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## MyoCell, a cell-based, autologous skeletal myoblast therapy for the treatment of cardiovascular diseases

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### Abstract

Cell therapy is fast emerging as a potential therapeutic option in cardiovascular therapeutics. Because of their inherent myogenic differentiation potential, skeletal myoblasts (SkMs) have been extensively assessed in preclinical and clinical studies for their feasibility, safety and effectiveness for myocardial repair. Bioheart Inc is developing MyoCell, autologous SkMs delivered by MyoCath and MyoStar catheter delivery systems for the treatment of cardiovascular diseases such as myocardial infarction and congestive heart failure. MyoCell is undergoing phase II/III clinical development and has so far demonstrated safety and efficacy, including improvements in cardiac function in phase I/II clinical trials.

### Introduction

Cardiovascular pathologies in general and heart failure in particular remain the leading cause of morbidity and mortality in developed countries. In 2005, an estimated 80,700,000 American adults (one in every three) suffered from one or more types of cardiovascular pathologies and one out of every 2.8 deaths in 2004 in the US was caused by this disease [954444]. Ischemic heart disease is characterized by extensive loss of functioning myocytes. In large infarcts, the intrinsic repair mechanism of the heart fails to compensate for the lost myocytes, which initiates a vicious cycle of remodeling. The conventional surgical intervention, coronary artery bypass grafting (CABG), and pharmacological treatments (eg,  $\beta$ -blockers and ACE inhibitors) provide only symptomatic relief and fail to address the underlying cause of the problem, the characteristic extensive myocyte loss. Heart transplantation, which is considered to be the gold standard in treatment for heart failure, is limited by the disparity between the number of donors and recipients, graft rejection and the undesired effects of immunosuppression. The intrinsic repair mechanism in the heart through scar formation subsequent to an infarction episode is similar to that observed in other tissues. The massive myocyte death by apoptosis and necrosis provokes a local inflammatory response that typically triggers the release of inflammatory cytokines and recruitment of inflammatory cells to the area of infarct [951489], [951493]. Because of the release of cytokines and inflammatory cells, matrix metalloproteins are elevated and lead to the formation of granulation tissue. Failure of the terminally differentiated

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**Associated patent.**

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cardiomyocytes to enter the cell cycle and the inadequacy of resident cardiac stem and progenitor cells to generate myocytes to compensate for the massive loss allows scar tissue formation, which limits left ventricle contractile function [951489], [951493].

**Therapeutic MyoCell**

**Originator** Bioheart Inc

**Status** Phase III Clinical

**Indications** Congestive heart failure, Ventricular tachycardia

**Actions** Angiogenesis stimulator, Cardioprotectant

**Technologies** Autologous stem cell, Injectable formulation, Muscle stem cell

**Synonyms** Autologous cell-based therapy (MyoCath/MyoStar, cardiovascular diseases), autologous cell-based therapy (SR-200/MyoStar, cardiovascular diseases), autologous skeletal myoblast therapy (MyoCath/MyoStar, cardiovascular diseases), autologous skeletal myoblast therapy (SR-200/MyoStar, cardiovascular diseases), MyoCell VT, tissue regeneration therapy (MyoCath/MyoStar, cardiovascular diseases), tissue regeneration therapy (SR-200/MyoStar, cardiovascular diseases)

Stem cell-based therapies for *de novo* myocardial regeneration by augmentation of cardiomyocyte numbers either via mobilization or engraftment of stem cells are under development [951496], [951498], [951502], [951506]. For example, ACY-001, which is undergoing phase I clinical development by Arteriocyte Inc, is a therapy that uses autologous hematopoietic stem cells as an adjunct to bypass therapy to stimulate angiogenesis [648482]. Despite promising results from experimental animal studies and clinical trials, there are parameters that require optimization to establish stem cell therapy as a clinical option. Donor cells from different sources, such as bone marrow, skeletal muscle, myocardium, cord blood and embryos, and with different therapeutic potential have been used for transplantation into the heart [651633], [951973], [951979], [952132], [952154], [952193], [952204]. Most of these studies have demonstrated engraftment of donor cells and regeneration of the heart structures, including cardiomyocytes and blood vessels, and most of these changes have been associated with improved heart function. The intrinsic similarity of structure, electrophysiology and contractile properties of fetal cardiomyocytes allow them to functionally integrate with the host myocardium after engraftment [951979], [952132]. Nevertheless, poor availability because of the difficulty in obtaining donor fetal hearts and ethical issues involved in their use has hindered their clinical application. The use of embryonic stem cell-derived cardiomyocytes is gaining popularity and has demonstrated that engraftment behavior of these cells is similar to the endogenous cardiomyocytes [651633], [952193]. In addition, the use of resident cardiac stem and progenitor cells has produced encouraging data [952193], [952204]. However, bone marrow-derived stem cells and skeletal myoblasts (SkMs; muscle progenitors that can undergo mitosis and differentiate into mature muscle) are the best studied in experimental animal models and clinical trials. Numerous studies have examined their potential therapeutic effects on myocardial function and regeneration [952205], [952210], [952212], [952213]. While many of these studies have added to the mounting evidence for extensive myocardial regeneration [951506], [951973], some have shown poor or even no differentiation of bone marrow stem cells into cardiomyocytes [952214], [952217]. SkMs have many advantages over other cell types, including availability without ethical issues, ease of *in vitro* expansion to large numbers without loss of differentiation potential, increased resistance to ischemia and inherent myogenic ability. In addition, transplantation of autologous SkMs eliminates the risk of cell graft rejection without the use of immunosuppressants.

Therapy with SkMs has gained recognition with the demonstration that SkMs survive and form contractile myofibers in the infarcted heart post engraftment. SkMs isolated from various animal species, such as mice, rat, rabbit, sheep and pig, have been characterized and extensively studied for their reparability in experimental settings using small and large animal models. To summarize the results of preclinical studies, SkM transplantation is safe and effective in generating myocytes in the infarcted heart. Nevertheless, unlike earlier reports that the engrafted SkMs had cellular features implying their possible differentiation into cardiomyocytes or cardiac-like cells, it has now been established that SkMs do not transdifferentiate to adopt cardiac phenotypes and fail to develop electromechanical links with the host myocytes [952218], [952228], [952232]. Despite these observations, the functional consequences of SkM transplantation are improvement in systolic and diastolic functions, and attenuated ventricular remodeling by the graft serving as a scaffold to prevent left ventricular dilatation. These beneficial effects have been largely observed on echocardiographic and sonomicrometric determinations, validated by MRI and pressure-volume loops and supported by relevant data depicting angiomyogenic responses in the heart [952359]. SkM-induced hypertrophy of remnant myocyte cells in the infarcted area of rats aided in the recovery of the contractile force of the hibernating myocardium or attenuated the upregulation of renin-angiotensin and endothelin systems [952252]. SkM transplantation was as efficient as neonatal cardiomyocytes in rabbits [952263] and bone marrow cells in cardiac repair in rats [952267], [952268].

The preclinical data paved the way for early clinical trials of the autologous myoblast cell therapy, which have confirmed the feasibility and safety of this strategy; however, development was suspended following a phase II clinical trial (called MAGIC: myoblast autologous raft in ischemic cardiomyopathy) conducted by MG Biotherapeutics LLC/Genzyme Corp because the likelihood that the trial would result in the hypothesized improvements in heart function was low [651895]. Menasche *et al* first reported SkM transplantation by epicardial injections as an adjunct procedure to CABG in a 72-year old patient with history of myocardial infarction (MI) [952299]. The procedure was uneventful and without any complication. A 5-month follow-up revealed left ventricular ejection fraction (LVEF) improved from 21 to 30%. At 17 months after cell engraftment, when the patient died, the heart tissue was processed for histological and immunohistological studies, which showed islands of myofibers in the infarcted heart [952302]. Encouraged by the results of their pioneering study, Menasche *et al* then conducted SkM transplantation on nine more patients [952304]. Unlike the first trial, the cell engraftment procedure was not uneventful as five patients developed ventricular tachycardia associated with the cell transplantation procedure. However, the combined procedure of CABG and cell therapy yielded some encouraging data; LVEF increased from 23.8 to 32.1%, wall thickness improved and there was an average improvement in New York Heart Association (NYHA) class from 2.7 to 1.6 [952304]. This was followed by multiple clinical trials to transplant SkMs as an adjunct of CABG or implantation of left ventricular assist device (LVAD). In a trial that involved the use of human heart tissue to assess the fate of SkMs post engraftment, an interesting approach was adopted, which involved using SkM transplantation in patients with ischemic heart disease undergoing implantation of LVAD as a bridge to heart transplantation [952309]. Later, the hearts of these patients were removed during transplantation and subjected to histochemical and immunohistochemical analysis. The cells survived in the infarcted heart and underwent myogenic differentiation. Since publication of this report [952309], more than 200 patients have received SkM engraftment in various medical centers around the world.

