

# Myoblast Transplantation for Cardiac Repair: A Clinical Perspective

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The incidence of heart failure is achieving epidemic proportions. Adult human myocytes cannot regenerate because these cells do not reenter the cell cycle. In patients with heart failure, myoblast transplantation is emerging as a potential therapeutic option to augment the function of remaining myocytes. Both skeletal myoblasts and autologous bone marrow cell transplantation, after intensive preclinical experimental animal studies, have entered phase I safety studies in humans. Most of these clinical trials have involved small groups of patients and cell transplantation was carried out as an adjunct to coronary revascularization. Preliminary results show that the procedure is safe and leads to improved myocardial function. This paper reviews and summarizes the outcome of these phase I trials involving skeletal myoblast transplantation.

**Key Words:** cardiac, clinical, heart failure, human, myoblast, transplantation

## INTRODUCTION

Cardiovascular diseases (CVD), principally heart disease and stroke, remain the leading cause of morbidity and mortality for both men and women globally. In the United States alone, approximately 5 million patients suffer from heart failure. More than 61 million Americans have some form of CVD, including high blood pressure and coronary artery disease [1]. The incidence is likely to increase and in particular with "the graying of America" the proportion of elderly will increase. Although medical management has much improved, once a patient develops end-stage heart failure the prospect is dismal. These statistics serve to focus attention on the urgent need to develop additional therapeutic measures to manage this serious problem. The concept of whole-organ heart transplantation has been successful but has major limitations such as donor supply and immunosuppressive complications. Currently, artificial hearts and left ventricular assist devices are cumbersome and expensive and not a viable option. The concept of cellular transplantation to augment the function of the failing heart has much experimental and now increasing clinical evidence to suggest more detailed clinical evaluation [2,3]. However, the type of the cell, the number of the cells, the delivery strategy, and the time of injection still remain the subjects of intense investigation. This review

will discuss the current status of myoblast transplantation in the clinical arena and highlights the progress and pitfalls of the approach.

## TREATMENT FOR HEART FAILURE

The etiology of heart failure is multifactorial. Management of heart failure includes medical and surgical options [4–7]. The surgical options include revascularization, correction of valvular dysfunction, implantation of assist devices, and heart transplantation. Each of the aforementioned techniques has its limitations. The search for an alternative to pharmacological treatment is mandatory for patients with advanced disease. Dynamic cardiomyoplasty, after extensive evaluation, has largely been abandoned [8,9]. As the majority of patients in most regions of the world have heart failure due to an ischemic etiology that largely results in left ventricular impairment initially, the use of left ventricular assist devices for the management of heart failure holds promise. The concept of using left ventricular assist devices (LVAD) as a bridge to transplantation has gained wide acceptance [10]. Other options for LVAD use include a bridge to recovery and an alternative to transplantation. However, the use of LVAD is associated with considerable morbidity and mortality and they are extremely expensive. The current

LVAD available are not suitable for long-term implantation and to a large extent the elderly and patients with comorbid conditions may be excluded. Heart transplantation remains the only definitive treatment for the resultant end-stage heart failure; however, donor hearts remain scarce, transplant atherosclerosis develops uniformly in the transplanted heart, and immunosuppression is associated with considerable complications. Thus heart transplant cannot be viewed as a viable surgical option in the vast majority of patients with end-stage heart failure. Xenotransplantation currently, and for the foreseeable future, is not available.

The 21st century has seen rapid advances in the emergence of both molecular and cellular-level approaches to diagnose and treat diseases. In the area of heart failure and coronary artery disease such approaches have focused on cell transplantation for cellular myocardial reconstruction and gene therapy for angiogenesis [11–13]. These new approaches supplement the insufficiency of the intrinsic repair mechanism of cardiac muscle.

### THE ABILITY OF THE ADULT MYOCYTE TO RESPOND TO IRREVERSIBLE INJURY IS LIMITED

The limited regenerative ability of the adult myocardium is related to the fact that the adult cardiac cell is a postmitotic cell. In humans cardiomyocytes withdraw from the cell cycle early in postnatal life and become terminally differentiated [14]. The mechanism and the timing of this switch are not certain, although it is currently believed that the transition is gradual [15]. Division and proliferation of the terminally differentiated ventricular cardiomyocytes in the adult mammal do not occur after injury such as that caused by a myocardial infarction [14]. This, together with the lack of tissue-specific stem cells, renders the heart less capable of regenerating muscle cells in the event of irreversible injury. However, recently the aforementioned limitations for myocardial repair have been called into question [16]. A number of studies have shown that some cardiomyocytes can reenter the cell cycle in response to ischemic injury. The number, however, is small and is probably insufficient to repair the damage completely or sufficiently to restore overall function [17,18]. In addition recent publications suggest that there is a subpopulation of cardiac muscle stem cells in the adult heart that may transform into myocytes and vascular structures in response to myocardial injury [19]. Thus, the damaged heart has some potential to repair itself.

