Title:

Cardiovascular surgery for realization of regenerative medicine

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Abstract

Regenerative medicine is emerging as a new approach to the treatment of severe cardiovascular diseases that are resistant to conventional therapies. While the type of cell transplanted (e.g., pluripotent stem cells, bone marrow-derived stem cells, skeletal myoblasts, or cardiac stem cells) influences the outcome of stem cell transplantation, the method of transplantation is also important, as the efficiency of engraftment after simple needle injection is poor. Scaffold-free cell sheet transplantation technology is one of the most promising methods in this regard. Although the results of clinical trials of stem cell therapy have been marginal to date, further elucidation of the actual mechanisms of cardiac repair following cell therapy would enhance the potential for full-scale implementation of stem cell therapy. In addition to stem cell therapy, the field of cardiovascular regenerative medicine includes interspecific chimera technology, drug delivery systems using biodegradable materials, and gene therapy. Integration of these new modalities with conventional therapies will be important to realizing the goal of cardiovascular regenerative medicine tailored to the condition of each individual patient. Cardiovascular surgery would be an excellent means of carrying out this strategy and could potentially resolve the health problems of the increasing

number of advanced cardiovascular patients. Herein, we review the recent basic and clinical research associated with the realization of regenerative medicine in the field of cardiovascular surgery. (220 words)

Introduction

Cardiovascular disease remains the leading cause of death worldwide. In Japan, over 57,000 cardiovascular surgeries for treatment of advanced cardiovascular diseases are performed annually[1]. However, the surgical treatment of severe heart failure is limited by the shortage of donors for heart transplantation and by the risk for serious complications, such as infection or cerebral thrombosis, after the implantation of ventricular assist devices. This health problem has prompted research into new therapeutic approaches, including cardiac regeneration[2]. Numerous valuable outcomes over more than a decade of basic research are now on the horizon of translation to clinical application ("from bench to clinic"), and the expectations from society are considerable. The field of cardiovascular surgical research, as the practical setting of such translational research, is now gathering significant attention from basic researchers. The results of basic research must be proven by preclinical experiments in animal disease models that mimic human diseases before the techniques can be applied clinically, and cardiovascular surgeons, as experts in human surgical treatments, are therefore the personnel best-suited to make practical contributions to advancing regenerative medicine research.

In this review, we introduce recent basic and clinical research related to the field of cardiovascular surgery, including the different therapeutic approaches to, drawbacks of, and future expectations for new regenerative therapies for cardiovascular diseases.

Cell transplantation

Acute ischemic injury and chronic cardiomyopathies lead to permanent loss of cardiac tissue and, consequently, heart failure. Cell transplantation is thought to be an ideal therapeutic method for replacing lost myocardium[3,4]. Of the available cell sources, stem cells are now widely preferred for research or clinical trials concerning cardiac cell therapy[2,5]. The discovery of various stem cell populations possessing cardiogenic potential and the subsequent development of methods to isolate and expand these cells have begun to shape the notion of restorative therapy. In spite of the great deal of knowledge gained through numerous basic research studies, significant barriers to true cardiac regeneration remain, and the field still lacks results sufficiently conclusive to support full-scale implementation of such therapies. Very few of the transplanted tissue stem cells seem to differentiate into mature cardiovascular cell types, suggesting that transplanted cells exert paracrine effects by which humoral factors induce or support

favorable processes, including angiogenesis, prevention of apoptosis, and promotion of healing, in the injured myocardium rather than differentiating into renewed myocardium[2]. In this section, we introduce the present research achievements in stem cell therapy using various cell types, including clinical trials employing said cell sources, as well as the transplantation technologies that best support effective engraftment of the transplants. The characteristics of each stem cell population with respect to therapeutic potential are summarized in Table 1.

Pluripotent stem cells

Embryonic stem cells (ESCs) can be removed from the inner cell mass of the blastocyst and expanded in vitro practically indefinitely[6]. In culture, ESCs remain undifferentiated and pluripotent. When allowed to differentiate, ESCs can give rise to most somatic cell lineages[7]; their regenerative capacity is thus theoretically limitless. The advantages of these properties of ESC are especially significant for the heart as opposed to other organs, such as endocrine or sensory organs, as the heart functions as an assembly of many types of cells, including cardiomyocytes and others, and as numerous ($>10^8$) heart-composing cells might be required to fully repair a damaged human heart. The differentiation of ESCs can be driven towards cardiomyocytes or

other vascular cell types by culture as monolayers or embryoid bodies in various growth media[8-11]. These cells can then be transplanted into the heart. This approach to repairing cardiac tissue has been tested in preclinical studies with encouraging results[10,12]. In fact, of the various stem cell populations studied so far, ESCs have demonstrated perhaps the greatest capacity for cardiac cell differentiation and long-term cell survival[13].