MyoCell, being developed by Bioheart Inc, uses autologous skeletal muscle-derived stem cells for engraftment into the infarcted heart [745596] and represents a leap forward in stem cell technology. After nearly two decades of intense research, MyoCell is being investigated for the treatment of MI and congestive heart disease and is currently in phase II/III clinical

development [872586], [954700]. Moreover, the use of MyoCath and MyoStar catheter systems, an autologous SkM delivery system from Biologics Delivery Systems Group, Cordis Corporation, allows target-specific delivery of autologous SkMs into the infarcted myocardium [745596].

## Synthesis and SAR

Bioheart has developed a standard operating procedure to process autologous skeletal muscle biopsies obtained from the patient for purification of SkMs [853498], [952338]. The procedure involves mincing of the muscle biopsies followed by enzymatic digestion. The digestate is then filtered to obtain a single-cell suspension which is plated in cell culture dishes in SkM basal growth medium supplemented with 15 to 20% fetal bovine serum, recombinant human EGF (10 µg/ml) and dexamethasone (2 µg/ml). The cells are passaged three to five times to achieve the required cell number. After 17 to 19 days of culture, the cells are then assessed for purity, viability and sterility before transfer into 30-ml bags in a specially formulated injectate/transport medium. The medium is formulated to support cell survival for 96 h under controlled conditions [853498], [952338].

The MyoCath and MyoStar delivery systems comprise a 115-cm long injection catheter with a deflectable tip that can be controlled with a hand piece. The hand piece enables control of needle depth and advancement and has ports for infusing SkMs and heparin/saline wash to prevent blocks [892763], [952338].

## Preclinical development

Delivery of human SkMs to the left ventricle of an anesthetized pig was demonstrated using the NOGA catheter injection system (Biosense Webster) and replicated using Bioheart catheters. Catheter administration of 20 injections of 0.1, 0.2, 0.3, 0.5 or 1.0 ml containing  $1 \times 10^8$ /ml human SkMs caused no significant changes in heart rate, ECG or temperature. Histological examination demonstrated that human SkMs were observed throughout the apex and lateral wall of the left ventricle [892764].

A model of chronic, stable heart failure was induced in dogs by coronary artery micro-embolization to investigate the effect of autologous SkM transplantation [920362]. The MyoCath catheter was used to percutaneously inject  $5.4 \pm 2.1 \times 10^8$  SkMs, saline or fibroblasts (as a non-contractile control) into the infarcted region of six, five and three dogs, respectively, while surgical injection of SkMs or saline was administered to five and three dogs, respectively. Left ventricular peak systolic pressure increased by 7, 10, 11, 9 and 8% at weeks 2, 4, 6, 8 and 10, respectively ( $p = 0.089, 0.031, 0.001, 0.027$  and  $0.013$ , respectively), after percutaneous SkM injection, but no other changes in left ventricular systolic pressure, arterial pressure or heart rate were significant. Administration of percutaneous autologous SkMs significantly increased LVEF from 41 to 47% at 10 weeks, compared with a decrease from 43 to 40% in saline control animals. Pressure-volume loop changes that indicated normalization of systolic function were observed 10 weeks after SkM administration, but not after saline administration. End-diastolic dimension was significantly decreased by 7.3 and 7.5% in surgical and percutaneous autologous SkM groups, respectively, while regional stroke work increased by 9.8 and 13%, respectively [920362].

An increase in diastolic blood pressure was induced in dogs ( $n = 13$ ) via daily coronary micro embolisms. SkMs ( $n = 8$ ) or saline ( $n = 5$ ) were injected into the infarcted region. There was a significant increase in maximum/minimum rate of left ventricular pressure difference ( $dP/dt_{max}$ ), mean aortic pressure and end-systolic elastance from chronic heart failure values following SkM injection compared with saline ( $10.3 \pm 8.3$  versus  $-0.2 \pm 6.3\%$ ,  $8.7 \pm 5.1$  versus  $1.9 \pm 3.0\%$ , and  $9.5 \pm 8.1$  versus  $-4.6 \pm 14.9\%$ , respectively) [952358].

A swine model of chronic MI was used to compare percutaneous and surgical transplantation of autologous SkMs [920350]. Following induced MI, six pigs were transplanted with a median of  $407 \pm 115 \times 10^6$  SkMs by direct surgical injection, six received percutaneous SkM transplant and four received culture without cells (two via surgery and two via percutaneous access). There was a significant increase in LVEF in animals administered SkMs compared with control animals, with no difference between the percutaneous and surgery groups. Morphology, bromodeoxy-uridine staining and MY-32 antibody labeling were utilized to detect myoblasts in 11 out of 12 transplanted animals. The identified cells did not contain markers for cardiomyocytes, but did co-localize with antibodies to smooth muscle actin, suggesting that some of the transplanted cells contributed to the formation of new blood vessels. The area of smooth muscle-actin-positive blood vessels was significantly higher in the transplanted animals than in control animals. Transplanted animals achieved significant reductions in infarct-area fibrosis (collagen volume fraction of 30.9 and 32.3% in surgical and percutaneous groups, respectively) compared with control animals (45.9%) [920350].

## Toxicity

No specific studies on the toxic nature of SkMs in general and MyoCell in particular have been published so far. In pigs and dogs administered SkMs of approximately  $1 \times 10^8$  and above, no toxic effects were observed [892764], [920362].

## Metabolism and pharmacokinetics

No metabolism and pharmacokinetics data of MyoCell were available at the time of publication.

## Clinical development

### Phase I

The feasibility and safety of MyoCell was investigated in patients ( $n = 5$ ) with NYHA class greater than 2 who had had an anterior wall MI [853498]. SkMs were administered via endoventricular injection using the NOGA-guided catheter system and MyoStar injection catheters in the infarcted myocardium. Subsequent to electromechanical mapping of the left ventricle,  $16 \pm 4$  injections each containing  $16.6 \times 10^6$  SkM cells were made endocardially in the areas exhibiting low voltage and linear local shortening. Patients were followed up for 6 months. At 3 months, angiography but not nuclear or MRI assessment demonstrated a statistically significant increase in LVEF from  $36 \pm 11$  to  $41 \pm 9\%$ . At 6 months LVEF was increased, but this was not significant. MRI analysis demonstrated significant increase in wall thickening at the injected segments [853498]. Despite it being challenging to derive a firm conclusion, the study results highlighted the feasibility of endovascular cell delivery in the clinic.

