Cell transplantation for cardiac regeneration: where do we stand?

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During the last decade transplantation of cells into the heart has emerged as a novel therapy for the prevention and treatment of heart failure. Although various cell types have been used, most experience has been obtained with the progenitor cells of skeletal muscle, also called myoblasts, and a wide array of bone marrow-derived cell types. The first preclinical studies demonstrated an improvement in global and regional heart function that was attributed mainly to a direct contractile effect of the transplanted cells. Furthermore, it was suggested that multiple cell types are able to form true cardiomyocytes and truly 'regenerate' the myocardium. More recent studies have questioned these early findings. Other mechanisms such as paracrine effects on the infarct and remote myocardium, a reduction in adverse remodelling and improvement of mechanical properties of the infarct tissue likely play a more important role. On the basis of encouraging preclinical studies, multiple early-phase clinical trials and several randomised controlled trials have been conducted that have demonstrated the feasibility, safety and potential efficacy of this novel therapy in humans. This review summarises the available evidence on cardiac cell transplantation and provides an outlook on future preclinical and clinical research that has to fill in the remaining gaps. (*Neth Heart J* 2008; 16:88-95.)

Keywords: cell transplantation, heart failure, myoblasts, bone marrow cells, review

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In recent years, cell transplantation has emerged as a possible treatment for many types of cardiac disease.¹ Transplanting cells that are able to differentiate and replace dead or dysfunctional cardiomyocytes and vasculature, or can induce regeneration via paracrine mechanisms could be a definitive therapy for cardiac ischaemic injury and heart failure. The goal of this paper is to outline the present status of cardiac cell transplantation, thereby focussing on basic as well as clinical studies and give an outline of future research in this field.

Cell transplantation in basic research

The initial cell types used for intracardiac cell transplantation were cell types that could easily be harvested and cultured in an autologous way, thereby surpassing the need for immunosuppression. The two cell populations used most widely in the first basic research studies and the first randomised clinical studies were 1) skeletal muscle progenitor cells or myoblasts² and 2) bone marrow-derived cells (BMCs) including bone marrow-derived circulating cells.³ Other cell types include cardiomyocytes, cardiac stem cells,⁴ embryonic stem cells,⁵ other types of circulating progenitor cells,⁶ as well as less frequently used cells such as umbilical cord blood cells,⁷ tissue-derived fibroblasts,⁸ smooth muscle cells⁹ and adipose tissue-derived cells.¹⁰

Myoblasts

Myoblasts were initially chosen for cardiac repair in basic and clinical studies because they are able to convert into myocytes, contract and self-renew. Furthermore, a large number of autologous myoblasts can be obtained from a small muscle biopsy, no ethical dilemmas accompany the use of myoblasts, they do not possess significant risk of tumour formation and, finally, myoblasts are highly resistant to ischaemia.

Animal studies showed that after injection, they are able to survive and proliferate and replace up to ~75% of the infarct area with myocardial tissue within three weeks.² Initially, there were several reasons for believing that the newly formed muscle resembled true myocardium. Most importantly, some of the newly formed muscle fibrils converted to a 'slow twitch'

type,^{2,11,12} which is more adapted to the continuous cardiac contraction, suggesting that the cardiac environment alters the development of the myoblasts. This was also observed in human studies.¹³ Furthermore, some groups reported that the transplanted myoblasts showed a cardiac-like phenotype^{14,15} including the presence of typical cardiac proteins such as troponin I and cardiac-myosin heavy chain.¹⁶

The efficacy of myoblast transplantation after myocardial infarction (MI) has been shown in different animal models, including mouse,¹⁷ rat,^{18,19} rabbit,²⁰ sheep²¹ and dogs.²² Myoblast transplantation improved global parameters of function such as ejection fraction and developing left ventricular pressure and regional parameters such as regional stroke work at the infarct site,^{2,20} wall thickening²³ and myocardial tissue velocities.²¹ Myoblast grafting also improved diastolic properties of the infarct region, with increased compliance of the infarct tissue.²⁴ Furthermore, myoblast transplantation prevented deleterious morphological changes that are characteristic for left ventricular remodelling.¹⁸