To date, no human trials of the use of ESCs for cardiac repair have been attempted. There have been 3 main concerns regarding the use of ESC transplantation as a treatment modality. First, the differentiating cell mass contains cells from 3 germ layers, the ectoderm, mesoderm, and endoderm, and therefore possesses the capacity to differentiate along any or all of these lineages. This potential increases the risk of teratoma formation at the transplantation site. Although such teratomas are believed to be largely benign in vivo, some teratoma cells have been found to express markers similar to those found in malignant tumors[14]. We recently reported that the transplantation of cell sheets reassembled with defined mouse ESC-derived cardiovascular populations resulted in functional improvement in a rat sub-acute myocardial infarction (MI) model, and no tumors formed within the 3-month observation period after transplantation[12]. Transplantation of such fully differentiated cells alleviates some of the risk of teratoma formation. Other reports of the

transplantation of differentiated cells have shown increased engraftment and functional improvement[10,13]. While no long-term studies assessing the real risk of teratoma formation have yet been performed, the theoretical concern remains an important obstacle. The second issue concerns immunity. ESCs have been reported to express specific human leukocyte antigen (HLA) subclasses[15]; this raises worries about graft rejection and might necessitate immunosuppression, which could increase the risk of surgical wound infection after cell transplantation surgery. Finally, the origin of ESCs has raised considerable ethical concerns and led to heated debates among scientists and the wider public.

The discovery that reprogramming adult somatic cells with genes that confer ESC pluripotency generates ESC-like cells, called induced pluripotent stem cells (iPSCs), may resolve the ethical and immunogenic issues associated with the use of ESCs[16,17]. Mouse iPSCs can be differentiated into cardiovascular cell populations almost identical to those produced from mouse ESCs, indicating that the regenerative capacity of iPSCs is almost equal to that of ESCs[18]. Furthermore, a potent differentiation protocol based on high-density monolayer cultures and chemically defined factors, and modifications thereof, have been reported to produce cardiomyocytes from human iPS cells with an efficiency of 40–70%[10,19]. The application of this technique would strongly promote

cardiac regenerative therapy utilizing human iPS cells. Recently, methods for generating human iPS cell lines without genomic integration by using episomal vectors[20] or human artificial chromosome vectors[21] have been reported. These may reduce tumorigenesis due to mutations, which could otherwise limit the clinical application of iPSCs. Based on the results of these basic studies, iPSCs are currently thought to be one of the most promising sources of cells for cardiac regeneration. However, further careful exploration of the feasibility of this new therapeutic modality will be required before its clinical application.

Bone marrow-derived stem cells

There are various cell subsets within the bone marrow niche that possess stem cell properties; these include hematopoietic progenitor/stem cells, mesenchymal stem cells, and endothelial progenitor cells. Each of these cell populations has both advantages and disadvantages for use in cardiac regeneration (Table 1). Although the capacities of these cells to differentiate into cardiomyocytes are rather low compared with those of ESC/iPSCs, their potential for cardiac restoration has been confirmed in many preclinical studies.

Bone marrow hematopoietic stem cells (or circulating peripheral-blood progenitor

cells) are a well characterized and abundant source of progenitor cells. A number of remarkable studies have shown that direct transplantation or mobilization from endogenous reservoirs of bone marrow-derived cells significantly improves cardiac function. Some of these studies actually demonstrated regeneration of contracting cardiomyocytes and vascular beds[22,23]. However, other investigations found limited or no differentiation of bone marrow cells into cardiovascular cell types[24]; this suggests a beneficial effect independent of direct tissue regeneration, such as neovascularization due to paracrine effects. The observed improvements in cardiac function prompted a number of clinical trials using autologous bone marrow cells to treat patients with heart failure or MI. These studies used circulating hematopoietic progenitor cells, or bone marrow mononuclear cells (MNCs), which also contain hematopoietic stem cells. While the results of small early studies were encouraging, larger, randomized, placebo-controlled and blinded studies have shown mixed results[25,26]. The results of the REPAIR-AMI trial (the largest of the randomized, placebo-controlled trials) were positive in that left ventricular function improved and the combined clinical endpoint of death, MI, or revascularization within 2 years was reduced[27]. In contrast to the improved left ventricular function found by the REPAIR-AMI trial, a recent, randomized, double-blind, placebo-controlled study (the LateTIME trial) in which autologous bone

marrow MNCs were transplanted into patients with MI 2–3 weeks after successful percutaneous coronary intervention showed no beneficial effect on left ventricular ejection fraction (LVEF)[28]. These apparently conflicting results may be attributable to the cell preparation or the timing of cell administration. Further large-scale trials are clearly necessary to assess the role of infused bone marrow cells in cardiac repair in order to improve the therapeutic efficacy of this promising technique.