A second small study was designed to carry out a more in-depth investigation of hemodynamic changes using pressure-volume loop analysis following MyoCell administration [920357]. Patients ( $n = 5$ ) had an NYHA class greater than 2, reduced LVEF and a previous anterior wall MI. MyoStar injection catheter was used to deliver 15 endocardial injections (apart from in one patient who only received ten injections) each containing approximately  $10 \times 10^6$  cells. Patients were evaluated at 6 and 12 months using angiography, left ventriculography and right and left heart catheterization. The mean NYHA class was  $2.4 \pm 0.9$ ,  $1.8 \pm 0.4$  and  $2.2 \pm 0.4$  at baseline, 6 and 12 months; however, none of these differences were significant. There was a significant increase from baseline in cardiac output at 6 and 12 months (from  $4.6 \pm 0.9$  to  $5.6 \pm 1.6$  and  $5.4 \pm 1.5$  l/min, respectively), which was because of non-significant reduced end-systolic volume and increase in heart rate. Ejection fraction was significantly increased at 6

months (from  $33 \pm 7$  to  $41 \pm 11\%$ ) and  $dP/dt_{\max}$  was significantly increased at 12 months (from  $1025 \pm 236$  to  $1281 \pm 328$  mmHg/s). The end-systolic pressure-volume relationship was significantly reduced at 6 and 12 months (from  $190 \pm 95$  to  $150 \pm 107$  and  $157 \pm 75$  ml, respectively) [920357].

A stand-alone trans-catheter-based SkM engraftment procedure was conducted in patients ( $n = 6$ ) with coronary artery disease and myocardial dysfunction because of prior MI, and an NYHA class of 2 or above [952338]. The trial also included six patients, as a matched control, who were not administered SkMs. The muscle biopsies obtained from the patients were processed according to the standard operational procedures of Bioheart, which produced high-purity desmin-positive SkMs ( $70 \pm 21\%$ ). The viability of the cells was  $96.6 \pm 1.7\%$  at the time of injection. Endovascular engraftment of  $210 \pm 150 \times 10^6$  SkMs was conducted using the MyoCath injection catheter with an average of  $19 \pm 10$  injections. After the 12-month follow-up, there was no change in left ventricle end-diastolic diameter in the SkM-treated group, while the control group demonstrated significant myocardial remodeling with left ventricle end-diastolic diameter increasing from  $68.3 \pm 4.9$  to  $72.3 \pm 5.0$  mm. LVEF significantly improved 12 months after SkM injection from  $24.3 \pm 6.7$  to  $32.2 \pm 10.2\%$ , while in the control group, LVEF decreased. Wall motion score index significantly improved following inotropic challenge in the SkM-treated group compared with baseline and the control group. The patients receiving SkM engraftment had significantly improved NYHA class (from  $3.17 \pm 0.41$  to  $1.67 \pm 0.82$ ) during the 12-month follow-up, while NYHA class remained the same in the control group ( $3 \pm 0$ ). There was a significant increase in the walking distance for the SkM group in the 6-min walk test [952338].

The phase I, multicenter, non-randomized MYOHEART (myogenesis heart efficiency and regeneration trial, NCT00054678) US clinical trial was initiated with a primary objective to evaluate the safety of MyoCell engraftment using MyoCath in patients ( $n = 20$ ) who had previously experienced MI at least 12 weeks before the initiation of the trial [954669]. Patients were divided into four cohorts of five patients each to receive escalating doses of 25, 75, 225 and  $675 \times 10^6$  SkM cells with increasing number of injection sites (between 2 and 27). The efficacy endpoints were changes in left ventricular wall thickness and function. There was general but insignificant improvement in LVEF, quality-of-life score and the 6-min walk test. It was observed that the new contractile muscle formed from immature myoblast injections served as a scaffold within the scar tissue [954669].

Similarly, a phase I/II European clinical trial in patients ( $n = 20$ ) with cardiac pathologies with 1-year follow-up was completed (no treatment schedule was available) [744962]. There was a significant 75% reduction in emergency hospitalizations and premature ventricular contractions dropped from 1.89% at baseline to 0.04% at 1 year. Annual mortality rates were 2.8% compared with 20% for historical controls. An overall 80% of patients had improved one heart failure class and 33% of patients improved two heart failure classes. At least 50% of the patients included in the study had greater than 4% improvement in LVEF and 15.3% had an improvement in motion score index under stress [744962].

Furthermore, a phase I, multicenter, non-randomized, open-label clinical trial (NCT00050765) in patients ( $n = 15$ ) who have experienced anterior, lateral, posterior or inferior wall MI, required CABG surgery and who have an implantable cardioverter defibrillator in place had apparently been completed at the time of publication [744574], [890268]. Patients were divided into three cohorts and escalating MyoCell injections of 2, 6 and 18 were administered to the akinetic myocardial scar and safety and cardiovascular effects of MyoCell implantation were to be assessed [744574]. No further data were available at the time of publication.

## Phase II

A the SEISMIC trial (NCT00375817) was a phase II, multicenter, randomized, open-label clinical trial sponsored by Bioheart, which was conducted in the US and Europe [744573], [954661]. The trial involved patients (n = 46; 30 MyoCell- and 16 control-treated) with congestive heart failure post-MI and was intended to assess the safety and cardiovascular effects of MyoCell implantation by a catheter delivery system. The primary safety endpoint was defined as serious adverse events at 3 and 6 months, while the secondary safety endpoints were hospitalizations, Holter monitoring, 12-lead ECG data, frequency of ventricular arrhythmias and safety of the use of MyoCath. The primary efficacy endpoint was a change in LVEF and secondary efficacy endpoints were quality-of-life assessment, 6-min walk, NYHA class, and improvements in global contractility, wall thickness, coronary perfusion and infarct size [954661]. Treatment-arm patients were administered 150 to  $800 \times 10^6$  SkMs cells in 6 to 32 injections using the MyoCath catheter. Over the 6-month period of the trial, 50% of the treatment-arm patients obtained some improvement in LVEF, while 57% of patients in the control group had a worsening of LVEF. Similar to the MYOHEART trial, the newly formed contractile muscle served as a scaffold within the scar tissue. At 6 months, 50% of the treated patients had improvement in NYHA class score compared with 25% of the control group [954661].

Data from treated patients (n = 26) and control patients (n = 14) in the SEISMIC trial demonstrated that at 6 months 84 and 16% of treated and control patients, respectively, had 6-min walking test scores that were unchanged or improved, while these scores were worse in 16 and 69% of treated and control patients, respectively [891855], [954682], [954700]. In 94% of treated patients NYHA scores were improved or unchanged, compared with 58% in the control group, while 42 and 6% of the control and treatment groups, respectively, had worse NYHA scores [891855], [954682], [954700].

## Phase II/III

The encouraging results led to the design of a multicenter, randomized, double-blind, placebo-controlled phase II/III clinical trial (MARVEL, NCT00526253) in patients (expected n = 390) with class II or III heart failure who had previously experienced MI. Patients were recruited at the time of publication and were to receive MyoCell autologous SkMs ( $400$  or  $800 \times 10^6$  cells) via injection catheter together with the NOGA XP Cardiac Navigation System [872586], [954700]. The primary endpoints were patient wellbeing and distance they can walk [954700].

## Side effects and contraindications

In a pilot clinical trial into the feasibility and safety of MyoCell administration, one out of five patients was hospitalized 6 weeks after treatment because of progressive heart failure and asymptomatic ventricular tachycardia. However, no adverse events were observed in the other patients [853498]. In the other clinical trial of five patients, there were no serious adverse events, however, two patients experienced single episodes of non-sustained ventricular tachycardia [920357].

In one trial of six patients administered  $210 \pm 150 \times 10^6$  SkMs, there were three incidents of ventricular tachycardia in two patients. At 1-year follow-up none of these patients demonstrated repeat ventricular arrhythmia. In addition, three of the control patients experienced ventricular tachycardia during the follow-up period [952338].

During the MYOHEART trial, one out of five patients administered the highest dose died and six patients experienced arrhythmias of which four were thought to be related to the treatment.

There was one case of arthralgia, one patient experienced dizziness and one patient suffered a pleural effusion [954669].

During the SEISMIC trial, six patients in the treatment arm experienced a total of 12 significant adverse events, including one death by multiple organ failure, one worsening of heart failure, one pericarditis, ventricular tachycardia in six patients (five of which were thought to be related to the treatment and all were resolved –all patients had experienced ventricular tachycardia prior to the investigation), one hematoma, one herpes zoster and one non-sustained ventricular tachycardia (this also occurred in one patient in the control arm) [954661]. The incidents of arrhythmias were the same in treatment and control groups [954682].

