the cells were given by the transendocardial rather than the intracoronary route of administration [18]. Similar benefit has been reported by Wang et al. when autologous mesenchymal cells were used to treat patients with idiopathic dilated cardiomyopathies [19].

Beneficial results treating patients with coronary heart disease and refractory angina with CD 34^+ cells by NOGA catheter have been reported in 167 patients who received 1×10^5 or 5×10^5 cells /hg of mobilized CD 34^+ cells or an equal volume of diluent [14]. Patients with refractory angina who received intramyocardial injections of autologous CD 34^+ cells (10^5 cells/hg) had significant improvements in angina frequency and exercise tolerance [14]. Mathiasen et al. showed similar benefit in treating patients with coronary artery disease (CAD) and refractory angina with bone marrow-derived mesenchymal cells over a 3-year follow-up with reduced hospital admissions for cardiovascular disease and excellent long-term safety [20]. Others have reported similar beneficial results in similar patients when bone marrow-derived stem cells were used [21,22].

Clinical studies of stem cell therapy in patients with ischemic cardiomyopathies have revealed several critical limitations and have raised important points to consider. One major limitation is that human stem cells become dysfunctional with age [8,15,23]. In addition, stem cells become less able or unable to replicate themselves in older individuals (i.e., in those $>$ 60 years of age) [8,23]. Furthermore, the absolute numbers of stem cells in the bone marrow and in the circulation are reduced in older adults. Similarly, the number and effectiveness of bone marrow-derived and circulating stem cells are also reduced in patients with severe diseases and risk factors for cardiovascular disease [24–28]. Therefore, notwithstanding its elegance, the human rescue system of stem cells is unable to repair damage in the hearts of those in whom repair is most often needed. Another potentially important limitation is that in many of the trials of stem cell therapy in patients with cardiovascular disease, the composition (i.e., the cellular make-up) and potency of the transplanted cell product is different for each patient because of the inherent heterogeneity of products isolated from individual patients. This likely contributes to variations in outcomes. While these realizations are sobering, they are also critical issues to consider when designing, implementing, and interpreting the results of any stem cell clinical trial involving patients with cardiovascular disease.

As we learn the capabilities and limitations of specific stem cell populations through preclinical and clinical research, we can use this information to design more effective stem cell therapies. As noted above, because the patients in these clinical studies are generally older and may have multiple co-morbidities, the stem cells isolated from them may be less potent. One approach that can be used to circumvent this issue is the use of allogeneic stem cells. Mesenchymal stem cells, which can be found in the bone marrow, adipose

tissue, or myocardium, may not be immunologically rejected when taken from one person and transplanted into another; thus, it appears to be feasible to use mesenchymal stem cells from youthful donors to treat aging individuals with cardiovascular diseases [29,30]. Indeed, when allogeneic mesenchymal cells from a healthy young donor were injected transendocardially into patients with ischemic or nonischemic cardiomyopathy, positive results were observed in those patients who received a high cell dose, including improved LV function and coronary blood flow and substantially reduced rates of death, progressive heart failure, and hospital readmission [29].

Another approach that could be used to improve cell therapy in patients who are older and may have multiple co-morbidities is to rejuvenate the patient's senescent stem cells. In a study by Madonna and colleagues [1], the induced overexpression of telomerase reverse transcriptase and myocardin in mesenchymal stromal cells from aged mice resulted in improvements in cell function both in vitro and in vivo. Likewise, ex vivo modification of senescent human cardiac progenitor cells with PIM-1 kinase has been shown to increase cellular proliferation and survival [31]. Furthermore, Sanada and colleagues [32] have shown in a mouse model that older hearts have more quiescent c-kit$^+$ cardiac stem cells than younger hearts, but that stem cell factor can be used to stimulate these cells and effectively reverse aging cardiomyopathy.

Studies have also indicated that the efficacy of stem cell treatments can possibly be enhanced by using specific cell combinations. In a porcine model of MI, Williams and colleagues [33] showed that a combination of mesenchymal and c-kit$^+$ stem cells improved LV function and reduced infarct size significantly more than either cell type did alone. The encouraging results with cardiosphere-derived cells in patients with ischemic cardiomyopathy also suggest that using a combination of stem cells may be more effective than using a single type of stem cell [4,5].