Alternative methods have been considered to compensate for the deficient intrinsic repair mechanism for the regeneration of myocardial tissue damaged by ischemia and infarction [20–22]. These include the transplantation of contractile cells into the myocardium to repopulate the area of injury and scarring [23,24].

### CELL TRANSPLANTATION FOR HEART FAILURE

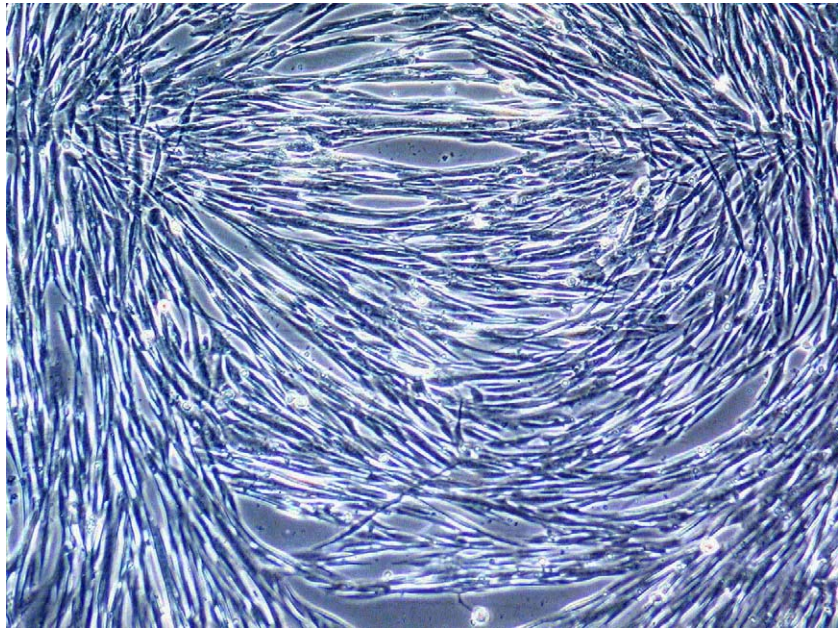
Myocardial cell transplantation is intended to compensate for the loss of cardiomyocyte number and aims to limit and/or reverse the consequences of contractile dysfunction of a damaged left ventricle [25–28]. These effects could be related directly to the injected cells or mediated indirectly by angiogenic or growth factors secreted by transplant cells [29–31]. More recently, transplantation of cells with inherent ability to secrete growth factors or genetically modulated cells carrying angiogenic growth factors is being assessed with the added consequence of angiogenesis concomitant with myogenesis [32–37]. These results are still relatively preliminary and await additional preclinical studies. However, the techniques for cardiac regeneration appear promising and offer alternative methods for the treatment of ischemic heart disease [38].

### THE CHOICE OF DONOR CELLS

In addition to other factors, choice of cells is influenced by the ultimate and desired aim of the procedure [39]. Various cell types such as embryonic, fetal, and adult; differentiated and undifferentiated; and myogenic and nonmyogenic have been explored in numerous animal studies as possible sources for implantation into the damaged heart. The earlier studies have used cells including embryonic stem cells, adult and fetal cardiomyocytes, fibroblasts, smooth muscle cells, mesenchymal stem cells, and skeletal myoblasts [40–46]. Of these, embryonic stem cells, fetal cardiomyocytes, mesenchymal stem cells, and myoblasts appear to have the greatest potential for use in cellular cardiomyoplasty. Grafting of adult and fetal cardiomyocytes is efficacious, producing viable and stable grafts after implantation in animals [47,48]. They form intercalated discs and gap junctions after grafting, suggesting electromechanical coupling of these cells with the host myocardium [49]. However, the use of embryonic stem cells and fetal cardiomyocytes in humans raises ethical and availability issues.