## Patent summary

In its 2008 annual filing to the SEC, Bioheart stated that it relies primarily on one US patent for MyoCell, one US patent for its original catheter device MyoCath and a number of patents for its second-generation catheter MyoCath II. The company's "primary MyoCell patent", US-05130141, was in-licensed in February 2000 from Dr Philip K Law and his company Cell Transplants International. Bioheart says it has no patent protection for MyoCell outside of the US. The initial MyoCath patent, US-05972013, was in-licensed from Comedicus Inc in January 2000. Technology used in MyoCath II was in-licensed from TriCardia LLC in April 2006 (see US-06241710, US-06547769, US-06855132 and US-06949087) [745603].

Bioheart's license agreement with Dr Law in 2000 extended to "future developments related to heart muscle regeneration and angiogenesis", so may now include Dr Law's WO-00228470, WO-03085092, WO-2004014302 and/or WO-2005020916: A European equivalent to WO-00228470 was granted as EP-01324802 in 2006. Law's later filings were still pending grant by October 2008.

Bioheart has also filed its own claims on catheter-based delivery of cells into the heart: WO-00204063 (granted as US-06979321), WO-00247753 (still pending grant by October 2008) and WO-00205866 (granted as EP-01301228). In June 2003, Bioheart sold catheter-related intellectual property rights (including rights under the Comedicus license and filings that would later be granted as US-07033345) to Advanced Cardiovascular Systems (ACS) [954725]. As part of the sale, Bioheart was granted a license to continue using this intellectual property, co-exclusively with ACS (now Abbott Laboratories).

In August 2005, Bioheart announced the imminent grant of US-06988004, covering electrical stimulation technology that could be used to increase blood supply to transplanted MyoCell muscle cells [745599].

In February 2006, Bioheart announced a patent licensing agreement with Cleveland Clinic of Cleveland, Ohio, to additionally enhance its MyoCell technology [745601]. The agreement provides Bioheart with the worldwide exclusive rights to five pending US patent applications and corresponding foreign filings related to the repair of scarred heart tissue damaged from a heart attack. The pending patents cover methods of repairing damaged heart tissue by transplanting muscle stem cells that have been gene-modified to overexpress therapeutic proteins capable of recruiting other stem cells within a patient's own body to the cell transplanted area [745601].

## Current opinion

There is a general perception that temporal changes observed in the indices of cardiac performance correlate well with the extent of donor cell engraftment. Even a small degree of SkM engraftment may form a more compliant scar such that stiffness decreases and diastolic



properties are enhanced. A higher number of the engrafted SkMs is therefore required to fully replace the scar and reverse the reduction in contractile function [952236]. Moreover, the site of SkM delivery with respect to the infarct significantly influences the outcome of the procedure. While centrally delivered SkMs dose-dependently contributed to increased left ventricular end-systolic volume, end-diastolic volume and mass attenuated negative left ventricle remodeling was observed after engraftment of SkMs in the periphery of the infarct [952241]. These observations necessitate site-specific engraftment of cells rather than random injections into the free wall of the left ventricle. MyoCath and MyoStar in this regard can ensure that the cells are engrafted at a site where engraftment is the most beneficial.

Other SkM therapies in development include autologous SkM therapy (Advanced Cell Technology Inc) involving transplantation of autologous cells delivered via catheters to treat heart failure, which has reached phase I clinical development [832822], and skeletal muscle-derived pluripotent stem cell therapy (Cybios LLC) under preclinical development for MI [736075]. In the light of preclinical data and results from clinical trials, improvement in the indices of left ventricular remodeling and contractile function can be achieved by transplantation of SkMs. The magnitude of SkM engraftment and the functional outcome of the procedure show significant correlation. However, massive early cell death and poor donor cell survival in the early stage after implantation significantly lowers the effectiveness of the procedure. Hence there is a requirement to adopt remedial measures that can promote the survival of SkMs at the site of the cell graft. Despite the proliferative potential of SkMs, their rate of division is not enough to compensate for the apoptotic loss of cell graft during the acute phase after engraftment. Various strategies, including genetic modification of cells, pretreatment with growth factors and cytokines, and the use of immunosuppressants, have been developed to address this issue, albeit with limited success.

Pharmacological preconditioning and cell transduction with pro-survival genes may be the more logical options to achieve this goal. SkMs are excellent carriers of therapeutic genes and genetically modified SkMs, using viral and nonviral vectors, are therapeutically more effective in cardiac repair [952270], [952272]. This property of SkMs can be exploited for a combined gene and cell therapy strategy to deliver therapeutic genes to the cardiomyocytes, which are more resistant to gene transduction. Genetically modified SkMs thus serve as a reservoir of the transgene product of interest to achieve non-fluctuating therapeutic levels in the myocardium and enhance therapeutic effectiveness of the cells. Genetic modification of SkMs not only improves their angiogenic potential, it also improves the survival of transplanted SkMs in an ischemic heart [952273], [952279], [952280]. A study using human SkMs transduced with adenoviral-VEGF-165, which are under preclinical development by the National University of Singapore to treat MI, peripheral vascular disease and heart failure [744120], [744123], [744124], demonstrated that the cells were able to express VEGF-165 for 30 days [744120]. A significantly higher angiomyogenic response was observed in the animal hearts treated with genetically modified cells compared with the cell medium-injected or non-transduced SkM-injected controls [744120]. The proposal by Bioheart, under license from the Cleveland Clinic, to study the effectiveness of MyoCell SDF-1, a therapy utilizing autologous cells genetically modified to express additional growth factors [889936], is a step in the right direction and has demonstrated promising results in preclinical studies [927830]. None of the previous clinical trials have adopted this approach, which may yield better prognosis. Alternatively, a combined therapeutic strategy of using growth factor treatment with SkM engraftment is expected to improve donor cell survival in the ischemic heart.

Alternative cell sources for the treatment of cardiac pathologies that are currently in clinical development include the above-mentioned ACY-001; autologous endothelial progenitor (CD34+) cell therapy (Kobe Institute of Biomedical Research and Innovation), which was safe and effective in patients with chronic critical limb ischemia in a phase I/II clinical trial

[736534]; t2c-001 (t2cure GmbH/Johann Wolfgang Goethe-Universität Frankfurt am Main), an autologous bone-marrow-derived endothelial progenitor cell therapy, which improved outcomes in patients with MI [871766]; as well as CD-133-rich autologous bone marrow stem cell therapy (Institute for Regenerative Medicine and Stem Cell Therapy), which is undergoing phase II clinical trials [752716]. However, as mentioned above, studies have suggested, for instance, poor differentiation of bone marrow stem cells into cardiomyocytes and SkMs have advantages such as availability without ethical issues and ease of *in vitro* expansion to large number without loss of differentiation potential.

Pharmacological preconditioning combined with SkM engraftment resulted in higher survival of the preconditioned cells when subjected to oxidant stress and post engraftment in the experimental heart model of acute coronary artery ligation [952296]. These are important findings and may go a long way in preventing donor SkM apoptosis post engraftment. SkM engraftment has additive benefits when conducted together with ACE inhibitor administration [952248]. Because of the persistence of therapeutic benefits, SkM transplantation better preserves left ventricular function than ACE inhibitors alone [952252].