In recent years, the conversion of myoblasts into true cardiomyocytes has been strongly debated.²⁵ One of the main reasons is that fusion of transplanted myoblasts with native cardiomyocytes has been demonstrated to occur at the transplant-recipient interface,²⁶ which probably explains the earlier reports of myoblast conversion. Other critics claimed that it is possible that part of the beneficial effect on regional and global function can be attributed to other mechanisms than synchronous contraction of transplanted myoblasts with the myocardium. For example, changes in the viscoelastic properties of the wall, a reduction in wall thinning, or secretion of cytokines that modulate remodelling of remote myocardium could all have contributed.

Several factors argue against a direct contractile contribution of myoblasts. First and foremost, there is a marked difference in the electromechanical innervation of skeletal and cardiac muscle. In skeletal muscle, each myofibril is separately innervated by its own neuronal motor unit. In cardiac muscle, the action potential is propagated via cell-cell contact through channels called gap junctions. In vitro, myoblasts were shown to be able to form gap junctions with cardiomyocytes.²⁷ However in vivo, results were controversial: the presence of gap junctions in transplanted myoblasts has been observed in some studies at the site of donor and host cell contact¹⁶ and in another study, a small population of transplanted myoblasts was observed that showed calcium transients in synchrony with host cardiomyocytes, suggesting electromechanical coupling.²⁸ However, at the same sites myoblasts and cardiomyocytes were observed that had fused into one single, hybrid cell, with twice the DNA content that probably accounted for this observation. These results argue against effective functional coupling between myoblasts and host myocardium. However, not all questions have

been answered. Other 'atypical' donor-derived junctions can hypothetically play a role and future studies should shed more clarity on this subject.

Bone marrow-derived cells

Large-scale myocardial regeneration using BMCs was first reported in 2001.²⁹ In this study, infarcted mouse hearts were injected with BMCs expressing the stem cell antigen c-kit (the haematopoietic stem cell fraction) and the authors reported that up to ~68% of the infarct was regenerated by donor-derived cardiomyocytes and vascular structures. The new muscle restored contractility in the infarct scar, thereby improving global heart function. Although the regeneration of new myocardium with improvement in function by this specific type of BMCs was repeatedly reported afterwards, the ability of these cells to directly regenerate new cardiomyocytes was questioned more recently, for three reasons. First, the discovery of fusion events between native cardiomyocytes and the transplanted cells,³⁰ which can explain some of the earlier reports of transdifferentiation; second, the possibility of false-positive results when using only histological methods such as immunohistochemistry for detection of newly formed myocardium;³¹ and third, the recognition that the plasticity of these cells is less than previously assumed.³²

Another bone marrow-derived cell population, the endothelial progenitor cells, also called angioblasts, were shown to incorporate into the infarct vasculature,³³ thereby reducing cardiomyocyte apoptosis and improving cardiac function. This effect was dose dependent and caused regeneration of new myocardium by inducing cardiomyocyte cycling.³⁴ However, several authors have questioned this process of direct incorporation of BMCs into the vascular wall and instead proposed a different mechanism.³⁵ The development of new vasculature or collateral vessels could be induced by pro-arteriogenic factors secreted by the transplanted BMCs, possibly by the presence of large numbers of monocytes in these fractions. Furthermore, recent studies using more sophisticated analytical methods questioned the reported in vitro transdifferentiation of these cells into cardiomyocytes.³⁶

Other types of BMCs caused less controversy such as the mesenchymal stem cell fraction or the bone marrow fraction used in clinical trials: the mononuclear cells. Mesenchymal stem cells, cells which normally support haematopoiesis, were shown to be able to differentiate into cardiomyocytes in vitro³⁷ and in vivo which was accompanied by an improvement in global heart function and induction of angiogenesis in several small³⁸ and large³⁹ animal studies.