Therefore, clinical trials assessing the use of select adult stem cells for treating patients with ischemic cardiomyopathies have produced encouraging results that suggest these therapies are safe and may potentially improve clinical outcomes, including LV function, infarct size, and the occurrence of future adverse clinical events. However, the studies performed to date have been relatively small, and the follow-up for these studies has been limited to only a few months to years. Furthermore, when designing future studies, clinicians should continue to build on what is now known about stem cell biology and should try new approaches based on this information, such as using allogeneic mesenchymal stem cells from young donors, rejuvenated autologous stem cells, and/or specific combinations of stem cells that have been shown to produce better effects in relevant preclinical evaluations. In addition, because some patient-specific factors, such as baseline bone marrow composition and LVEF [13,15], have been shown to be associated with improved outcomes after stem cell therapy, it may be beneficial to select more targeted study populations in future studies.

Every human being has an elegant system of rescue stem cells that potentially may enhance the repair process after injury. We must find ways to maximize the benefits of this system and the body's stem cells by producing cell therapies that can repair and regenerate organs and possibly even delay the aging process. As with most medical breakthroughs, the development of successful stem cell therapies will be achieved through small, incremental improvements. Clinical trials are a vital part of this process because proof of the safety and efficacy of stem cell therapy can come only from studies in humans with cardiovascular disease. Ultimately, we have the opportunity to develop a more personalized approach to stem cell therapy for heart disease. It is evident that we are on the right path of discovery in this field, and we should, therefore, have the fortitude to continue. As Shakespeare also admonished, let us not "lose the good we oft might win, by fearing to attempt."[‡]

GENE THERAPY

The other area in which clinical trials are a critical method to enhance our understanding of the mechanisms involved in native tissue repair is targeted gene therapy. There is strong preclinical evidence that the major mechanism by which stem cells affect their benefit is via paracrine release of a variety of trophic substances (e.g.SDF-1) that then activate resident stem cells [34,35]. This hypothesis was confirmed by the work of Dzau [34] who showed that the supernatant from stem cells in culture was as effective as transplantation of the cells alone. This has led to the hypothesis that delivery of sufficient quantities of targeted genes to the area of injury or dysfunction could potentially be as effective as stem cell delivery in driving native tissue repair.

Preclinical research has identified several types of potential target genes, which can be delivered as synthesized human genes and cause no immune stimulation, and therefore have a minimal risk equivalent to autologous stem cells. Some of the very first trials of regenerative medicine for cardiovascular disease tested

[‡]Measure for Measure, Act I, sc. 4, lines 435–436.

pro-angiogenic genes like vascular endothelial growth factor (VEGF) [35,36] and fibroblast growth factor FGF) [37] for both peripheral vascular disease and refractory angina, largely relying on delivery via percutaneous injections into the gastrocnemius muscle. The results of these trials were not as positive as anticipated, perhaps related to a variety of factors such as dose, route, and vector used.

Subsequently, several new target genes have moved to clinical trials, primarily for heart failure, including those with direct inotropic stimulation of beta adrenergic signaling, including calcium handling proteins such as SERCA-2a [38,39] and adenyl cyclase [40]. Other target genes now in clinical trial include those involved in stem cell homing and have other beneficial cardiac effects such as stromal cell-derived factor 1 (*SDF-1*) [41,42] and Neuregulin (NRG-1) [43]. These genes have been delivered by variable methods including intracoronary, intramyocardial, and most recently, retrograde via the coronary sinus. Importantly, the transfection rates achieved by these methods of gene delivery have ranged from 40% to 50%, and evidence of expression of the genes ranging 10−25 days. The encouraging results of early phase trials with these targeted genes (unpublished) have led to recent completion of a Phase IIB trial with SERCA-2a in 240 patients, and the Neuregulin gene in nearly 300 patients. Phase III trials of both genes are planned to begin enrollment by publication of this book. Similarly, a Phase IIB trial is planned for the *SDF-1* gene in patients with ischemic cardiomyopathy, with potential rolling enrollment to a Phase III trial.

The entire field of gene therapy for cardiovascular disease is reviewed in detail in this book, and demonstrates the potential of this alternative or complementary approach to tissue repair. Collectively, these trials, and those with cell therapy, demonstrate the importance of clinical trials in the rapid progress in field of enhancing tissue repair for cardiovascular diseases.

TISSUE ENGINEERING

One of the limitations of current approaches to stem cell therapy is the very short retention time (days to at most a few weeks), where cells remain in the target tissue after being transplanted. A great deal of progress has been made in the field of tissue engineering to design new methods to increase cell retention. These include remarkable work on the development of scaffolds using both natural and synthetic materials, including extra cellular matrix, to hold either stem cells or genes for slow and programmed release. In addition, scaffolds are being developed with cells implanted for delivery to the target tissue.