Based on available clinical results, there are no specific side effects related to SkM transplantation except its much purported pro-arrhythmogenicity. The data from preclinical and clinical studies suggest that MyoCell administration itself would be safe and may not initiate any undesired events except pharmacologically treatable arrhythmias in some cases. However, as MyoCell transplantation is intended to be a clinically relevant therapeutic option, it would necessitate 'on the shelf availability' of the cells for immediate use. Such an arrangement would not only be cost effective and logistically more pertinent, this would also help to avoid unnecessary delay in donor cell availability, which can be caused by the isolation, purification and propagation of SkMs *in vitro*. Purification of SkMs for every patient is a labor-intensive strategy. Moreover, on the shelf availability of SkMs will offer greater flexibility to the doctors to decide in terms of the time of cell administration after infarction episode. Encouraged by the results of a xenomyoblast engraftment in a porcine heart model using transient immunosuppression [744124], [952313], Law *et al* showed the first allomyoblast engraftment in three patients was safe and demonstrated the feasibility of non-autologous SkMs [954841]. Alternatively, MyoCell technology can be exploited to isolate the SkMs of an individual and deposit in cell banks for any probable future use. Similarly, an encouraging outcome of the ongoing clinical trials will demand larger quantities of viable, sterile, genetically well-defined and functionally demonstrated SkMs in a ready-to-use form. Such a development will warrant more economical and efficient production of SkMs.

Despite the beneficial mechanical effects of SkM engraftment on the infarcted heart, failure of the cell graft to express significant amounts of N-cadherin and connexin-43 prevents the development of an efficient and functional electromechanical integration with the host myocardium. This is thought to contribute to the occurrence of postoperative ventricular tachycardia in some of the early-phase clinical trials and brought up the safety concerns related to the use of SkMs as donor cells [952299], [952341]. Therefore, more attention is directed at the electrophysiological consequences of SkM transplantation. In an interesting *in vitro* study using a co-culture system, clustering of SkMs resulted in slowing down of conduction velocity [952344], while SkMs modified for connexin-43 expression had improved electrical impulse propagation and decreased arrhythmogenesis [952344]. Nevertheless, the *in vivo* experimental studies have produced data with discrepancies and inconsistencies [952348], [952352], [952354]. Studies suggested that induced arrhythmia was primarily caused by infarction and not by SkM transplantation [952354]. Fouts *et al* used optimal mapping in the canine left ventricular wedge preparation and reported that arrhythmia inducibility was not increased by SkM transplantation [952354]. Postoperative adverse events reported following SkM engraftment are those commonly encountered in heart failure patients undergoing CABG.

Moreover, the choice of injection site has greater bearing on arrhythmogenic outcome. SkMs transplanted in the infarct border zone cause more frequent arrhythmias than central scar injections. This would be consistent with the fact that conduction velocity, which is already decreased in the border zone, is additionally slowed by the clusters of engrafted cells, thereby enhancing electrical instability. However, despite these observations and studies suggesting that SkMs are responsible for cardiac arrhythmias, one should remain conscious about the arrhythmogenic nature of the scar tissue itself, in and around which the cells are transplanted.

In addition to all these issues, the strategy to deliver SkMs to the heart requires special consideration. SkM delivery to the endoventricle with the help of a catheter-based system may be clinically more relevant and more advantageous compared with intravenous or intracoronary injections. The technology of Bioheart involved in the preparation and development of MyoCell is based on the use of autologous SkMs delivered to the heart, via the MyoCath and MyoStar catheter systems, for the treatment of MI and congestive heart failure. Catheter-based delivery of the cells will be less invasive and permit heart cell therapy to be used as a sole therapy. As discussed earlier, clinical trials are already underway to assess the feasibility, safety and efficacy of MyoCell to repair the infarcted heart. However, based on current study results, it is premature and would be wrong to draw clinically meaningful conclusions pertaining to the efficacy of SkM transplantation before adequately powered clinical trials have been completed.

In summary, the data from all these studies clearly show the feasibility, safety and effectiveness of SkM engraftment in general and a potential for MyoCell to reach the market in particular. At the time of publication, Bioheart planned to file for European marketing approval for the use of MyoCell in class III heart failure patients that continued to deteriorate despite treatment with currently available therapies [947459]. The use of MyoCell is feasible because of the ease of accessibility and *in vitro* propagation to achieve the number of cells required for engraftment. Except for the issue of postoperative episodes of arrhythmias, which remained clinically manageable and treatable in most of the cases, there has never been a serious safety concern regarding the use of MyoCell for humans. Moreover, similar to other stem cell preparations, the issue of its effectiveness remains debatable, with limited improvement in the contractile function indices, but reduced left ventricular remodeling.

## Acknowledgements

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## Deals

**In December 2006, a memorandum of understanding between Bioheart and Korea Green Cross for the joint development and commercialization of MyoCell was signed [932231].**

## Development status

Developer	Country	Status	Indication	Date	Reference
Bioheart Inc	North America	Phase III	Congestive heart failure	27-MAR-08	889936
Bioheart Inc	Western Europe	Phase III	Congestive heart failure	25-MAR-05	744573
Bioheart Inc	South Korea	Phase II	Congestive heart failure	31-MAR-07	932231

Developer	Country	Status	Indication	Date	Reference
Bioheart Inc	US	Discovery	Ventricular tachycardia	20-NOV-06	745596

## Literature classifications

### Chemistry

Study type	Result	Reference
Synthesis	SkMs are obtained by mincing muscle biopsies, digesting in enzymes and filtering to obtain a single-cell suspension. This can be plated in culture dishes with growth medium to increase cell numbers before the cells are stored for use.	853498

### Biology

Study type	Effect studied	Model	Result	Reference
<i>In vivo</i>	Efficacy	Dogs with chronic, stable heart failure administered $5.4 \pm 2.1 \times 10^8$ SkMs percutaneously.	Left ventricular peak systolic pressure increased significantly by 10, 11, 9 and 8% at weeks 4, 6, 8 and 10, respectively. LVEF increased from 41 to 47% 10 weeks after skeletal myoblast administration and decreased from 43 to 40% in control animals.	920362
<i>In vivo</i>	Efficacy	Swine model of chronic MI administered a median of $407 \pm 115 \times 10^6$ SkMs by percutaneous or surgical transplantation.	LVEF significantly increased in animals administered SkMs percutaneously or surgically compared with control animals. Transplanted animals had significantly reduced fibrosis in the infarct area compared with control animals.	920350

### Clinical

Effect studied	Model	Result	Reference
Safety and efficacy	Phase I clinical trial in patients (n = 5) with NYHA class greater than 2 and previous anterior wall MI administered $16 \pm 4$ injections each containing $16.6 \times 10^6$ SkM cells by trans-catheter delivery.	LVEF increased from 36 to 41 (at 3 months) and 45% (at 6 months) with increased wall thickness. No serious adverse events were observed. One patient had non-sustained ventricular tachycardia.	853498
Efficacy	Phase I clinical trial in patients (n = 5) with NYHA class greater than 2, reduced LVEF and a previous anterior wall MI administered 15 endocardial MyoStar injections each containing approximately $10 \times 10^6$ cells.	The mean NYHA class was $2.4 \pm 0.9$ , $1.8 \pm 0.4$ and $2.2 \pm 0.4$ at baseline, 6 and 12 months, respectively. Cardiac output increased from $4.6 \pm 0.9$ l/min at baseline to $5.6 \pm 1.6 \pm 1.6$ and $5.4 \pm 1.5$ l/min at 6 and 12 months, respectively. Ejection fraction was significantly increased at 6 months (from $33 \pm 7$ to $41 \pm 11\%$ ) and $dP/dt_{max}$ was significantly increased at 12 months (from $1025 \pm 236$ to $1281 \pm 328$ mmHg/s). The end-systolic pressure-volume relationship was significantly reduced at 6 and 12 months (from $190 \pm 95$ to $150 \pm 107$ and $157 \pm 75$ ml, respectively). No serious adverse events were observed apart from two patients experiencing single episodes of non-sustained ventricular tachycardia.	920357
Efficacy and safety	Phase I clinical trial (MYOHEART) in patients (n = 20) with MI administered 2 to 27 injections of 25, 75, 225 and $675 \times 10^6$ SkM cells.	There was general but insignificant improvement in LVEF, quality-of-life score and the 6-min walk test. One out of five patients at the highest dose died and six patients experienced arrhythmias of which four were thought to be related to the treatment. Arthralgia, dizziness and pleural effusion were observed in one patient each.	954669
Efficacy and safety	Phase II clinical trial in patients (n = 46) with congestive heart failure post MI administered 6 to 32	Over a 6-month period of the trial, 50% of the treatment-arm patients obtained some improvement in LVEF, while 57% of patients in the control group had a worsening of LVEF. At 6 months, 50% of the treated	954661

Effect studied	Model	Result	Reference
	injections of $150$ to $800 \times 10^6$ SkM cells using the MyoCath catheter.	patients had improvement in NYHA class score compared with 25% of the control group. Observed adverse events following treatment were ventricular tachycardia in six patients, one death by multiple organ failure, one worsening of heart failure, one hematoma and one herpes zoster.	