### SUITABILITY OF MYOBLASTS FOR TRANSPLANTATION

Unlike heart muscle, skeletal muscle has the ability to regenerate and repair after injury due to the presence of satellite cells. They proliferate and differentiate when activated in response to muscle injury. Skeletal myoblasts are mononucleated unipotent progenitor cells that can be expanded *in vitro* (Fig. 1). The advantages of using autologous skeletal myoblasts are availability, the lack of immunologic barriers to the transplantation process, which precludes the need for immunosuppression to allow donor cell acceptance by the host, and the diminished risk of tumorigenesis. At the same time, the satellite cells can be genetically modified *in vitro* to deliver

FIG. 1. Human skeletal myoblast *in vitro* culture.

angiogenic cytokines and growth factors to encourage angiomyogenesis. Animal studies have shown that grafted myoblasts form myotubes in the myocardium and eventually mature to become well-formed myofibers with contractile apparatus. These results have been confirmed by results in humans [50,51]. These grafted cells acquire a fatigue-resistant slow-twitch muscle phenotype that is better suited to perform a cardiac-type work load. Furthermore, it has also been shown that the transplantation of myoblasts results in a significant functional improvement in damaged hearts [25,28,52–54]. However, there have been contradicting reports on the differentiation of myoblasts into cardiomyocyte-like cells with intercalated discs [55]. The ability of the skeletal myoblasts to make meaningful electromechanical connections for the transmission of electrical impulses with the host cardiomyocytes through gap junctions is questionable [56].

## SKELETAL MYOBLASTS IN HUMAN STUDIES

### The European Experience

As a result of encouraging animal studies, the first clinical application of cell transplantation as an adjunct to coronary artery bypass grafting (CABG) was performed by Menasche and colleagues using cultured autologous skeletal myoblasts in a 72-year-old male patient [57]. The patient was in New York Heart Association (NYHA) class III [58,59] with a mean left ventricular ejection fraction (LVEF) of  $21 \pm 2\%$  by echocardiography. Follow-up at 5 months showed the patient was in NYHA class II with an improvement in the LVEF to 30%. The procedure was performed without any complications. The promising

results of this study paved the way for phase I clinical trials. These have been carried out in humans with two different cell types: bone marrow-derived adult stem cells [33,60,61] and skeletal muscle myoblasts [57,62,63]. Patients were selected based on a previous history of myocardial ischemia, left ventricular dysfunction, the presence of a nonviable and akinetic postinfarction scar, and an indication for CABG.

Since their first landmark study, Menasche and colleagues have reported nine more patients as a part of phase I trials [64]. The patients (mean age  $60 \pm 3$  years) were diagnosed with severe left ventricular dysfunction (LVEF, 35%). An average of  $8.74 \times 10^8$  autologous myoblasts (range  $5 \times 10^8$  to  $1.15 \times 10^9$ ) (86% CD56<sup>+</sup>; range 67–96) were injected into akinetic, nonrevascularizable, and nonviable scar as assessed by dobutamine echocardiography and positron emission tomography (PET). The average number of sites injected was 38 (range 27–57). The cell transplantation procedure was event free, without any perioperative complications. The nine operative survivors were followed for up to 8 months. The results showed an improvement in NYHA class for all the patients, from NYHA class  $2.7 \pm 0.2$  to  $1.6 \pm 0.1$  ( $P < 0.02$ ), in parallel with documented improvement in LVEF from  $24 \pm 1$  to  $34 \pm 1\%$  ( $P = 0.02$ ). The improvement in cardiac function restoration was observed during the first 3 months posttransplantation. This improvement has not deteriorated since. These positive findings have resulted in a randomized multicenter trial.

Siminiak and colleagues in Poland, in August 2001, documented the first reported case report of autologous myoblast transplantation for the treatment of post-my-

cardial infarction-depressed myocardial function [62]. The procedure was carried out as an adjunct to CABG in a 55-year-old female patient who suffered a transmural anterior wall myocardial infarction. Echocardiographic functional assessment revealed extensive akinesia of the apex and apical segments of the anterior wall and the septum. The absence of the viability in the akinetic region was shown by low-dose dobutamine echocardiography. Transplantation of  $1.2 \times 10^6$  autologous myoblasts prepared from the patient's own skeletal muscle biopsy samples was carried out into the akinetic area of the left ventricle during routine CABG. Echocardiography at 4 weeks posttransplantation revealed significant improvement in segmental contractility, especially in the apical region. A more detailed account of the phase I study involving one female and nine male patients was presented by Siminak *et al.* during the American Heart Association Scientific Sessions 2002 [65]. All the patients underwent autologous myoblast transplantation as an adjunct to CABG. Six-month follow-up showed improved segmental contractility and restored myocardial function.