Cell transplantation in clinical research

Myoblasts

Since the first clinical myoblast transplantation in 2000,⁴⁰ seven phase I safety and feasibility trials have

Table 1. Skeletal myoblast trials in chronic ischaemic disease.

Study	Cells	Controls	Follow-up	Results
Hagege et al., ⁴¹ n=9	8.7x10 ⁸ myoblasts, transepical. during CABG, 3 to 228 months after MI	No controls	18 to 58 months	- EF increased (4.4%; echo) - Enhanced regional contractility - Improvement in NYHA class - VTs in 5 patients requiring ICD implantation
Pagani et al., ⁴³ n=5	3.0x10 ⁸ myoblasts, transepical during LVAD implantation, 9 to 26 years after infarction	No controls	68 to 191 days	- Histology of explanted tissue demonstrates skeletal myofibres parallel to host tissue - VTs in 3 patients responding to β -blockers and amiodarone
Herreros et al., ⁴² n=12	2.0x10 ⁸ myoblasts, transepical during CABG, 3 to 168 months after MI	No controls	3 months	- EF increased (18%; echo) - Enhanced regional contractility - Increased regional viability
Siminiak et al., ⁴⁴ n=10	0.04-5.0x10 ⁷ myoblasts, transepical during CABG, 4 to 10 ⁸ months after MI	No controls	12 months	- EF increased (6.8%; echo) - Enhanced regional contractility
Smits et al., ⁴⁶ n=5	2.0x10 ⁸ myoblasts, transendocardial via EMM-guided catheter, 2 to 11 years after infarction	No controls	6 months	- EF increased (9.0%; LV angio) - Enhanced regional contractility (MRI) - VTs in 1 patient
Dib et al., ⁴⁵ n=30	0.1-3.0x10 ⁸ myoblasts, transepical during CABG (n=24) or LVAD implantation (n=6)	No controls	11-45 months	- EF increased (8.0%; echo) at 2 years - Reduced EDV and ESV - VTs in 3/24 CABG patients - Histology of explanted tissue demonstrates skeletal myofibres
Siminiak et al., ⁴⁷ n=10	17-106x10 ⁶ myoblasts, catheter based-transvenous approach, 5 to 96 months after infarction	No controls	6 months	- EF increased in 6/9 successfully transplanted patients

CABG=coronary artery bypass grafting, MI=myocardial infarction, EF=ejection fraction, NYHA=New York Heart Association, VT=ventricular tachycardia, ICD=implantable cardioverter defibrillator, LVAD=left-ventricular assist device, LV angio=left ventricular angiogram, EMM=electromechanical mapping, EDV and ESV=end-diastolic and -systolic volumes, respectively.

been performed (table 1).⁴¹⁻⁴⁷ The first phase II, multi-centre, double-blind, placebo-controlled, dose-ranging, randomised study using myoblast transplantation as an adjunct to CABG, the MAGIC trial, was recently stopped because of lack of efficacy. Preliminary results from this trial suggest a modest increase in ejection fraction, as assessed by nuclear imaging and a reduction in left ventricular volumes.⁴⁸ The final results of this trial have not yet been reported. Four of the phase I studies involved myoblast transplantation as an adjunct to CABG,^{41,42,44,45} one involved endoventricular injections of myoblasts⁴⁶ and one study used a coronary sinus approach.⁴⁷ Generally, patients had signs of congestive heart failure class 2-3 (NYHA), with two- to three-vessel coronary disease and an

ejection fraction ranging between 20 and 40%. Myoblasts were injected into akinetic, scar regions. From these studies, some conclusions can be drawn.

First, all studies involving CABG with adjunctive myoblast implantation documented increased ejection fraction and three studies showed increased regional function by echocardiography in myoblast-injected segments. However, since myoblast-injected hearts were also revascularised in those studies, this functional improvement cannot be exclusively attributed to the injected myoblasts.