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## References

648482. Arteriocyte Inc. Arteriocyte initiates phase one clinical trial with \$1.4 million NIH STTR grant. Press Release. 2006 January 19;
651633. Passier R, Mummery C. Cardiomyocyte differentiation from embryonic and adult stem cells. *Curr Opin Biotechnol* 2005;16(5):498–502. [PubMed: 16099156]
651895. Genzyme Corp. Genzyme ends enrollment in phase 2 trial of cardiac cell therapy. Press Release. 2006 February 23;
736075. Hashemi S, Kayadjanian N, Sharp LL, Osman HE, Griesbeck J, Guan X, Lu M, El-Kalay MA, Zhou R, Zhang H, Litt H, et al. Adult pluripotent stem cells: *In vitro* studies and cell engraftment in a porcine model of myocardial infarction. *Circulation* 2006;114(18 Suppl)Abs 2035
736534. Kawamoto A, Katayama M, Handa N, Kinoshita M, Baba R, Takano H, Kihara Y, Morioka S, Fukushima M, Asahara T. Safety and efficacy is sustained up to one year after transplantation of autologous CD34+ cells in no-option patients with chronic critical limb ischemia. *Circulation* 2006;114(18 Suppl)Abs 2192
744120. Ye L, Haider HK, Jiang S, Ling LH, Ge R, Law PK, Sim EK. Reversal of myocardial injury using genetically modulated human skeletal myoblasts in a rodent cryoinjured heart model. *Eur J Heart Fail* 2005;7(6):945–952. [PubMed: 16202652]
744123. Niagara MI, Haider HK, Ye L, Koh VSW, Lim YT, Poh KK, Ge R, Sim EK. Autologous skeletal myoblasts transduced with a new adenoviral bicistronic vector for treatment of hind limb ischemia. *J Vasc Surg* 2004;40(4):774–785. [PubMed: 15472608]
744124. Haider HK, Ye L, Jiang S, Ge R, Law PK, Chua T, Wong P, Sim EK. Angiomyogenesis for cardiac repair using human myoblasts as carriers of human vascular endothelial growth factor. *J Mol Med* 2004;82(8):539–549. [PubMed: 15175859]
744573. Bioheart Inc. NCT00375817: Safety and effects of implanted (autologous) skeletal myoblasts (MyoCell) using an injection catheter = SEISMIC trial. 2006. Clinicaltrials.gov
744574. Bioheart Inc. NCT00050765: Autologous cultured myoblasts (biowhittaker) transplanted via myocardial injection. 2006. Clinicaltrials.gov

744962. Bioheart Inc. Bioheart phase I/II data presented on human trial utilizing MyoCell (adult stem cells – myoblasts). Press Release. 2004 December 10;
745596. Bioheart Inc. Company website: Bioheart Inc. Company World Wide Web Site. 2006 November 20;
745599. Bioheart Inc. Bioheart granted US patent for inducing angiogenesis by electrical stimulation of muscle. Press Release. 2005 August 02;
745601. Bioheart Inc. Bioheart Inc and Cleveland Clinic sign major patent licensing agreement. Press Release. 2006 February 20;
745603. Bioheart Inc. Bioheart Inc and Tricardia LLC sign patent licensing agreement. Press Release. 2006 April 27;
752716. Establishing the first multi-center trial for a intramyocardial stem cell application. Company Presentation. 2006 December 14;
832822. Advanced Cell Technology Inc. Advanced Cell Technology completes acquisition of Mytogen, Inc. Transaction significantly accelerates stem cell clinical development activities; phase II clinical trials to begin shortly. Press Release. 2007 September 24;
853498. Smits C, van Geuns J, Poldermans D, Bountiokos M, Onderwater EE, Lee CH, Maat AP, Serruys PW. Catheter-based intramyocardial injection of autologous skeletal myoblasts as a primary treatment of ischemic heart failure: Clinical experience with six-month follow-up. *J Am Coll Cardiol* 2003;42(12):2063–2069. [PubMed: 14680727]
871766. Maidhof, R. IDDB Meeting Report; Cell therapy for cardiovascular diseases – fourth international conference; New York, NY, USA. 16-18 January 2008; 2008 Jan 16-18.
872586. Bioheart Inc. NCT00526257: To assess safety and efficacy of myoblast implantation into myocardium post myocardial infarction (MARVEL). 2007. Clinicaltrials.Gov
889936. Bioheart Inc. Bioheart Inc announces financial results for full-year 2007. Press Release. 2008 March 27;
890268. Bioheart Inc. Bioheart product pipeline. Company World Wide Web Site. 2008 March 28;
891855. Bioheart Inc. Final data from the SEISMIC trial suggest safety, efficacy of autologous stem-cell therapy for treating congestive heart failure. Press Release. 2008 April 01;
892763. Sherman W, Martens TP, Viles-Gonzalez JF, Siminiak T. Catheter-based delivery of cells to the heart. *Nat Clin Pract Cardiovasc Med* 2006;3:S57–S64. [PubMed: 16501633]
892764. Law PK, Weinstein J, Leonhardt H, Hain S, Williams S, Fang Q, Jordan T, Goodwin T. Human myoblast transfer into the heart. *Abstr Soc Neurosci* 2001;27(1):633.
920350. Gavira JJ, Perez-Ilzarbe M, Abizanda G, Garcia-Rodriguez A, Orbe J, Paramo JA, Belzunce M, Rabago G, Barba J, Herreros J, Panizo A, et al. A comparison between percutaneous and surgical transplantation of autologous skeletal myoblasts in a swine model of chronic myocardial infarction. *Cardiovasc Res* 2006;71(4):744–753. [PubMed: 16843451]
920357. Steendijk P, Smits PC, Valgimigli M, van der Giessen WJ, Onderwater EE, Serruys PW. Intramyocardial injection of skeletal myoblasts: Long-term follow-up with pressure-volume loops. *Nat Clin Pract Cardiovasc Med* 2006;3:94–100. [PubMed: 16446778]
920362. He KL, Yi GH, Sherman W, Zhou H, Zhang GP, Gu A, Kao R, Haines HB, Harvey J, Roos E, White D, et al. Autologous skeletal myoblast transplantation improved hemodynamics and left ventricular function in chronic heart failure dogs. *J Heart Lung Transplant* 2005;24(11):1940–1949. [PubMed: 16297802]
927830. Bioheart Inc. Bioheart reports promising results from SDF-1 modified MyoCell therapy in heart failure study. Press Release. 2008 July 24;
932231. Bio-industry. *Pharma Koreana* 2007;17(3):22–25.
947459. Bioheart Inc. Bioheart announces 35 leading US heart failure centers engaged in phase II/III Marvel trial of myogenic cells for treating advanced heart failure. Press Release. 2008 September 30;
951489. Ren G, Dewald O, Frangogiannis NG. Inflammatory mechanisms in myocardial infarction. *Curr Drug Targets Inflamm Allergy* 2003;2(3):242–256. [PubMed: 14561159]
951493. Frangogiannis NG. The mechanistic basis of infarct healing. *Antioxidant Redox Signal* 2006;8(1112):1907–1939.