Mesenchymal stem cells (MSCs) are a subset of stem cells found in the stroma of the bone marrow that can differentiate into osteoblasts, chondrocytes, and adipocytes[29] and also into small numbers of cardiomyocytes[30]. MSCs are thought to be either less immunogenic than other lines[31] or inherently immunomodulatory[32], alleviating the need for immunosuppression or autologous therapy. Preclinical studies of transplantation of MSCs into post-infarct animals demonstrated improved left ventricular function, reduced infarct size, and increased survival rate[30,31,33]. A clinical study of MSCs in patients with MI demonstrated improvement of left ventricular function[34]. The disadvantage of MSCs for this clinical application is their broad differentiation capacity. MSC populations remain significantly heterogeneous and are therefore less predictable after transplantation. Some studies have indicated that transplanted MSCs had differentiated into osteoblasts inside ventricular tissue[35].

Another bone marrow cell subset is the endothelial progenitor cells (EPCs). In the past, angiogenesis was thought to occur exclusively though the proliferation of mature endothelial cells at injury sites. This concept has changed with the discovery that bone marrow-derived EPCs reach injury sites and incorporate into the microvasculature (vasculogenesis)[36]. This finding drastically altered our understanding of vascular growth and became a new therapeutic approach. EPCs can be identified by their ability to acquire endothelial cell characteristics, i.e., the expression of cell surface makers such as cluster of differentiation molecule 133 (CD133), CD34, the vascular endothelial growth factor receptor 2 kinase (VEGFR-2; also designed as KDR), and vascular endothelial cadherin (VE-cadherin), both in vitro and in vivo. Of these, $CD34^+$ and $CD133^+$ cells are the most widely recognized and utilized[37]. EPCs are mobilized from the bone marrow during states of injury, such as trauma, MI, or cancer[38-40]. The research into their use began with attempts to enhance their mobilization or incorporate EPCs directly into the vasculature of injured sites. VEGF, granulocyte colony stimulating factor (G-CSF), and statins (3-hydroxy-3-methylglutaryl coenzyme-A reductase inhibitors) have been reported to increase the mobilization of EPCs from the bone marrow[41-43]. Preclinical studies of the use of EPCs to treat experimental hind-limb ischemia demonstrated significant improvements in blood-flow recovery and limb

salvage[36]. Furthermore, injection of EPCs into infarcted myocardium improved left ventricular function[44]. The results of a randomized multicenter clinical trial in patients with acute ischemia (REGENT trial) showed no significant improvement in left ventricular function after treatment with EPCs. However, there was a trend in favor of EPC therapy in the patients with the most severely impaired left ventricular function and in those with longer delays between the onset of symptoms and revascularization[45]. EPCs have already been used in the field of interventional cardiology. Drug-eluting stents (impregnated with various chemicals that inhibit neointimal thickening) reduce the restenosis rate but increase the risk of in-stent thrombosis, a potentially fatal event. GENOUS stents are coated with anti-CD34 antibodies, which work to trap circulating EPCs and augment the process of luminal endothelialization; this may prevent restenosis[46]. A prospective study showed that implantation of the EPC-capture stent is safe and effective, with satisfactory immediate results and mid-term outcomes and no evidence of stent thrombosis[47]. However, EPCs have several disadvantages as a therapeutic material. First, this cell population is heterogeneous: EPCs circulating in the peripheral blood span the full range of differentiation from angioblasts to mature endothelial cells. Second, the stem cell pool of EPCs is quite limited, and ex vivo expansion would be the only way to attain sufficient numbers of EPCs for the treatment

of a significant injury or ischemic event[48]. Finally, the circulating pool of EPCs is reduced in patients with such common comorbidities of cardiac ischemic disease as diabetes mellitus, hypertension, and hypercholesterolemia[49]. Although further research is required to overcome these problems and to enhance the therapeutic efficiency of EPCs in ischemic tissue, these cells remain promising as a potential therapeutic material.