One of the most exciting approaches to tissue engineering is the concept of organogenesis, or creation of whole organs potentially to be used for elective, off-the-shelf transplantation. Research in this area has demonstrated both the complexity of individual tissues and cells within each organ, the challenging requirements of creating equipment to sustain and test these constructs while maintaining sterility including bioreactors, as well as which cell to use, and lessons showing the astounding plasticity of transplanted cells [44]. Each of these approaches in tissue engineering is reviewed in detail in this section of the book.

The goal of using stem cell and gene therapy to enhance tissue repair of acute and chronic diseases to create new treatments to reduce the significant morbidity of cardiovascular diseases has come a long way in the past decade. The field of regenerative medicine is an excellent example of bidirectional flow, or translational medicine, between the basic and clinical sciences. Shortcomings in clinical trials have driven a good deal of basic research which has led to further understanding of the important mechanisms involved in cell homing and viability, as well as strategies to enhance cell homing such as hypoxia and gene transfection. Another focus of both basic and clinical science is on reversing a potential significant limitation of cell therapy which is the adverse effects of aging on stem cell number and function. Finally, basic research has led to the identification of many new sources and types of cells, including importantly the safety and use of allogeneic cells, which are now being evaluated in clinical trials using new routes of delivery.

This book is designed to provide the reader with an in-depth review of the current state of this field, as well as a look at the many future directions. We await the results of the clinical trials and basic research that together will advance the clinical implementation of these therapies. We believe that we are on the threshold of a new era in regenerative medicine taking advantage of the lessons learned in the past decade, and the strategies moving into clinical trials. We thank all of the authors and contributors who have worked so hard to produce the chapters of this book, and without whom this book would not have been possible.

DEDICATION

This book is dedicated both to all of the basic and clinical science investigators who have helped enhance the knowledge in this field, and to the thousands of patients who have participated in clinical trials of stem cell and gene therapy, and to whom we express our collective and enormous gratitude.

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CHAPTER

1

Introduction and Overview of Stem Cells

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One of the most exciting advances in medicine today has been the use of stem cell and gene therapy for repair in both acute and chronic illness. Stem cells have been shown to be the primary mechanism for tissue repair everywhere in the body, and not limited to cardiovascular disease. Some even believe that diseases such as atherosclerosis represent a failure of stem cells. However, because cardiovascular disease has become the leading cause of death and morbidity in the world [1–3], this area has seen the greatest evaluation by clinical trials of cell therapy in the field known as regenerative medicine. This introductory chapter is designed as a guide to understanding the new sources and types of stem cells now being examined in clinical trials, as well as the surface markers that help identify each type of cell.

WHAT IS REGENERATIVE MEDICINE?

Regenerative medicine can be defined as the use of stem cell or gene therapy to repair or recover damaged organs, tissues, or vessels. Stem cells have been the predominant therapy used to date, as they are the most immediately mobilized response to acute injury, but the use of targeted gene therapy is also being explored vigorously (see Part II on Gene Therapy). There are many examples in nature of tissue regeneration, including the newt, which can regenerate an entire limb within 6 weeks of amputation, and the zebrafish, which can restore amputation of the distal cardiac apex within 10–14 days [4]. The liver is the human organ most capable of regeneration [5].

Attempts to induce the heart to regenerate run contrary to the long-held tenant that the heart is a postmitotic, terminally differentiated organ [6,7]. However, programmed cell death, or apoptosis, occurs in the heart as in every other organ and tissue. Estimates of the loss, and therefore turnover, of the heart during a lifetime range between 50% and 3–4 fold [8]. Millions of people are living beyond 80 years of age without evidence of cardiac dysfunction, which by definition, requires the capacity of the myocardium to regenerate.

Many important lessons have been learned over the past decade, but it has been more challenging than anticipated to identify the mechanisms that will enable us to achieve significant regeneration of new functional cardiomyocytes and vasculature. Our understanding of the mechanisms involved in cell therapy has evolved as has our understanding of stem cell biology, including identification of factors such as chemokines, cytokines, adhesion molecules, and matrix metalloproteins that are involved in tissue repair (see Chapter 39). This knowledge has led to an increasing number of clinical trials that have also helped to move the field forward. However, questions remain including identification of the optimal cell type and number, the method and timing of delivery, and ways to potentially precondition cells or target tissue to enhance responses. In addition, extensive research is now focused on ways to enhance cell retention in the target tissue and to gain a better understanding of the importance of the extracellular matrix in this process (see Part II on Tissue Engineering). Here, we provide an introduction to the terminology and basics of stem cells used to drive native tissue repair and regeneration and a firm foundation of the current status of knowledge in this field.