951496. Pouzet B, Vilquin JT, Hagege AA, Scorsin M, Messas E, Fiszman M, Schwartz K, Menasche P. Intramyocardial transplantation of autologous myoblasts: Can tissue processing be optimized? *Circulation* 2000;102(19 Suppl 3):III210–III215. [PubMed: 11082389]
951498. Suzuki K, Murtuza B, Suzuki N, Smolenski RT, Yacoub MH. Intracoronary infusion of skeletal myoblasts improves cardiac function in doxorubicin-induced heart failure. *Circulation* 2001;104(12 Suppl 1):I213–I217. [PubMed: 11568058]
951502. Orlic D, Kajstura J, Chimenti S, Limana F, Jakoniuk I, Quaini F, Nadal-Ginard B, Bodine DM, Leri A, Anversa P. Mobilized bone marrow cells repair the infarcted heart, improving function and survival. *Proc Natl Acad Sci USA* 2001;98(18):v10344–10349.
951506. Rota M, Kajstura J, Hosoda T, Bearzi C, Vitale S, Esposito G, Iaffaldano G, Padin-Iruegas ME, Gonzalez A, Rizzi R, Small N, et al. Bone marrow cells adopt the cardiomyogenic fate *in vivo*. *Proc Natl Acad Sci USA* 2007;104(45):17783–17788. [PubMed: 17965233]
951973. Kajstura J, Rota M, Whang B, Cascapera S, Hosoda T, Bearzi C, Nurzynska D, Kasahara H, Zias E, Bonafe M, Nadal-Ginard B, et al. Bone marrow cells differentiate in cardiac cell lineages after infarction independently of cell fusion. *Circ Res* 2005;96(1):127–137. [PubMed: 15569828]
951979. Leor J, Patterson M, Quinones MJ, Kedes LH, Kloner RA. Transplantation of fetal myocardial tissue into the infarcted myocardium of rat. A potential method for repair of infarcted myocardium? *Circulation* 1996;94(9 Suppl):II332–II336. [PubMed: 8901770]
952132. Matsushita T, Oyamada M, Kurata H, Masuda S, Takahashi A, Emmoto T, Shiraishi I, Wada Y, Oka T, Takamatsu T. Formation of cell junctions between grafted and host cardiomyocytes at the border zone of rat myocardial infarction. *Circulation* 1999;100(19 Suppl):II262–II268. [PubMed: 10567314]
952154. Caspi O, Huber I, Kehat I, Habib M, Arbel G, Gepstein A, Yankelson L, Aronson D, Beyar R, Gepstein L. Transplantation of human embryonic stem cell-derived cardiomyocytes improves myocardial performance in infarcted rat hearts. *J Am Coll Cardiol* 2007;50(19):1884–1893. [PubMed: 17980256]
952193. Dawn B, Stein AB, Urbanek K, Rota M, Whang B, Rastaldo R, Torella D, Tang XL, Rezazadeh A, Kajstura J, Leri A, et al. Cardiac stem cells delivered intravascularly traverse the vessel barrier, regenerate infarcted myocardium, and improve cardiac function. *Proc Natl Acad Sci USA* 2005;102(10):3766–3771. [PubMed: 15734798]
952204. Torella D, Indolfi C, Goldspink DF, Ellison GM. Cardiac stem cell-based myocardial regeneration: Towards a translational approach. *Cardiovasc Hematol Agents Med Chem* 2008;6(1):53–59. [PubMed: 18220721]
952205. Murry CE, Wiseman RW, Schwartz SM, Hauschka SD. Skeletal myoblast transplantation for repair of myocardial necrosis. *J Clin Invest* 1996;98(11):2512–2523. [PubMed: 8958214]
952210. Tambara K, Sakakibara Y, Sakaguchi G, Lu F, Premaratne GU, Lin X, Nishimura K, Komeda M. Transplanted skeletal myoblasts can fully replace the infarcted myocardium when they survive in the host in large numbers. *Circulation* 2003;108:II259–II263. [PubMed: 12970243]
952212. Amado LC, Saliaris AP, Schuleri KH, St John M, Xie JS, Cattaneo S, Durand DJ, Fitton T, Kuang JQ, Stewart G, Lehrke S, et al. Cardiac repair with intramyocardial injection of allogeneic mesenchymal stem cells after myocardial infarction. *Proc Natl Acad Sci USA* 2005;102(32):11474–11479. [PubMed: 16061805]
952213. Pochampally RR, Neville BT, Schwarz EJ, Li MM, Prockop DJ. Rat adult stem cells (marrow stromal cells) engraft and differentiate in chick embryos without evidence of cell fusion. *Proc Natl Acad Sci USA* 2004;101(25):9282–9285. [PubMed: 15197249]
952214. Murry CE, Soonpaa MH, Reinecke H, Nakajima H, Nakajima HO, Rubart M, Pasumarthi KB, Virag JI, Bartelmez SH, Poppa V, Bradford G, et al. Haematopoietic stem cells do not transdifferentiate into cardiac myocytes in myocardial infarcts. *Nature* 2004;428(6983):664–668. [PubMed: 15034593]
952217. Balsam LB, Wagers AJ, Christensen JL, Kofidis T, Weissman IL, Robbins RC. Haematopoietic stem cells adopt mature haematopoietic fates in ischaemic myocardium. *Nature* 2004;428(6983):668–673. [PubMed: 15034594]



952218. Reinecke H, Poppa V, Murry CE. Skeletal muscle stem cells do not transdifferentiate into cardiomyocytes after cardiac grafting. *J Mol Cell Cardiol* 2002;34(2):241–249. [PubMed: 11851363]
952228. Leobon B, Garcin I, Menasche P, Vilquin JT, Audinat E, Charpak S. Myoblasts transplanted into rat infarcted myocardium are functionally isolated from their host. *Proc Natl Acad Sci USA* 2003;100(13):7808–7811. [PubMed: 12805561]
952232. Reinecke H, MacDonald GH, Hauschka SD, Murry CE. Electromechanical coupling between skeletal and cardiac muscle. Implications for infarct repair. *J Cell Biol* 2000;149(3):731–740. [PubMed: 10791985]
952236. Atkins BZ, Hueman MT, Meuchel JM, Cottman MJ, Hutcheson KA, Taylor DA. Myogenic cell transplantation improves *in vivo* regional performance in infarcted rabbit myocardium. *J Heart Lung Transplant* 1999;18(12):1173–1180. [PubMed: 10612375]
952241. McCue JD, Swingen C, Feldberg T, Caron G, Kolb A, Denucci C, Prabhu S, Motilall R, Breviu B, Taylor DA. The real estate of myoblast cardiac transplantation: Negative remodeling is associated with location. *J Heart Lung Transplant* 2008;27(1):116–123. [PubMed: 18187097]
952248. Pouzet B, Ghostine S, Vilquin JT, Garcin I, Scorsin M, Hagege AA, Duboc D, Schwartz K, Menasche P. Is skeletal myoblast transplantation clinically relevant in the era of angiotensin-converting enzyme inhibitors? *Circulation* 2001;104(12 Suppl 1):I223–I228. [PubMed: 11568060]
952252. Toh R, Kawashima S, Kawai M, Sakoda T, Ueyama T, Satomi-Kobayashi S, Hirayama S, Yokoyama M. Transplantation of cardiotrophin-1-expressing myoblasts to the left ventricular wall alleviates the transition from compensatory hypertrophy to congestive heart failure in Dahl salt-sensitive hypertensive rats. *J Am Coll Cardiol* 2004;43(12):2337–2347. [PubMed: 15193703]
952263. Thompson RB, Emani SM, Davis BH, van den Bos EJ, Morimoto Y, Craig D, Glower D, Taylor DA. Comparison of intracardiac cell transplantation: Autologous skeletal myoblasts versus bone marrow cells. *Circulation* 2003;108:II264–II271. [PubMed: 12970244]
952267. Huwer H, Winning J, Vollmar B, Welter C, Lohbach C, Menger MD, Schafers HJ. Long-term cell survival and hemodynamic improvements after neonatal cardiomyocyte and satellite cell transplantation into healed myocardial cryoinfarcted lesions in rats. *Cell Transplant* 2003;12(7):757–767. [PubMed: 14653622]
952268. Agbulut O, Vandervelde S, Al Attar N, Larghero J, Ghostine S, Leobon B, Robidel E, Borsani P, Le Lor'h M, Bissery A, Chomienne C, et al. Comparison of human skeletal myoblasts and bone marrow-derived CD133+ progenitors for the repair of infarcted myocardium. *J Am Coll Cardiol* 2004;44(2):458–463. [PubMed: 15261948]
952270. Ye L, Haider HKh, Jiang S, Tan RS, Toh WC, Ge R, Sim EK. Angiopoietin-1 for myocardial angiogenesis: A comparison between delivery strategies. *Eur J Heart Fail* 2007;9(5):458–465. [PubMed: 17188570]
952272. Elmadbouh I, Haider HKh, Jiang S, Idris NM, Lu G, Ashraf M. *Ex vivo* delivered stromal cell-derived factor-1 $\alpha$  promotes stem cell homing and induces angiomyogenesis in the infarcted myocardium. *J Mol Cell Cardiol* 2007;42(4):792–803. [PubMed: 17350033]
952273. Suzuki K, Murtuza B, Smolenski RT, Sammut IA, Suzuki N, Kaneda Y, Yacoub MH. Cell transplantation for the treatment of acute myocardial infarction using vascular endothelial growth factor-expressing skeletal myoblasts. *Circulation* 2001;104(12 Suppl 1):I207–I212. [PubMed: 11568057]
952279. Sugimoto T, Inui K, Shimazaki Y. Gene therapy for myocardial angiogenesis: With direct intramuscular gene transfer of naked deoxyribonucleic acid encoding vascular endothelial growth factor and cell transplantation of vascular endothelial growth factor transfected H9c2 myoblast. *J Thorac Cardiovasc Surg* 2003;51(5):192–197.
952280. Lu Y, Shansky J, Del Tatto M, Ferland P, McGuire S, Marszalkowski J, Maish M, Hopkins R, Wang X, Kosnik P, Nackman M, et al. Therapeutic potential of implanted tissue-engineered bioartificial muscles delivering recombinant proteins to the sheep heart. *Ann Ny Acad Sci* 2002;961:78–82. [PubMed: 12081871]
952296. Niagara MI, Haider HKh, Jiang S, Ashraf M. Pharmacologically preconditioned skeletal myoblasts are resistant to oxidative stress and promote angiomyogenesis via release of paracrine factors in the infarcted heart. *Circ Res* 2007;100(4):545–555. [PubMed: 17234963]

