

Stem Cell Cardiomyoplasty: State-of-the-Art

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Abstract

Congestive heart failure (CHF) remains the most common diagnosis made in cardiology wards today. No long-term therapeutic option for end-stage CHF is available except for orthotopic heart transplant. Cellular-based therapy has emerged as a potential new therapy for patients with advanced heart failure. Different cell types are being explored in preclinical and clinical studies with encouraging results. Critical issues, such as types of cells, ideal number of cells, route of delivery, timing and targets of delivery, remain to be optimised to maximise the benefits of cell therapy. In this review, we seek to summarise the latest data and postulate future directions in this potentially exciting field.

Ann Acad Med Singapore 2004;33:451-60

Key words: Cardiomyoplasty, Heart failure, Myocardial repair, Stem cells

Introduction

Congestive heart failure (CHF) remains the most common diagnosis made in cardiology wards today. At present, no long-term therapeutic option for end-stage CHF is available except for orthotopic heart transplant. There remains a severe shortage of donor hearts for transplant. Since 1990 when the heart transplant programme in Singapore began, only 24 heart transplants have been performed.

Coronary artery disease and its sequelae of myocardial infarction (MI), acute coronary syndrome and stable angina remain a leading cause of cardiac mortality and morbidity. Despite rapid advances in revascularisation procedures, a proportion of patients develop irreversible loss of functioning myocytes. This is followed by fibrous scar formation and left ventricular remodelling. Eventually, there is a progressive reduction in left ventricle (LV) function and overt CHF develops.

Although orthotopic heart transplantation is the preferred treatment for end-stage heart failure, chronic organ shortage and complications with immunosuppression have led to a search for alternate options. Recently, cellular-based therapy has emerged as a potential new therapy for patients with advanced heart failure. Cell therapy using foetal and neonatal cardiomyocytes,¹⁻⁸ embryonic stem (ES) cell-derived cardiomyocytes,⁹⁻¹³ smooth muscle cells,¹⁴⁻¹⁶ fibroblasts,¹⁷⁻¹⁹ skeletal myoblasts²⁰⁻²⁶ and more recently bone marrow stem cells (BMSCs)²⁷⁻³⁶ have been explored in preclinical and clinical studies.

Early cell transplantation results are encouraging, with reports of significant improvement of cardiac performance and claims of myocardial regeneration. To date, more than 100 patients with MI have been reported to show improved cardiac performance after receiving cell therapy.^{24-26,32-35,37-41} However, some basic questions remain to be answered before cellular transplant can be adopted on a wider scale. Who should be considered for stem cell therapy? What are the optimal cell types and cell numbers for injection? What is the most appropriate cell delivery method? Can injections be repeated? What are the underlying mechanisms in the observed improvements? What are the long-term outcome and possible complications? In this review, we seek to summarise the latest data and perhaps postulate future directions in this potentially exciting field.

Cell Types for Transplant: Current Status

The mammalian heart has previously been considered a terminally differentiated organ that is incapable of self-renewal. Recent evidence suggests that myocardium may have some capacity to undergo limited self-repair following acute injury such as MI or chronic failure resulting from pressure/volume overload. Some cardiomyocytes in the distressed heart have been found to re-enter the cell cycle and proliferate to compensate for apoptosed or necrosed myocytes.⁴²⁻⁴⁵ This natural regenerative process is believed to be inadequate in repairing major injuries with massive cell death. The idea of augmenting this self-repair

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process by transplanting exogenous cells that are structurally and functionally congruent with the myocardium is hence logical.

The regenerative cells may be derived from undifferentiated stem cells or more differentiated somatic cells. Totipotent stem cells derived from embryos are the most primitive cells and they are able to develop into all the 200 unique cell types that make up the complete human organism.⁴⁶ Stem cells derived from adult tissues are more limited in their differentiation capacity but can develop into multiple cell types, including cell lineages of different organs. For example, stem cells in bone marrow that replenish blood cells or liver stem cells that regenerate hepatocytes have potential to transdifferentiate into myocytes and endothelial cells in the myocardium.^{29,47} On the other hand, lineage committed cells, such as cardiomyocytes and skeletal myoblasts, can be used primarily to replace the injured myofibres.^{1,20}

Cardiomyocytes

The rationale of using cardiomyocytes for grafting into myocardium has been reported in various animal models with promising results.^{1-6,8} These cells can be derived from foetal, neonatal or adult hearts. Adult cells are limited in practicality since they are post-mitotic and incapable of cell division *ex vivo*. Because of limited numbers, they are unlikely to be useful for cell therapy even though they may be derived autologously. Foetal and neonatal cells retain limited ability to enter the cell cycle; hence, these cells can be expanded in culture to yield a satisfactory number of cells for transplantation. Foetal and neonatal cells, but not adult cells, have also been demonstrated to survive in the myocardium and form intercalated discs and gap junctions with the host cardiomyocytes following cell transplant.⁶ They could potentially improve myocardial function not only by passively preventing detrimental remodelling but also actively contributing to systolic force development. Presently, the ethical issues involved in procuring human foetal or neonatal cells effectively preclude their use in routine therapy.