Chachques and colleagues initiated a phase I study for autologous myoblast transplantation as an adjunct to routine CABG in 4 male and 1 female patient (mean age 63 years) in NYHA functional class III [66]. The study has now been extended to include 18 patients (90% males) with an average NYHA class 2.6 [67]. The patients were diagnosed with impaired left ventricular function (ejection fraction  $32 \pm 5\%$ ) and left ventricular posterior wall postischemic scars (akinetic and absence of metabolic viability). Autologous myoblasts were generated from  $12 \pm 3$  g skeletal muscle biopsy samples from the patients and expanded in  $17 \pm 4$  days up to  $300 \pm 20$  million. The propagation of the cells was carried out in complete human medium, using the patients' own sera. At the time of implantation, the purity of myoblasts was  $78 \pm 5\%$ , with  $95 \pm 3\%$  cell viability. The cells, suspended in 5 ml human albumin solution, were injected into  $12 \pm 4$  injection sites in and around the infarct region. There were no complications related to the cell transplantation procedure. The patients showed uneventful recovery and were discharged from the ICU 2 days after surgery. At follow-up (mean  $9 \pm 3$  months) no cardiac arrhythmias were observed. Echocardiographic studies showed an improvement in regional wall motion (from akinetic scars to hypokinetic ventricular wall). The infarct scar size appeared to be significantly reduced from  $21 \pm 5$  to  $8 \pm 3$  cm<sup>2</sup> ( $P < 0.05$ ). Myocardial viability tests showed regenerating nodes, with patients moving from mean heart failure class 2.6 to class 1.3.

In addition to the aforementioned phase I studies, clinical myoblast transplantation studies are under way in Spain (14 cases), The Netherlands (13 cases), Germany (Düsseldorf and Rostock), and Italy (5 patients) (unpublished data).

### The American Experience

In addition to myoblast transplantation as an adjunct to CABG, the procedure has also been carried out in association with LVAD implantation as the first part of a multicenter trial sponsored by Diacrin, Inc., in Massachusetts [51]. Five patients (median age 60 years) with a history of ischemic cardiomyopathy were selected for the study. These patients were on the waiting list for heart transplantation and were to receive an LVAD implantation as a "bridge to transplantation." They were on maximal inotropic support with a median LVEF of 15%. Muscle biopsies from the thigh were cultured and resulted in a myoblast purity of 43 to 97%. A total of  $300 \times 10^6$  cells were injected into each patient, with the exception of one patient who required urgent LVAD implantation before sufficient cells could be cultured. A total of 3 to 38 sites were injected into the infarcted areas or the border zones. These sites were marked with surgical clips. This permitted histological examination of the site after LVAD explantation. The second part of the trial is a dose-escalation phase II trial, with safety being evaluated at doses ranging from 10 million to 300 million cells.

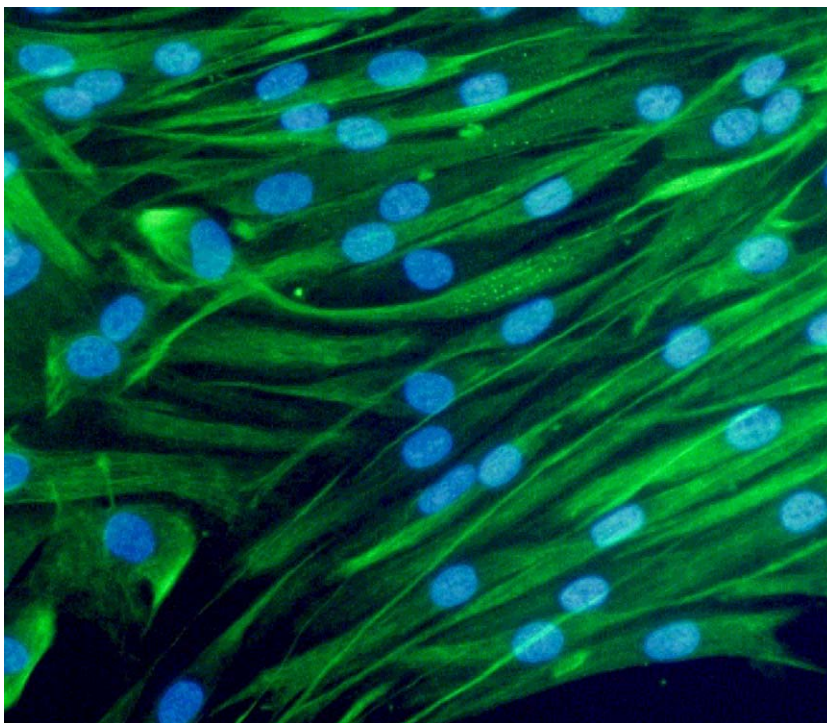
Dib and colleagues at the Arizona Heart Institute, Phoenix, presented a study involving transplantation of skeletal muscle cells into the scarred regions of the heart in 16 patients as an adjunct to CABG ( $n = 11$ ) or LVAD implantation ( $n = 5$ ) [68]. The study was part of the multicenter industry-sponsored trial from Diacrin and involved a dose-escalation study. Autologous myoblasts were purified and proliferated *in vitro* from each patient's thigh muscle biopsy samples. A total of 10 million to 300 million cells were injected directly into the scarred regions. There were no intraoperative or postoperative complications associated with the procedure. The follow-up evaluation by magnetic resonance imaging, echocardiography, and PET showed successful survival of the transplanted cells within the heart at the site of the graft, which registered improvement in LVEF from 21 to 29% at 3 weeks follow-up.