Second, the injection procedure itself appeared to be safe. Although, in the first study by Menasche, five of nine patients⁴¹ developed ventricular tachycardia (VT) after the procedure, the first results of the MAGIC

Table 2. Bone marrow trials in acute myocardial infarction

Study	Cells	Controls	Follow-up (months)	Results
Strauer et al., ⁴⁹ n=10 vs. 10 controls	0.9-2.8x10 ⁷ MNCs, intracoronary, 7 days after MI	Nonrandomised, no sham harvest or infusion	3	- EF unchanged (LV angio) - Reduced ESV - Enhanced regional contractility - Improved perfusion (SPECT) - Decrease infarct size (LV angio)
TOPCARE-AMI, ⁵⁰ n=29 and n=30	2.1x10 ⁸ MNCs or 1.6x10 ⁷ CPCs intracoronary, 5 days after MI	No controls	4	- EF increased (8%; LV angio) - Reduced ESV - Enhanced regional contractility - Improved perfusion (SPECT) - Enhanced CFR (Doppler) - Decrease infarct size (MRI)
Fernandez-Aviles et al., ⁵¹ n=20 vs. 13 controls	7.8x10 ⁷ MNCs, intracoronary, 14 days after MI	Nonrandomised, no sham harvest or infusion	6	- EF increased (5.8%; MRI) - Reduced ESV - Enhanced regional contractility
Bartunek et al., ⁵² n=19 vs. 16 controls	1.3x10 ⁷ CD133+ cells, intracoronary, 12 days after MI	Nonrandomised, no sham harvest or infusion	4	- EF increased (7.1%; LV angio) - Reduced ESV - Enhanced regional contractility - Improved perfusion (SPECT)
Chen et al., ⁵³ n=34 vs. 35 controls	4.8-6x10 ¹⁰ MSCs, intracoronary, 18 days after MI	Randomised, sham harvest and saline infusion	6	- EF increased (18%; LV angio) - Reduced ESV and EDV - Enhanced regional contractility - Increased viability (FDG) - Decrease infarct size (LV angio)
BOOST, ⁵⁴ n=30 vs. 30 controls	2.4x10 ⁹ MNCs, intracoronary, 6 days after MI	Randomised, no sham harvest or infusion	18	- No change in EF, volumes, regional contractility or infarct size (MRI)
Janssens et al., ⁵⁵ n=33 vs. 34 controls	4.8x10 ⁸ MNCs, intracoronary, 24 hours after MI	Randomised, double blinded, sham harvest and infusion	4	- No change in EF, volumes or regional contractility (MRI) - Decrease infarct size (MRI)
REPAIR-AMI, ⁵⁶ n=101 vs. 103 controls	2.4x10 ⁸ MNCs, intracoronary, 4 days after MI	Randomised, no sham harvest, medium infusion	4	- EF increased (5.5%; LV angio) - ESV and EDV unchanged - Enhanced regional contractility - Increased viability (FDG)
ASTAMI, ⁵⁷ n=50 vs. 50 controls	6.8x10 ⁷ MNCs, intracoronary, 6 days after MI	Randomised, no sham harvest or infusion	6	- No change in EF, volumes or infarct size (MRI)

MNCs=bone marrow-derived mononuclear cells, MI=myocardial infarction, EF=ejection fraction, LV angio=left ventricular angiogram, EDV and ESV=end-diastolic and -systolic volumes, respectively, CPCs=circulating progenitor cells, CFR=coronary flow reserve, MRI=magnetic resonance imaging, MSC=bone marrow-derived stromal cells, SPECT=single photon emission computed tomography, FDG=fluorodeoxyglucose.

trial did not show a higher incidence of adverse events in the treated group.⁴⁸ In most study protocols, implantable cardioverter defibrillators (ICDs) are now prophylactically implanted.

Third, histological analysis of explanted human hearts after transplantation or at autopsy showed that the injected myoblasts differentiated to myotubes, even at 17.5 months after delivery.^{43,45} These myotubes

showed alignment parallel to the host tissue and were embedded in scar tissue.