WHAT DEFINES A STEM CELL?

A stem cell can be defined by two specific characteristics:

1. Endless self-renewal, with no limitations or maximum number of replications.
2. Capability to differentiate into any tissue type and cell line in the body. The bone marrow provides

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two primary types of stem cells: those that will form all of the hematopoietic cells in the body, including red blood cells, white blood cells, platelets, lymphocytes, macrophages, etc. [9], and those from ectoderm, endoderm, and mesoderm origins that can differentiate into all tissue types in the body. Most stem cells have a predominant form(s) of cell that they differentiate into, which helps define them in the laboratory. For example, mesenchymal stem cells are characterized by differentiating into bone, cartilage, and adipose tissue [10,11], but they can also differentiate into functioning cardiomyocytes, blood vessels, and even neuronal tissue. This differentiation capacity is true of mesenchymal cells whether they originate from the bone marrow, adipose tissue, umbilical cord, or even placenta.

Types of Stem Cells

Stem cells may be classified in many different ways. The most traditional classification of stem cells is based on their plasticity or developmental versatility. They may thus be classified as *totipotent* (can give origin to an entire organism), *pluripotent* (can give rise to all tissues), and *multipotent* (can give rise to a limited range of cells within a tissue).

Stem cells may also be classified according to their origin.

Embryonic: These pluripotent cells may carry a high risk of development of unusual tumors called teratomas [12]. Research on embryonic stem cells is currently limited to basic science studies to help understand mechanisms of stem cell function. There are no clinical cardiac trials of embryonic stem cells. Their use has been associated with a significant controversy because of the moral and ethical issues surrounding their use.

Adult stem cells: These are multipotent cells that are undifferentiated cells in a differentiated tissue. They are used in widespread clinical investigations.

Stem cells are commonly classified according to cell surface markers. Many surface markers help identify individual types of stem cells. This feature aids in separating specific cell types by flow cytometry and other methods to obtain pure cell cultures. However, not every stem cell has a specific set of surface markers. For example, mesenchymal stem cells may have several different surface markers rather than one defining characteristic set of markers [13]; however, their unique feature of adherence to plastic surfaces helps define this cell type. Table 1.1 provides a list of the common surface markers that help define a specific type of stem cell.

Stem Cell Sources

Another common way to classify stem cells is according to their source. Although stem cells were originally thought to be restricted primarily to adult bone marrow and embryos, the number of cell sources (from different tissues) has expanded significantly.

Bone Marrow

The bone marrow, although no longer the sole source of adult stem cells, is by far the greatest reservoir of stem cells. Its composition includes largely mononuclear cells, of which 95% are hematopoietic precursors, and an array of natural killer–type cells, as well as T and B lymphocytes [7,14,15]. In addition, other types of stem and progenitor cells, including endothelial progenitor cells (EPCs) [16], make up 1–2% of the total cell composition, and mesenchymal stromal cells (MSCs) constitute only 0.01–0.2%. Clinical trials have examined the use of the entire bone marrow mononuclear cell (BMMNC) population, as well as selected cell populations such as CD34 + EPCs, CD133 + smooth muscle progenitors, or, more recently, MSCs [17,18].

One of the lessons learned from the use of autologous bone marrow is of particular importance for treating patients with cardiovascular disease, who are often over the age of 60 years—the number and functional capacity of bone marrow cells decreases with each decade of life [19]. This is due in part to the well-documented reduction in telomere length of both hematopoietic and other stem cells with advancing age [20–22] (see Chapter 5), which may limit the capacity for these cells to respond as effectively as needed for optimal tissue repair. Clearly, there is often a disparity between chronologic and physiologic age, and no absolute age should be considered an exclusion for autologous cell therapy.

The bone marrow is the source of several other cells that have been developed for clinical use, including the multipotent adult progenitor cell (MAPC) [23] as well as the derivation into cardiac MSCs [24] (see below for details). Another fraction of cells from the bone marrow is characterized by the presence of an enzyme (aldehyde dehydrogenase; ALDH) and not by cell surface markers; this population is highly enriched for mesenchymal and EPC activities [25,26].

Adipose Tissue

Perhaps, one of the most unexpected sources of stem cells is adipose tissue. Several studies have shown that there are 500–2000 times more stem cells per gram of tissue in abdominal adipose tissue than there are in bone marrow in age-adjusted comparisons