952299. Menasche P, Hagege AA, Scorsin M, Pouzet B, Desnos M, Duboc D, Schwartz K, Vilquin JT, Marolleau JP. Myoblast transplantation for heart failure. *Lancet* 2001;357(9252):279–280. [PubMed: 11214133]
952302. Hagege AA, Carrion C, Menasche P, Vilquin JT, Duboc D, Marolleau JP, Desnos M, Bruneval P. Viability and differentiation of autologous skeletal myoblast grafts in ischaemic cardiomyopathy. *Lancet* 2003;361(9356):491–492. [PubMed: 12583951]
952304. Menasche P, Hagege AA, Vilquin JT, Desnos M, Abergel E, Pouzet B, Bel A, Sarateanu S, Scorsin M, Schwartz K, Bruneval P, et al. Autologous skeletal myoblast transplantation for severe postinfarction left ventricular dysfunction. *J Am Coll Cardiol* 2003;41(7):1078–1083. [PubMed: 12679204]
952309. Pagani FD, DerSimonian H, Zawadzka A, Wetzel K, Edge AS, Jacoby DB, Dinsmore JH, Wright S, Aretz TH, Eisen HJ, Aaronson KD. Autologous skeletal myoblasts transplanted to ischemia-damaged myocardium in humans. Histological analysis of cell survival and differentiation. *J Am Coll Cardiol* 2003;41(5):879–888. [PubMed: 12628737]
952313. Haider, HKh; Jiang, SJ.; Ye, L.; Aziz, S.; Law, PK.; Sim, EK. Effectiveness of transient immunosuppression using cyclosporine for xenomyoblast transplantation for cardiac repair. *Transplant Proc* 2004;36(1):232–235. [PubMed: 15013354]
952338. Ince H, Petzsch M, Rehders TC, Chatterjee T, Nienaber CA. Transcatheter transplantation of autologous skeletal myoblasts in postinfarction patients with severe left ventricular dysfunction. *J Endovasc Ther* 2004;11(6):695–704. [PubMed: 15615560]
952341. Siminiak T, Kalawski R, Fiszler D, Jerzykowska O, Rzeźniczak J, Rozwadowska N, Kurpisz M. Autologous skeletal myoblast transplantation for the treatment of postinfarction myocardial injury: Phase I clinical study with 12 months of follow-up. *Am Heart J* 2004;148(3):531–537. [PubMed: 15389244]
952344. Abraham MR, Henrikson CA, Tung L, Chang MG, Aon M, Xue T, Li RA, O'Rourke B, Marban E. Antiarrhythmic engineering of skeletal myoblasts for cardiac transplantation. *Circ Res* 2005;97(2):159–167. [PubMed: 15976318]
952348. Fernandes S, Amirault JC, Lande G, Nguyen JM, Forest V, Bignolais O, Lamirault G, Heudes D, Orsonneau JL, Heymann MF, Charpentier F, et al. Autologous myoblast transplantation after myocardial infarction increases the inducibility of ventricular arrhythmias. *Cardiovasc Res* 2006;69(2):348–358. [PubMed: 16376327]
952352. Mills WR, Mal N, Kiedrowski MJ, Unger R, Forudi F, Popovic ZB, Penn MS, Laurita KR. Stem cell therapy enhances electrical viability in myocardial infarction. *J Mol Cell Cardiol* 2007;42(2):304–314. [PubMed: 17070540]
952354. Fouts K, Fernandes B, Mal N, Liu J, Laurita KR. Electrophysiological consequence of skeletal myoblast transplantation in normal and infarcted canine myocardium. *Heart Rhythm* 2006;3(4):452–461. [PubMed: 16567294]
952358. He KL, Zhou H, Yi GH, Becker EM, Kao R, Harvey J, Gu A, Zhang GP, Mohri S, Wang J, Burkhoff D. Impact of autologous skeletal myoblast transplantation on hemodynamics and left ventricular function in repeated coronary microembolization-induced heart failure in awake dogs. *J Am Coll Cardiol* 2003;41(6 Suppl 1)Abs 1135-84
952359. Atkins BZ, Hueman MT, Meuchel J, Hutcheson KA, Glower DD, Taylor DA. Cellular cardiomyoplasty improves diastolic properties of injured heart. *J Surg Res* 1999;85(2):234–242. [PubMed: 10423324]
954444. American Heart Association. Web Site. Dallas, Texas, USA: 2008 Sep 30. American Heart Association. Heart disease and stroke statistics – 2008 update.
954661. Serruys PW. Myocardial repair by percutaneous cell transplantation of autologous skeletal myoblasts as a stand alone procedure in post myocardial infarction chronic heart failure patients. *Conf Cell Ther Cardiovasc Dis* 2007 January;3
954669. Sherman W. MYOHEART US phase I study interim analysis. *Conf Cell Ther Cardiovasc Dis* 2007 January;3
954682. Serruys PW. SEISMIC: Safety and effects of implanted (autologous) skeletal myoblasts (MyoCell) using an injection catheter. *ACC* 2008 April 1;57

954700. Sherman W. Cell therapy in the cath lab for heart failure: A look at MyoCell therapy and the SEISMIC trial. *Cath Lab Digest* 2008;16(5):1–11.
954725. Advanced Cardiovascular Systems Inc/Bioheart Inc. Ex-10.8: Asset purchase agreement between Advanced Cardiovascular Systems Inc and Bioheart Inc. Form S-1. 2003 June 24;
954841. Law PK, Fang G, Chua F, Kakuchaya T, Bockeria LA. First-in-man myoblast allografts for heart degeneration (abstract). *Int J Med Implants Devices* 2003;1:100–155.