Skeletal myoblasts

In adult skeletal muscle, a stem cell population called "satellite cells" exists beneath the basal membrane of muscle tissue, where they lie dormant until stimulated by muscle injury to proliferate[50]. Skeletal myoblasts (SMs) are derived from the satellite cells. SMs have been considered an attractive source for cardiac repair for several reasons. First, these cells are further differentiated than ESCs and therefore less prone to teratoma formation. Second, they can be harvested from the host, expanded ex vivo, and autologously re-transplanted, thus avoiding the need for immunosuppression[51]. Third, SMs are resistant to ischemia, an obstacle to the function of stem cells in injured myocardium, and are therefore especially appropriate for cardiac repair[52]. Finally, SMs have the capacity to differentiate into non-muscle cell types in vitro[53]. Most

transplantation experiments in animal cardiac disease models produced improved left ventricular function and decreased remodeling[51,52,54,55]. However, skeletal myoblasts do not fully differentiate into cardiomyocytes in vivo after transplantation, and the myotubules generated do not operate in synchrony with the surrounding myocardium[55], possibly due to a lack of connexin activity and electrical coupling with the surrounding myocardial cells. However, the improvement in left ventricular function in animal models prompted a series of clinical investigations. Early clinical studies were aimed at assessing the feasibility and safety of SM implantation[52,56,57]. Although these studies proved the therapy feasible and showed that SMs survive in the heart, only marginal benefit was recognized. Larger scale clinical trials were undertaken to assess the benefit of myoblast therapy. The most notable to date was the Myoblast Autologous Grafting in Ischemic Cardiomyopathy (MAGIC) trial, which randomized patients to receive injection of either SMs or culture medium. The results of this trial have been disappointing in that no significant benefit of SM transplantation has been observed[58]. Further clinical studies are ongoing and may show different results. Several barriers to the use of SMs still remain. The first limitation is the arrhythmogenic potential of the engrafted SMs. The MAGIC trial showed a higher number of arrhythmic events in the myoblast-treated patients[58]. Animal experiments have shown that the electrical coupling

of SMs to host cardiomyocytes increases when the SMs are induced to overexpress connexin 43, indicating that their arrhythmogenicity may be a surmountable obstacle[59]. The second limitation is the relatively poor engraftment of the injected cells into the host myocardium. Cellular mortality of over 90% within the first few days after injection has been reported in mice[60]. Some studies in humans have shown similarly high rates of cell death, with only scarce surviving cells[52]. Clinical research on autologous myoblast-sheet transplantation for treatment of severe heart failure is now ongoing in Japan[61]. As the cell sheet transplantation method is thought to improve the survival of the transplants over needle injection (mentioned below), this strategy is a promising means of resolving the survival problem, and better clinical outcomes would be expected. The third limitation of SM use is that the engrafted cells differentiate into myotubules rather than cardiomyocytes and therefore do not constitute a true regenerative therapy.

Cardiac stem cells

The modest (rather unsatisfactory) functional effects of the transplantation of bone marrow cells or SMs in human studies have stimulated further research into the natural and endogenous regenerative mechanisms of cardiac tissue. The heart has traditionally

been considered a post-mitotic organ, and mature cardiomyocytes withdraw from the proliferative cell cycle. However, contradictory data have accumulated, as cardiomyocyte proliferation and cell cycling have been observed under pathological conditions such as hypertension or MI[62,63] and even in the healthy heart[64]. Moreover, estimates of the death rates of adult cardiomyocytes suggested the existence of a pool of cardiac progenitor cells[65]. This evidence prompted a search for such resident cardiac cells. Several different cell populations with stem cell characteristics were subsequently discovered in the adult heart. The first cell population with stem cell properties is called the side population (SP); these cells have also been identified in various other organs, including bone marrow, skeletal muscle, and adipose tissue[66]. Isolated cardiac SP cells can differentiate into cardiomyocytes, endothelial cells, or smooth muscle cells, suggesting that they represent cardiac and vascular progenitor cells[67]. The second putative resident progenitor population constitutes cells expressing the stem cell factor receptor c-Kit (also designed as CD117), which are located in small clusters within the adult heart. c-Kit$^+$ cells have regenerative potential after transplantation and give rise to cardiomyocytes, endothelial cells, and smooth muscle cells. c-Kit$^+$ cell transplantation after ischemic injury significantly improves cardiac function[68]. The third cell type in the heart with stem cell properties consists of cells expressing stem cell antigen 1 (Sca-1).