Bone marrow-derived cells

In contrast with myoblasts, research into BMCs has moved much faster from preclinical studies to clinical trials (table 2). These studies mainly focused on cell therapy for acute MI, in contrast with myoblast studies that mainly involved chronic ischaemic heart disease. Most of the earlier acute MI clinical trials that have been completed were observational and used non-randomised control groups, all applying intracoronary injections.⁴⁹⁻⁵² These earlier studies showed the feasibility and safety of the procedure and suggested an improvement in both global and regional function as well as an improvement in regional perfusion of the infarct area. Thus far, five randomised, controlled studies have been reported.⁵³⁻⁵⁷ The first large study using bone marrow MNCs was the BOOST trial, which included a total of 60 patients.⁵⁴ MNCs were injected into the infarct coronary artery six days after infarction in 30 patients and functional improvement at six months, as measured by MRI, was significantly greater after cell transplantation (ejection fraction increased from 50.0 to 56.7% vs. 51.3 to 52% in controls). However, the control group did not receive a bone marrow aspiration and sham injection, so that a possible role for cytokine release during aspiration or a preconditioning effect of the repeated balloon inflation during injection of cells cannot be excluded. Recently, the 18-month outcomes of this study were reported. Rather disappointingly, no long-term benefits of BMC transplantation could be demonstrated in comparison with controls, suggesting that BMCs may accelerate recovery of function but do not provide a sustained, long-term effect. The first double-blind trial was reported in March 2006.⁵⁵ This trial included 33 patients with BMC transplantation and 34 patients with placebo injection 24 hours after acute MI. This trial showed no improvement in global LV function and no reduction in LV volumes or regional perfusion or metabolism, which is corroborated by recent observations in a swine model of acute MI.⁵⁸ However, contrast-enhanced MRI showed a significantly larger reduction in infarct volumes after BMC transplantation compared with controls suggesting a beneficial effect on infarct remodelling. However, in this trial cells were injected rather early, 24 hours after infarction, in contrast with other studies (four days to three weeks after infarction), which might explain these differences. More recently the largest, randomised, placebo-controlled, multicentre trial of intracoronary infusion of BMC after percutaneous coronary intervention for acute myocardial infarction was published involving 204 patients.⁵⁶ At four months, the absolute improvement in left ventricular ejection fraction (LVEF), measured by angiography, although modest, was greater in the treated group than in controls (5.5 vs. 3.0%). Furthermore, the rate of adverse clinical

events was significantly lower at one year. In contrast, in the smaller ASTAMI trial using three non-invasive imaging methods, including MRI, no significant improvement in LVEF at six months in the treated group was found.⁵⁷ However, the study had a modest power of 80% to detect an absolute change of 5% in LVEF. Furthermore technical differences in the characteristics of the infused BMCs might also explain these different outcomes.

Several trials have investigated BMC transplantation for chronic MI. All studies reported beneficial effects of BMC transplantation on global function, a reduction in LV volumes as well as improvement in regional function, perfusion and metabolism. Larger multicentre trials have to confirm the benefits of BMCs in patients with a chronic MI.

The future

Promising new cell types: cardiac stem cells and embryonic stem cells

It appears that the survival of adult, neonatal or foetal cardiomyocytes is extremely short and very little engraftment occurs.⁵⁹ Furthermore, these cells are difficult to increase to sufficient numbers in vitro. However, other cells can be isolated from the myocardium with more regenerative capacity. For example, cardiac stem cells (CSCs) can be isolated from mouse, rat and dog as well as human myocardium and are able to form all three major cardiovascular cell lineages: cardiomyocytes, endothelium and smooth muscle.⁶⁰ These cells can be identified by different stem cell markers such as Sca-1 and c-kit. However, it is estimated that their number is very limited: about 1 CSC per ~8000 to ~40,000 myocardial cells.⁶¹ Their in vivo regenerative potential has been described in mouse, rat and dog studies: when putative CSCs were injected into hearts directly or via intracoronary administration after MI, cells were reported to transdifferentiate in multiple cardiac lineages, thereby regenerating myocardium, preventing remodelling and ameliorating left ventricular function.⁵⁹ Despite their promising nature, especially the fact that they can be isolated from adult human hearts and thus form a source of autologous cells, many questions remain. The most important question is whether CSCs differentiate into true cardiomyocytes in vivo or just share some phenotypic characteristics.