Recently, Bioheart has initiated the multicenter Food and Drug Administration-approved MYOHEART clinical trial to be carried out at Mount Sinai Hospital (New York, NY), Duke University, and the American Cardiovascular Research Institute (Atlanta, GA). Similarly, Genzyme has announced a MAGIC (myoblast autologous grafting in cardiomyopathy) multicenter trial in Europe and America. Some other institutions involved in myoblast transplantation include the University of California at Los Angeles, Temple University at Philadelphia, the University of Michigan at Ann Arbor, and the Cleveland Clinic.

### The Asian Experience

Our group in Singapore carried out the first autologous myoblast transplantation on a beating heart as a part of a phase I clinical study. A 55-year-old male patient presented with acute myocardial infarction. The apex, ante-

**FIG. 2.** Fluorescent immunostaining of human skeletal myoblast for desmin expression *in vitro* using FITC-labeled antibodies.



rior wall, and septum of the left ventricle were akinetic and the LVEF was 31%. After informed consent and as part of an Institutional Review Board-cleared clinical trial, the patient received  $3.78 \times 10^8$  autologous myoblasts at 20 different sites in and around the infarct region during CABG. The cells were >98% pure for desmin expression, with >99% viability at the time of injection

(Fig. 2). A 6-month follow-up revealed a perfusion defect involving the anterior wall and the apex with partial reversibility on  $^{99m}\text{Tc}$ -tetrafosamine nuclear scan. Our findings were in agreement with previously published reports and demonstrated the safety, viability, and benefit of autologous myoblast transplantation as an adjunct to off-pump CABG (Fig. 3). Although further

**FIG. 3.** Autologous human myoblast transplantation in a human patient as an adjunct to coronary artery bypass surgery on a beating heart.



patients need to be evaluated, the benefits of reduced risks associated with off-pump CABG cardiopulmonary bypass offer an attractive technique for delivering the cells for transplantation.

More recently, Zhang and colleagues from Nanjing Medical University, People's Republic of China, have reported a phase I study including three patients with a history of coronary heart disease [63]. The patients underwent coronary artery bypass grafting and implantation of autologous satellite cells. Satellite cells were isolated from muscle biopsies of the right vastus lateralis muscle after enzymatic treatment. While the heart was under hypothermic cardioplegic arrest, 4 ml of cell suspension divided into approximately 40 doses was injected into the ventricular wall of the ischemic area in less than 5 min. All patients survived the procedure and had an uneventful recovery. There were reports of occasional arrhythmias. However, these did not require treatment. No arrhythmia was observed during longer follow-up. Beneficial results noted at 4 months after the operation include an increase in left ventricular ejection fraction and decreased left ventricular diastolic diameter, as well as improved ventricular wall thickness observed by 2-D echocardiography. There was significant improvement in perfusion ( $^{99m}\text{Tc}$  MIBI) and metabolic activity ( $^{18}\text{F}$ -deoxyglucose) at the cell implantation sites. The same group has reported a fourth patient with heart failure (NYHA class III–IV) and LVEF 37.1% [69]. The patient underwent routine CABG and autologous skeletal myoblast transplantation. At 5 months follow-up, the patient showed dramatic improvement to NYHA class II. The LVEF improved to 48.6% by 2-D echocardiography and the left ventricular systolic diameter changed from 48 mm (before cell transplantation) to 45 mm (after cell transplantation). There was marked improvement in the perfusion and metabolic activity. These results confirm the safety and early benefit of cellular cardiomyoplasty using autologous satellite cells.