Sca-1$^+$ cells home to infarcted myocardium and differentiate into cardiomyocytes around the injured area[69]. Finally, enzymatic digestion of heart tissue obtained via endomyocardial biopsy yields round cardiac progenitor cells that form so-called cardiospheres in suspension[70]. Cardiosphere-derived cells (CDCs) can also differentiate into cardiomyocytes, endothelial cells, and smooth muscle cells and exhibit remarkable capacities for proliferation and differentiation[70]. Once isolated, this cell population can be induced to differentiate into spontaneously contracting aggregates of cardiomyocytes that can then be transplanted into injured myocardium[71]. The injection of CDCs has produced functional improvement in preclinical studies[71]. Small numbers of CDCs have been observed to integrate into the injured myocardium, but this extent of cardiac tissue regeneration is insufficient to explain the functional improvement.

Cardiac stem cells appear to exist in specialized niches that support the growth and maintenance of the stem cell pool[72]. Putative niches have been thought to be localized throughout the myocardium[68]. However, there is recent evidence that the adult heart contains a resident stem cell population that originates from the epicardium and has the potential to differentiate into cardiomyocytes after MI[73]. Although the different cardiac stem cell pools are small relative to the number of mature resident cardiomyocytes, they are believed to be the source of the new cells in damaged myocardium[74]. It is unclear

whether the various cardiac stem cells are distinct types or represent different stages of a single cell lineage. Furthermore, the cardiac stem cell pool appears to diminish with age, possibly contributing to the poor efficiency of regeneration in elderly people[74]. This highlights the need to discover how to rejuvenate this senescent stem cell population, as it is largely the elderly who suffer increased mortality from cardiac ischemia.

Several phase-1 clinical trials using cardiac stem cells have been performed to date[75]. The CArdiosphere-Derived aUtologous stem CElls to reverse ventricUlar dySfunction (CADUCEUS) study was a proof-of-principle study of intracoronary delivery of CDCs in patients with recent MI and left ventricular dysfunction[76]. In CADUCEUS, patients underwent endomyocardial biopsy sampling about 4 weeks after MI to generate autologous CDCs that were subsequently injected into the coronary artery. The rates of serious adverse effects and arrhythmias did not differ between patients in the CDC group and controls receiving conventional medical therapy. MRI analysis of patients treated with CDCs showed reduced scar mass and increased viable heart mass and regional contractility. However, changes in end-diastolic volume, end-systolic volume, and LVEF 6 months after transplantation did not differ between the groups. The Stem Cell Infusion in Patients with Ischemic cardiOmyopathy (SCIPIO) trial was performed in patients with post-infarction left ventricular dysfunction before coronary artery

bypass grafting (CABG). Intracoronary administration of autologous c-Kit$^+$ cardiac stem cells about 4 months after surgery improved LVEF, and no adverse effects related to cell administration were reported[77]. In Japan, the results of the AutoLogous Human CArdiac-Derived Stem Cell to Treat Ischemic cArdiomyopathy (ALCADIA) trial, which tested combined therapy with autologous CDC transplantation and topical administration of basic fibroblast growth factor (bFGF), are now under evaluation[78]. Further investigations incorporating larger numbers of patients, longer follow-up times, and a true placebo arm will be needed to confirm the safety and efficacy of cardiac stem cell therapy.

Effective cell delivery: beyond needle injection

The results of this research have led to the conclusion that stem cells may be beneficial in the treated hearts but act primarily through paracrine mechanisms rather than through direct differentiation as initially expected. However, the low rates of grafted cell survival and engraftment diminish their potential and are serious technical limitations of stem cell therapy[79]. Over 70% of injected cells have been reported to die progressively during the first 48 hours after needle injection due to the hypoxic, inflammatory, and/or fibrotic environment[80]. Another report indicates that only 5.4 to 8.8% of microspheres

directly injected into the beating myocardium remain shortly after the injection due to massive mechanical loss[81]. Therefore, new strategies such as combining the cells with bioengineering techniques have been developed and are the subjects of intense research, and early results suggest that these new strategies may improve the efficiency of stem cell therapies. Initial experiments were performed by combining the cells with injectable biomaterials such as collagen, fibrin, or gelatin. Matrigel and other factors that provide a favorable environment rich in cytokines and growth factors were also tested. In general, these early studies showed increased survival of the transplanted cells and greater improvements in the cardiac function of the treated hearts[82]. However, these approaches did not assure complete cell retention or adequate distribution of the grafted cells. New techniques such as the creation of microtissues, i.e., cell sheets or patches, are now being developed in order to enhance both cell survival and the homogeneous and organized distribution of the cells[83].