ES cells are derived from the inner cell mass of blastocyst-stage embryos.⁴⁸ They are totipotent cells that are able to give rise to every somatic cell type of the adult organism. Indeed, ES cell-derived embryoid bodies are known to form early-stage cardiomyocytes that recapitulate the ontogeny of cardiogenesis by sequential expression of cardiac-specific transcription factors and sarcomeric proteins. This is followed by myofibrillogenesis and their organisation into sarcomeric structures which lead to heart excitability and contractility.⁴⁹⁻⁵² When transplanted into the ventricular wall, these ES cells-derived cardiomyocytes have been reported to form stable intracardiac grafts for 7 to 32 weeks, significantly contributing to the improved

haemodynamics and enhanced neovascularisation in the infarcted myocardium.^{11-13,53}

Although potentially useful for cellular cardiomyoplasty, allogeneic cardiomyocytes, whether derived from foetal/neonatal tissues or ES cells, are hampered by ethical issues that may preclude their clinical application.^{54,55} Furthermore, the extent of myocardial differentiation of these implanted cells has been questioned since they failed to migrate from the site of injection and retained foetal-like phenotypes in isolated grafts even after extended periods.^{1,4,11,56} These allografts may have long-term issues with host immunological rejection and chronic immunosuppression management. There is also concern that the undifferentiated pluripotent ES cells in the cardiomyocyte preparations may form teratomas in the hearts of susceptible hosts,⁵⁷ implying that purification strategies may be necessary.⁵³ ES-derived cardiomyocytes may consist of mixed muscle subtypes that have distinct sinus nodal, pacemaker, atrial- and ventricular-like characteristics⁹ that could present unanticipated arrhythmogenic potential when transplanted into the left ventricle of the myocardium.^{58,59}

Skeletal Myoblasts

In contrast to postnatal cardiomyocytes, adult skeletal myoblasts retain the ability to re-enter the cell cycle and proliferate readily in culture. Myoblasts differentiate readily into mature striated cells that are well-suited for contractile work and their resistance to ischaemia renders them ideal for cardiac transplant.⁶⁰ Improved ventricular function was demonstrated in various animal models^{20-22,61,62} and in clinical patients^{24,25,63} following myoblast transplant, but lack of electromechanical coupling with the host cardiomyocytes suggest the observed cardiac improvements were unlikely to be mediated through synchronised contractility between the transplanted and host cells.⁶³

A phase I trial of autologous intramyocardial transplantation of skeletal myoblasts with patients having left ventricular ejection fraction (LVEF) <35% after MI was recently concluded.^{23,64} There were encouraging improvements at 2 months follow-up although concomitant coronary artery bypass graft (CABG) surgery could have biased the patient's clinical outcome. A setback from the trial was that 4 patients required an implantable cardio-defibrillator (ICD) after experiencing sustained ventricular tachycardia following cell transplant. Similar arrhythmogenic events needing ICD were also reported after percutaneous endocardial injection of skeletal myoblasts.²⁶ It is still unclear whether development of symptomatic cardiac arrhythmias is inherent to transplantation of skeletal myoblasts or a natural course of response following MI. Nevertheless, a major drawback of skeletal myoblasts for cardiomyoplasty seems to be their

Table 1. Cell Surface Characteristics of Bone Marrow Derived Stem Cells

Cell surface markers	Cell types developed	Differentiation conditions	Reference no.
CD31 ⁺	endothelial cells	pig myocardial ischaemia model, t.e. injection	36
CD34 ⁺	myocytes, endothelial cells, smooth muscle cells	Mouse MI model, i.v. injection	71
CD45 ⁻	myocytes	rabbit MI model, t.m. injection	72
CD117 ⁺	endothelial cells	mouse ischaemic hind limb model i.m. injection	70
Lin ⁻ /CD117 ⁺	myocytes, endothelial cells, smooth muscle cells	mouse MI model, t.m. injection	29
CD34 ⁺ /CD117 ⁺	endothelial cells	rat MI model, i.v. injection	73
CD34 ⁺ /CD117 ⁺ /SCA-1 ⁺	myocytes, endothelial cells	mouse MI model, i.v. injection	68
CD133 ⁺	endothelial cells	in vitro, defined media of FBS, VEGF, bFGF, IGF-1	74
Stro-1 ⁺ /CD106 ⁺	adiopocytes, chondrocytes, osteocytes	in vitro, defined media of FBS, dexamethasone, ascorbate, glycerophosphate	69
CD34 ⁺ /SH2 ⁺ /SH3 ⁺	adiopocytes, chondrocytes, osteocytes	in vitro, defined media of FBS, dexamethasone, ascorbate, glycerophosphate	75
CD14 ⁺ /CD34 ⁻ /CD117 ⁻	adiopocytes, chondrocytes, osteocytes, endothelial cells	in vitro, defined media of FBS, PDGF-BB, EGF, VEGF, dexamethasone, ascorbate, glycerophosphate	76