Time constraints and the logistic problem of generating autologous skeletal myoblasts for every patient can be overcome if allogeneic skeletal myoblasts from healthy young donors can be made available. The safety and feasibility of allogeneic myoblast transplantation were tested for the first time in human subjects on January 17, 2003, at the Bakoulev Center in Moscow, Russia [70]. Two patients (mean age 59) received between 1 and 1.2 billion skeletal myoblasts as an adjunct to CABG on nonbeating hearts. The patients received 5–7 mg/kg/day cyclosporin starting 5 days prior to until 2 months after cell transplantation. Despite cyclosporin discontinuation, immunorejection was not observed. At 3 months follow-up, subjects were in stable condition with angina at class I–II (CCS) instead of class IV. Echocardiography demonstrated 14.6 and 10.5% increases in LVEF. Nuclear imaging using single photon emission computed tomography demonstrated positive

dynamics, with an increase in LVEF, and a reduction in perfusion defects both at rest and during exercise. The results of this study support the concept of using allogeneic myoblasts as an alternative therapy for heart failure using only a short course (2 months) of immunosuppressive therapy.

## SOME IMPORTANT CONSIDERATIONS RELEVANT TO MYOBLAST THERAPY IN HUMANS

### Safety, Feasibility, and Efficacy

The safety and feasibility of cell transplantation have been repeatedly documented during multiple preclinical animal experiments and clinical phase I studies [57,71,72]. There have been no reports of perioperative complications in any of the studies involving myoblast transplantation. The only serious postoperative adverse event related to the procedure was the occurrence of ventricular arrhythmias [51,64].

Because the preliminary goal of initial studies has been to assess the safety of the procedure, most of the studies to date have lacked a control arm, i.e., a group of patients into whom no cells were injected. The sample size included in these early studies was small and the application of the cell transplantation approach in all these studies was conducted as an adjunct to standard revascularization procedures such as CABG and LVAD. This makes it impossible to interpret the outcome of the uncontrolled studies and to assess the benefits of the approach in real terms. However, the end-point measurements in these studies highlight improvement in the quality of life, reduced nitroglycerine consumption, enhanced exercise tolerance, improvement in NYHA class, improvement in wall motion by echocardiography, and significantly reduced perfusion defects (Table 1).

### Mortality

There have been no reported intraoperative deaths. Postoperative deaths have occurred (Table 1). By the end of the follow-up period (mean = 7.5 months, range 1.0 to 12.0), there have been three reported cases of unrelated postoperative deaths among patients who have undergone cell transplantation after CABG [64]. One early postoperative death was related to a mesenteric infarction, while the cause of death for the other two patients was unrelated to myoblast transplantation. In addition, another patient died 17.5 months after the procedure as a result of a stroke [50]. After a mean LVAD support time of 122 days (range 68 to 191), four of the five patients who had a LVAD implanted before cell transplantation underwent device explantation [51]. Three of them received heart transplant, while one died as a result of sepsis. One other patient remains on LVAD support. No deaths have been linked directly to the cell transplant procedure. One