Cellular patches are created by using biomaterials that act as delivery platforms or scaffolds for the cells, assuring their engraftment and interaction with the tissue. One class of materials used is hydrogel/extracellular matrix-based matrices, in which the cells are embedded in a soluble hydrogel matrix that condenses in response to a temperature change to form a cellularized patch that can be applied to the pericardium.

Patches of MSCs entrapped in a collagen-I matrix have been created and implanted into infarcted rat hearts, where they increased cell engraftment and functional improvement; these results appear to have been due to potentiation of the trophic effect of the MSCs by their increased survival in the tissue in the patch format[84]. Three-dimensional contractile loops of mixed collagen and neonatal cardiomyocytes, a more-sophisticated approach, have been successfully created. Implantation of these loops could support the contractile function of damaged heart[85].

Another promising approach is the creation of cell sheets with no scaffold support. This approach would avoid inflammatory reactions against the biomaterials constituting the scaffolds. Scaffold-free sheets have been constructed using culture dishes covalently grafted with the temperature-responsive polymer poly (N-isopropylacrylamide) (PIPAAm), which enables the preparation of cell sheets without enzymatic digestion[86]. The beneficial potential of this technique has been demonstrated by the transplantation of monolayers of adipose tissue-derived MSCs[33] or triple-layer cardiac tissue sheets bioengineered with ESC-derived defined cardiac cell populations (our recent report)[12] into infarcted hearts. Both methods demonstrated increased tissue neovascularization and positive attenuation of heart remodeling, resulting in improved cardiac function. The direct mechanical support of the transplanted cell sheets would be desirable for

more effective cardiac regeneration. However, no evidence of the reinforcement of contraction by the physical integration of the cell sheet and host myocardium was reported to date. To realize that, more increased survival of cell sheets would be essential, and supplemental strategies together with current cell sheet transplantation, such as vascularization of cell sheet, might be promising[12].

Transplantation of in-vitro-created 3D cardiospheres has recently been shown to improve both engraftment of cardiac progenitors and their in-vivo differentiation towards cardiac and vascular cells[87]. De-cellularized tissues have also been explored as scaffolds for cell transplantation. Tissues such as bovine pericardium[88] and omentum[89] have also been used to support cell types such as mesenchymal cells in order to enhance their paracrine effects. However, obtaining sufficient cardiac cells with no immunological risk and creating patches/organs that mimic the structure and function of the heart remain challenging.

Future directions for cardiac cell therapy

In spite of the knowledge described above, the application of stem cells have been much less studied in chronically than in acutely infarcted hearts[90]. Indeed, stem cell therapy for chronic MI is of capital importance due to the growing patient population.

Although the inhibition of tissue degeneration in the acute stage of the disease through paracrine effects such as angiogenesis is a main goal of cell therapy, the implementation of this strategy in hearts with chronic MI, in which the inflammation has receded and the angiogenic processes mostly ended, could also have therapeutic effects such as avoiding progression toward heart failure, reducing fibrosis, or regenerating the myocardium as a new contractile muscle mass. To date, cell therapy for chronic MI has been effective in both small and large animal models[33,55,89]. However, despite some exceptions, the effect on cardiac contractility is indirect rather than through true cardiac regeneration.

One future direction is the combination of stem cell therapy and conventional surgical procedures. Concomitant CABG and stem cell administration has been studied in patients with chronic myocardial ischemia, but the results were too marginal to justify full-scale therapeutic implementation[58,77]. We have previously reported that combined rat fetal cardiomyocyte injection and plication of left ventricular aneurysm resulted in sufficient functional improvement in a rat chronic MI model[4]. The combination of cell therapy and various surgical procedures other than CABG, such as left ventricular reconstruction or mitral repair for ischemic mitral insufficiency, might be a promising strategy in the future and could provide hope for many patients, especially those with

severe chronic heart failure who are ineligible for heart transplantation.

Another direction of future research is the further elucidation of the mechanisms of cardiac repair through cell therapy. Previous studies of stem cell therapy relied on injections of heterogeneous cell populations, which limited the insights they could provide into the cellular and molecular behaviors and mechanisms of action of transplanted cells. An understanding of the roles of each cell population as well as the various complex intercellular interactions in the heterogeneous populations transplanted would be a breakthrough in the improvement of cardiac cell therapy. Utilizing the mouse ES cell differentiation system to obtain defined cardiovascular populations, we recently found a major cellular mechanism, that is, cardiomyocytes are essential for sufficient cardiac restoration after sub-acute MI mainly through angiogenesis[12]. This approach to the elucidation of the regenerative mechanisms could be especially important in the context of chronic MI.