bFGF: basic fibroblastic growth factor; EGF: epidermal growth factor; FBS: foetal bovine serum; IGF: insulin growth factor; i.m.: intramuscular; i.v.: intravenous; MI: myocardial infarction; PDGF: platelet-derived growth factor; t.e.: transendocardial; t.m.: transmyocardial; VEGF: vascular endothelial growth factor

inability to transdifferentiate into cardiomyocytes⁶⁵ and their electromechanical isolation from the host cardiomyocytes.⁶⁶ This has emerged as a potential cause of pathological arrhythmia in initial clinical trials.

Bone Marrow Derived Stem Cells

BMSCs are a mixed population of cells derived from the bone marrow. These marrow cells have been broadly categorised into haematopoietic and mesenchymal lineages based on defined cell surface makers such as CD34 and CD45.⁶⁷ Additional surface makers, such as CD117, CD133, Sca-1 and Stro-1,^{29,37,68-70} are being investigated to further divide these cells into subpopulations (Table 1). BMSCs are ideal for myocardial transplantation since they are capable of developing into various mesodermal lineages that include smooth muscle, angioblasts and cardiac muscle,

which are the 3 major cell types in the heart. BMSC-derived haematopoietic stem cells (HSCs) or endothelial progenitor cells (EPCs), mononuclear cells (MNCs) and mesenchymal stem cells (MSCs) have been demonstrated to transdifferentiate into cardiomyocytes and endothelial cells following engraftment into the myocardium.^{28,29,31,68,77-79} Such differentiation is important in the regeneration of hypoperfused and necrosed tissues of infarcts. Regenerated myocardial tissue was found to constitute as much as 68% of the infarcted ventricle 9 days following transplantation of Lin⁻ CD117⁺ HSC in an acute mouse MI model.²⁹ However, it may be premature to attribute the functional improvement in myocardial performance after injury to these BMSC-derived cardiomyocytes since there is a wide variation in the reported cardiac transdifferentiation (0.02% to 50%) following engraftment.^{29,68}

Table 2. Human Clinical Stem Cell Transplant Trials Using Bone Marrow Derived Stem Cells

Trial author	Patients (n)	Route of transplantation	BMSC characteristics	Imaging performed for outcome	Follow-up (mo)	Outcomes
Assmus et al ³²	20	Intracoronary (Adjunct PTCA)	MNC or CPC 7.3x10 ⁶ -14.6x10 ⁶ (2.8% CD34, 1% CD133)	Echocardiography LV angiography PET	4	Improved LVEF Improved wall motion Enhanced perfusion Increased viability
Strauer et al ³³	10	Intracoronary (Adjunct PTCA)	MNC 2.8x10 ⁷ (2.1% CD34, 0.65% CD133)	Echocardiography LV angiography SPECT	3	Unchanged LVEF Improved wall motion Enhanced perfusion
Figulla et al ³⁸	11	Intracoronary (Adjunct PTCA)	MNC 5x10 ⁷	Echocardiography	3	Unchanged LVEF Unchanged perfusion
Stamm et al ³⁷	6	Intramyocardial (Adjunct CABG)	CD133 ⁺ MNC 1.5x10 ⁶	Echocardiography SPECT	3-9	Enhanced LVEF Enhanced perfusion
Hamano et al ⁴¹	5	Intramyocardial (Adjunct CABG)	MNC 3x10 ⁸ -2.2x10 ⁹	SPECT	1 & 12	Enhanced perfusion
Fuchs et al ³⁵	10	Transendocardial NOGA (Sole therapy)	Unfractionated BM 1.2x10 ⁷ -1.4x10 ⁸ (2.6% CD34)	Echocardiography SPECT	3	Unchanged LVEF Improved angina score Improved perfusion
Perin et al ³⁹	14	Transendocardial NOGA (Sole therapy)	MNC 25.5x10 ⁶ (2.4% CD34)	Echocardiography LV angiography SPECT	2 & 4	Improved LVEF Enhanced perfusion
Tse et al ⁴⁰	8	Transendocardial NOGA (Sole therapy)	MNC (3.2% CD34)	MRI	3	Unchanged LVEF Enhanced perfusion Improved wall thickening and wall motion