Table 1

Reference	Adjunct	Source	Patients (n)	Cell count	Purity	Sites injected	Results	Complications
Menasche <i>et al.</i>	CABG	Autologous	1	$800 \times 10^6$	65% CD56 <sup>+</sup>	33	Stabilized in NYHA class II LVEF improved to 30% Improved segmental contractility and perfusion	None
Menasche <i>et al.</i>	CABG	Autologous	10	$871 \times 10^6$	86±3% (range 67–97%)	37±3 (range 27–57)	LVEF increase (from 23.8±3.9 to 32.1±7.5%) New-onset echocardiographic systolic shortening Improvement in NYHA class (2.7±0.2 to 1.6±0.1)	4 patients with VT
Chachques <i>et al.</i>	CABG	Autologous	5 extended to 18	$300 \pm 20 \times 10^6$	82±5%	6±2	Improved regional fractional shortening (9±3 to 20±5%); reduced scar size Improvement in NYHA class	None
Siminiak <i>et al.</i>	CABG	Autologous	1	$1 \times 10^6$	—	8	Increase in segmental contractility seen on echocardiography	1 episode of sustained VT
Siminiak <i>et al.</i>	CABG	Autologous	10	$2 \times 10^7$	—	—	No peri operative complications Improved segmental contractility	Sustained VT in 2 patients; 1 death unrelated to cell transplantation
Nabil <i>et al.</i>	CABG	Autologous	11	$10–300 \times 10^6$	61–96% CD56 <sup>+</sup>	3–30	Improved LVEF from 21 to 29% MRI and PET scan showed evidence of viability	None
Sim <i>et al.</i>	CABG on beating heart	Autologous	1	$3.74 \times 10^8$	>98% desmin positive	20	Improved cardiac function on echocardiography Reduction of perfusion defect from 30 to 22% Improved LVEF from 30 to 37% at 6 months	None
Pagani <i>et al.</i>	LVAD		5	$300 \times 10^6$	43 to 97%	3 to 38	Myofiber staining for myosin heavy chain parallel to host myocardial fibers Increased blood vessel count ( $72 \pm 17$ cells vs $229 \pm 24$ cells, $P < 0.0001$ )	Atrial fibrillation (n=2); VT (n=2)
Law <i>et al.</i>	CABG	Allogeneic	2	$1.1 \times 10^8$ and $1.2 \times 10^8$	>98%	18 and 19	Echocardiography showed 14.6 and 10.5% increases in LVEF with no local hypokinetic regions <sup>99m</sup> Tc-tetrofosamine SPECT showed positive dynamics, reduced perfusion defects during exercise and rest	None
Zhang <i>et al.</i>	CABG	Autologous	3	—	—	30–40	Improved LVEF and left ventricular wall thickness on 2-D echocardiography Significant improvement on perfusion scan	Occasional arrhythmia during intensive care unit stay but not observed during the follow-up

CABG, coronary artery bypass grafting; LVAD, left ventricle assist device; LVEF, left ventricle ejection fraction; **MRI**, magnetic resonance imaging; PET, positron emission tomography; VT, ventricular tachycardia.

death has been reported by Siminiak *et al.* (2002) postoperatively [65]. The death, however, has been related to acute myocardial infarction and was unrelated to the cell transplantation procedure.

### Histology

Histological data of the grafted areas in the human patients have been reported by Hagege *et al.* and Pagani *et al.* [50,51]. The patient of Menasche's landmark study died 17.5 months after receiving myoblast transplantation. The heart was explanted postmortem and subjected to histological studies [50]. The results showed the presence of myofibrils that stained for skeletal muscle-specific myosin heavy chain in the injected areas. They were seen to be aligned in a direction parallel to host myocardial fibers. Slow-twitch isoforms and fast-twitch isoforms as well as coexpression of both forms were seen in percentages of 32, 35, and 33%, respectively. Percentages of 44, 55, and 0.6% are seen in human skeletal muscle. This switch of phenotype toward the slow-twitch isoform is thought to be caused by repeated stretch as a result of contraction of the adjacent myocardium or by incomplete elimination of the fibers expressing only the fast isoform. Compared to noninjected areas, areas where myoblasts were injected showed a significant increase in number of blood vessels ( $72 \pm 17$  cells vs.  $229 \pm 24$  cells,  $P < 0.0001$ ).

Multinucleated giant cells have also been seen in grafted segments in the myocardium, associated with noncellular material introduced during transplantation [51]. Aside from these cells, no sign of ongoing inflammation was reported. There was an absence of connexin-43 staining on immunohistochemistry, thus suggesting impaired electrophysiological coupling between the grafted cells and the surrounding host cardiomyocytes.

### Cell Survival

Myoblast transplantation is confronted with the problem of donor cell survival posttransplantation [73]. It has been shown in animals that up to 90% of grafted cells die within the first 24 to 48 h after transplantation [74,75]. In addition, total myoblast survival is probably <1% in humans. This poor cell survival has been attributed to inflammatory changes at the site of implantation. Inflammation is the result of trauma due to needle puncture, immune-mediated rejection of myoblasts, or release of immune modulators as a result of myoblast cell death [76]. In particular, natural killer cells have been shown to play a central role in the early death of grafted myoblasts [77]. The exposure of myoblasts to the culture medium containing animal proteins *in vitro* may lead to changes in the surface antigen characteristics of the myoblasts and expression of neoantigens [78]. To overcome this problem, Chachques and colleagues are the only group using human serum-supplemented myoblast culture medium for myoblast purification and culture

[67]. However, a recent investigation has revealed the poor ability of human serum-supplemented culture medium to support myoblast growth *in vitro* [79]. The presence of nonviable cells in the myoblast preparation for transplantation renders them vulnerable to immune rejection [76]. Other mechanisms proposed for low cell survival include mechanical cell damage during grafting and cell leakage from the sites of needle puncture.