The last direction of future research that we introduce in this section is the "bioartificial heart." Replacing the injured area with a cardiac sheet or patch might be useful when a relatively small area of the heart is affected. However, this approach would not suffice when the heart has become nonfunctional and organ transplantation is indicated. Bioartificial hearts would be the ideal option in these cases, as they would

theoretically avoid the problems of both organ transplants, such as donor shortages and immune rejection, and those of mechanical hearts, such as thromboembolism formation. In-vitro heart "reconstruction" using decellularized cadaveric hearts has been demonstrated to be feasible. It is possible to re-vascularize and re-muscularize a heart "skeleton" of which only the extracellular matrix has been preserved to create a new heart. Cardiomyocytes and endothelial and fibroblastic cells are perfused using a bioreactor, which provides a pulsatile flow and pacing that mimics physiological conditions. Surprisingly, such newly formed hearts can contract spontaneously[91]. Of course, many issues, such as methods for isolating a sufficient volume of cardiovascular cell populations without the risk of immune rejection (in this regard, iPS cells would be the ideal cell source) and preventing fatal arrhythmias, remain to be resolved before these bioartificial organs are developed to the point of clinical use. In any case, the demonstration of the possibility of creating such organs represents a great step forward in the treatment of cardiac diseases.

Other research related to cardiovascular regeneration

Interspecific chimera technology

There are many promising approaches to cardiac regeneration besides the cell therapies discussed above. The generation of human hearts from other animals by using interspecific chimera technology with blastocyst complementation is one such approach. The injection of rat wild-type iPSCs into $Pdx1^{-/-}$ (pancreatogenesis-disabled) mouse blastocysts was recently reported to result in the generation of normally functioning rat pancreases in $Pdx1^{-/-}$ mice[92]. This result indicates that organs derived from donor iPSCs can be generated in vivo in a xenogenic environment. The development of this technology for the generation of human hearts within size-matched animals, such as pigs, is ongoing in Japan ("NAKAUCHI Stem Cell and Organ Regeneration" project[93]).

However, several problems remain to be overcome. The first of these concerns xenotransplantation. As the vessels (including the aorta and other large vessels), blood cells, nervous system, and other associated tissues would be derived from animals, while the organ itself would be of human origin, the transplantation of these chimeric organs into humans would be partial xenotransplantation rather than full allo/auto-transplantation. The generation of animals with genetically human blood vessels and nervous systems would be one solution to the potential for immunological rejection of xenotransplanted organs. The second problem is that in the abovementioned experiment, the iPSC-derived cells were found not only in the pancreas but in all organs

and tissues, including the brain and gonads[92]. Without proper control of the differentiation of iPSCs, the generation of human organs in livestock animals will pose ethical issues. One approach to addressing this problem is the use of lineage-committed stem or progenitor cells in place of iPSCs. The introduction of such cells into an appropriate microenvironment at the appropriate developmental time point might allow restriction of differentiation to a particular organ.

Biomaterials for efficient drug delivery

The technology for realizing the beneficial effects of cell therapy must be further advanced before it can attain its full potential. The combination of cell therapy and local administration of proteins that induce paracrine effects such as angiogenesis is one possible method of enhancing the therapeutic potential of cell therapy. Tabata et al. have developed a system for sustained release of angiogenic cytokines, such as bFGF, from a biodegradable material, gelatin hydrogel; this system enables us to control the release of cytokines over the periods required for efficient clinical outcomes[94]. The addition of such sustained release of bFGF enhanced the functional benefit of the transplantation of CDCs in a porcine MI model[71] and is being used in the ongoing clinical trial ALCADIA mentioned above[78].

In addition to its use in cardiac regeneration, the sustained-release system is also applicable to the treatment of critical limb ischemia. We found that the sustained release of bFGF improved the resolution of foot ulcers or other clinical symptoms in patients with severe limb ischemia in an initial phase 1–2a study[95], and an advanced clinical trial is now ongoing. Drug delivery systems using biodegradable biomaterials could thus be a promising strategy for the advancement of cardiovascular regeneration.