Embryonic stem cells (ESCs) have several features that make them an excellent candidate for cardiac cell therapy: (1) Embryonic stem cells can form true cardiomyocytes⁶² with a distinct electrophysiological⁶³ and contractile phenotype⁶⁴ and (2) they have an unlimited proliferation potential.⁶⁵ Several studies have demonstrated the feasibility of transplanting ESCs in vivo, using mouse as well as human⁶⁶ ESCs. A major limitation is that these cells require a rigorous purification step to obtain a homogenous population of cardiovascular progenitor cells, thereby eliminating

cells that cause teratoma formation. However, very recently there was a breakthrough with the discovery of the transcription factor *isl1* as a marker of cardiovascular stem cells that give rise to the three major cardiac cell lineages: cardiomyocytes, endothelium and smooth muscle.⁶⁷ By selecting cells from a mixed embryonic stem cell culture, cells can be grown with a differentiation capacity limited to cardiac structures. Ongoing ESC research has to deal with the immune response against allogeneic cells and ethical issues that limit the use of human embryonic cells.

The ability to monitor cells and determine cell fate will be of critical importance in future studies. The use of intrinsic genetic differences in donor cells will be the most important method to detect cells unambiguously. Therefore the use of transgenic mice will play a more important role in the future. Transgenesis may also be an important tool to guide proliferation and differentiation of the injected cells.

In order to monitor cells non-invasively after transplantation multiple imaging modalities will be evaluated to determine cell fate during the course of the study, thereby identifying early determinants of the efficacy of cell transplantation. The two imaging modalities preferentially used nowadays are magnetic resonance imaging (MRI) and nuclear imaging using intracellular labels, such as iron-based labels for MRI.⁶⁸ These imaging modalities will further enable more precise regional functional assessment using, for example, methods such as strain rate imaging.

Finally, future studies will have to shed more light on the issue of what the best cell type for cardiac repair is and the ideal injection route for this type of cell. Therefore, comparative studies will have to be performed using multiple cell types and injection routes. Cells can be injected either as monotherapy or as a cocktail of various types.

Future focus in clinical research

With respect to myoblasts, the final results from the MAGIC trial are currently still pending. Although interpretation of the trial results is hampered by the early termination, the preliminary results are moderately positive. In the treatment arm a significant reversal of left ventricular remodelling was observed,⁴⁸ warranting further phase II studies, not only as an adjunct therapy to CABG but also as monotherapy.

With respect to bone marrow cells, there are currently several multicentre, randomised, controlled studies recruiting larger patient groups underway, mainly involving patients presenting with an acute MI treated with primary angioplasty. An example of such a study is the HEBE trial in the Netherlands.⁶⁹ In these studies, surrogate endpoints are chosen such as ejection fraction and gain in regional function. Despite the ongoing debate about the ideal imaging modality for assessing these parameters, MRI is probably the method of choice, especially since it accurately measures other useful parameters for secondary analysis such as

infarct size and remote myocardial mass. These trials will hopefully confirm the beneficial results found thus far.

Questions that still need to be answered by these studies are: what is the ideal time window for injection, how many cells are needed and is the intracoronary route superior to intramyocardial injection. Patients that present too late for angioplasty (e.g. 12 hours after the onset of symptoms) as well as patients with an ischaemic or dilated cardiomyopathy represent interesting future study populations since it is currently undetermined which patient groups are likely to benefit most from this therapy. Ultimately, robust clinical endpoints such as cardiac death as well as major adverse cardiac events should be the endpoints in larger studies. However, it is of importance to acknowledge that outcome studies in this field need more than 1000 treated patients to be appropriately powered, underscoring the need for collaboration to initiate multi-centre studies. ■

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