### Cardiac Arrhythmia

Cardiac arrhythmia as a postoperative complication has been reported after myoblast transplantation. Menasche reported that 4 of the 10 patients suffered from sustained monomorphic ventricular tachycardia (VT) that was resistant to treatment by amiodarone and beta-blockers and necessitated the implantation of an automatic internal cardioverter/defibrillator [13,64]. Pagani reports that 4 of 5 patients suffered from atrial fibrillation ( $n = 2$ ) or VT ( $n = 2$ ) [51]. The patient in the Poland trial also suffered from an episode-sustained VT that was resolved by treatment with amiodarone. Six of eight patients who developed arrhythmias had a prior history of arrhythmias. Most episodes of arrhythmias were clinically well tolerated and did not result in any deaths. The etiology of arrhythmia after myoblast transplantation is probably multifactorial and includes an inhomogeneous distribution of gap junctions, a difference in the isotypes of ion channels on skeletal muscle cells and cardiomyocytes, and the release of inflammatory mediators after needle puncture [80]. The presence of a nonmyogenic population present in the myoblast cell population that is transplanted may further aggravate the situation.

### FUTURE DIRECTIONS

The results of the phase I human studies, although encouraging, are still preliminary. A more concerted collaborative effort between the various research groups involved is likely to further the knowledge in the field. The development of strict inclusion and exclusion criteria, better establishment of the target population of patients who may benefit from cell transplantation, and more widespread trials will allow the real benefit of this concept to be established. The methods for end-point measurements of the studies should be made more uniform so that the results emanating from various centers may be more uniformly interpreted. To date, studies on myoblast transplantation have been carried out as an adjunct to routine surgical procedures such as CABG and mechanical-assist device implantation. This makes it hard to realize the true effectiveness of the cell transplantation approach. The beneficial effects could be related directly to the injected cells or indirectly to a combined effect of surgical manipulation and cell transplantation. Further investigative work needs to be done on the basic issues such as the ideal cell type, the optimal

number of cells, and the route of administration. The beneficial effects seen to date may be related directly to the injected cells or mediated indirectly by angiogenic or growth factors secreted by the transplanted cells. The most suitable time for cell transplantation after ischemic injury has also not been resolved [81]. If cells are transplanted too early after the injury, their survival could be impaired by the ongoing inflammatory response at the injured site; if they are injected too late, transplantation may not successfully prevent fibrosis from developing in the injured region.

The optimal mode of cell delivery continues to be evaluated. Without exception, in all the previous clinical studies reported, direct intramyocardial injection has been used for myoblast delivery. This is the simplest approach but is too invasive if considered in the perspective of the clinical scenario in which cell therapy will be used as the sole therapy. Furthermore, loss of cells due to leakage from the site of injection after direct injection needs to be prevented [67]. In addition to the surgical route of direct intramyocardial cell transplantation, intravenous, intracoronary, intra-arterial, and catheter-based delivery systems are currently being intensively evaluated as alternative approaches for cell delivery [61,82,83]. The results in the animal studies have shown the feasibility of an endovascular route of delivery to an infarcted area [84,85]. Cells can be delivered accurately with the assistance of NOGA electromechanical mapping. Catheter-based needle endomyocardial injection is associated with equivalent or superior injectate retention compared with open chest epicardial injection [86]. The percutaneous procedure raises the possibility of repeated grafting of cells without the need to perform an open chest procedure. The use of myoblasts from other than autologous sources may result in on-the-shelf availability of cells, which will help solve some of the logistic problems.

The mechanisms responsible for the beneficial effects seen with cell transplantation remain controversial. Elucidation of the exact mechanism of cell survival and exactly how the transplanted cells contribute to improvement in overall cardiac function remain the focus of intense investigation. In addition, the spectrum of cardiovascular pathologies that could benefit from myoblast transplantation needs to be assessed. Clinical trials must be designed to address these basic issues. Further to this, there is a need for blinded and placebo-controlled studies to assess the true efficacy of this approach. There is much theoretical and practical advantage to combining angiogenic gene therapy with myoblast transplantation using myoblasts as carriers of the exogenous genes encoding one or more angiogenic factors. In conclusion, the use of novel cell-based techniques to assist with cardiac regeneration holds much promise for the treatment of heart failure and could complement other available therapies.

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