Gene therapy

Another avenue of regenerative medicine is gene therapy, which is emerging as a potential treatment option in patients suffering from a wide spectrum of cardiovascular diseases, including coronary artery disease, peripheral vascular disease, vein graft failure, and in-stent restenosis[96]. Gene therapy, which is the direct introduction of transgenes into the vasculature or myocardium, may contribute to controlling the symptoms of cardiovascular disease and may also be able to reverse the pathological processes involved. However, before these objectives can be achieved, 3 goals must be accomplished: suitable vectors must be generated, a suitable gene or group of genes must be identified, and an appropriate delivery system must be developed. The optimal characteristics of these components may vary depending on the disease being targeted.

Gene therapy to induce calcium upregulation in patients with advanced heart failure was recently attempted. The Calcium Upregulation by Percutaneous Administration of Gene Therapy in Cardiac Disease (CUPID), a phase-2 trial, decreased the frequency of cardiovascular events in the patient group, who received intracoronary administration of adeno-associated virus type 1/sarcoplasmic reticulum Ca^{2+}-ATPase[97]. MicroRNAs (miRNAs), which are small, non-coding RNAs that regulate gene expression in a sequence-dependent manner, are also being investigated as a new modality of gene therapy for ischemic heart disease or vascular diseases[98].

In 2010, Ieda et al. reported that a combination of 3 developmental transcription factors (Gata4, Mef2c, and Tbx5) rapidly, efficiently, and directly reprogrammed postnatal cardiac or dermal fibroblasts into differentiated cardiomyocyte-like cells in vitro (direct reprogramming)[99]. This technology was recently applied to an in-vivo mouse MI model in which the 3 genes were delivered by a retroviral vector, resulting in the direct reprogramming of cardiac fibroblasts within the infarction site into cardiomyocyte-like cells and the attenuation of cardiac dysfunction[100]. Therefore, despite concerns over the ethics and safety of gene therapy (the latter related to the potential unexpected side effects of genomic integration), it is a promising segment of the broad field of cardiovascular disease research.

Conclusion

Herein, we introduced the status quo and future directions of stem cell therapy for treatment of cardiac disease and, more briefly, other approaches to cardiac regenerative research. We emphasize that we should not discuss which of these therapeutic modalities is to be preferred but rather consider them as components of an integrated medicine that would, as the summation of the new therapies introduced in this review and others that were not discussed, constitute a step towards the realization of cardiac regenerative therapy as a realistic option. In this context, the power of cardiovascular surgery as the integrator of basic research and clinical practice is virtually immeasurable.

Although much more work remains to be done, cardiac regenerative medicine, in conjunction with current treatment modalities, may help to further reduce the mortality and improve the quality of life of cardiovascular disease patients.

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Table Legends

Table 1:

Characteristics of stem-cell populations used for cardiac cell transplantation therapy.

Table

Stem cell type	Origin	Advantages	Disadvantages	Clinical trials
Pluripotent stem cells				
Embryonic stem cells (ESCs)	Inner cell mass of the preimplantation blastocyst	Theoretically unlimited self-renewal capacity; pluripotency	Ethical considerations; teratoma formation; graft-versus-host disease	None
Induced pluripotent stem cells	Most somatic cells (e.g., skin fibroblasts)	Same advantages as ESCs; free from ethical/immunological issues	Potential teratoma formation	None
Bone-marrow derived stem cells				
Hematopoietic stem cells (circulating progenitor cells, bone marrow mononuclear cells)	Bone marrow, peripheral blood	Easy to isolate; safe and feasible to transplant	Limited potential for differentiation into cardiomyocytes and vascular cells	TOPCARE-AMI[25], BOOST[26], REPAIR-AMI[27], LateTIME[28]
Mesenchymal stem cells	Bone marrow (adherent cells), adipose tissue	Easy to isolate and expand in culture; less immunogenic than other lines; multipotent	Great heterogeneity; heterotopic differentiation (e.g., ossification)	Report from Chen et al. (China)[34]
Endothelial progenitor cells	Bone marrow, peripheral blood	Mobilized from bone marrow or present in peripheral blood; important in vasculogenesis	Heterogeneity; small populations; reduced in individuals with cardiovascular comorbidities	REGENT[45]
Skeletal myoblasts	Mature skeletal muscle	Extensive scalability; resistance to ischemia; multipotent; no teratoma formation	Potential for arrhythmias; lack of cardiomyocyte differentiation	MAGIC[58], CAuSMIC[57]
Cardiac stem cells	Special niches in the myocardium	Resident in therecipient heart; robust cardiovascular differentiation potential; reduced tumor formation	Stem cell pool appears to undergo senescence; scalability largely unknown	CADUCEUS[76], SCIPIO[77], ALCADIA[78]

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