CABG: coronary artery bypass graft; CPC: circulating progenitor cell; LVEF: left ventricular ejection fraction; MNC: mononucleated cell; MRI: magnetic resonance imaging; NOGA™: Biosense Webster electromechanical cardiac mapping system; PET: positron emission tomography; PTCA: percutaneous coronary angioplasty; SPECT: single photon emission computed tomography

The extracardiac origin of the cardiomyogenic cells may be demonstrated by the colonisation of female myocardium with Y-chromosome positive cardiomyocytes following gender-mismatched heart or bone marrow transplants.^{9,80-85} Several clinical studies have demonstrated the safety and feasibility of intracoronary, transendocardial and transepical transplant of MNCs for myocardial repair after MI.^{32-35,37-41} Some of the studies were performed with the guidance of electromechanical mapping catheters that permit electrically defined pre-specified target zones. Preliminary results are promising with most trials reporting functional improvements with no adverse events in short-term follow-up. Improvement in regional indexes, such as wall thickness, wall motion and perfusion, with mixed outcomes in global contractile performance, were reported at 3 months follow-up (Table 2).

BMSCs are ideal for clinical cardiomyoplasty since MNCs can be readily isolated from direct bone marrow aspirate or collected from peripheral blood for autologous reinfusion with minimal manipulation. BMSCs may home to infarcted myocardium after being mobilised into the circulation, in a minimally invasive manner.⁸⁶ The simplicity

of MSC or EPC derivation from bone marrow and their high proliferation rates in culture also permit detailed physiological and biochemical characterisation before cell transplantation.

Our own data after isolating BMSCs from sternum of patients undergoing CABG suggests that BMSCs, in appropriate culture conditions, can be coaxed towards cardiomyogenic-like stem cells that express a wide range of cardiac-specific proteins.⁸⁷ These cells, although they show no spontaneous contractions, may be potentially very useful for transplantation into the infarcted myocardium to replace lost myocytes.

To Transplant Undifferentiated or Differentiated Stem Cells?

Transplantation of undifferentiated primitive BMSCs (or other primitive stem cells) into recipient tissues hinges on the hypothesis of milieu-dependent differentiation. Multipotent stem cells may undergo site-directed differentiation to become resident cells of the recipient organs. Stem cells may transdifferentiate into cardiomyocytes or endothelial cells in the regenerating

Table 3. Immunocytochemistry Analysis of Cardiomyogenic Mesenchymal Stem Cells Expressing Cardiac Markers

Antibody	Results
sarcomeric α -actinin	+++
sarcomeric α -actin	+++
skeletal/cardiac titin	+++
desmin	++
sarcomeric α -tropomyosin	+++
cardiac troponin I	+++
sarcomeric myosin heavy chain	+++
cardiac α/β myosin heavy chain	+
connexin-43	++
α -smooth muscle actin	+++
SERCA2 ATPase	+++
GATA-4	+
MyoD	-
skeletal muscle myosin heavy chain	-
slow muscle myosin heavy chain	-

Intensity of staining: "+" = positive; "++" = moderately positive; "+++" = strongly positive; "-" = negative

myocardium or myofibroblasts in the scar tissues following transplant.³⁰ Differentiation to tissues other than cardiomyocytes within the heart may lead to areas of arrhythmogenicity. This may be a compelling reason to differentiate the BMSCs into cardiomyocytes in culture before cell transplant. Another disadvantage of milieu-dependent induction of BMSCs is inefficiency in cardiomyocyte transdifferentiation, yielding insufficient quantity of cells to have any significance in repairing the injured myocardium.^{68,88} Lastly, there is no data on the long-term behaviour of the engrafted cells. There is concern that primitive BMSCs may become tumorigenic as demonstrated by the tendency of pluripotent ES cells to form teratomas in susceptible hosts.

Several methods have been used to induce differentiation of BMSCs into myogenic cells *ex vivo* that are more appropriate for cardiomyoplasty. A demethylating agent, 5-azacytidine (5-aza), has been reported to transdifferentiate murine BMSCs into myocytes^{28,31,89} that beat spontaneously.^{27,90} Demethylation of the myogenic transcription machinery has been suggested as the possible mechanism in the myogenic effect of 5-aza.⁹¹ However, there is inconsistency in transdifferentiation efficiency and widespread application of 5-aza may be hindered by its toxicity.⁹² Amphotericin-B has been reported to induce skeletal myogenesis of BMSCs⁶⁷ possibly by influencing the calcium flux process.⁹³ Our own data have suggested that insulin and dexamethasone may have cardiomyogenic effect.⁸⁷ Most of the BMSCs were found to express cardiac markers in culture (Table 3 and Figure 1) and readily engrafted into host cardiac fibres following cell transplantation. However, we were unsuccessful in inducing BMSCs using dimethyl sulphoxide and retinoic acid even

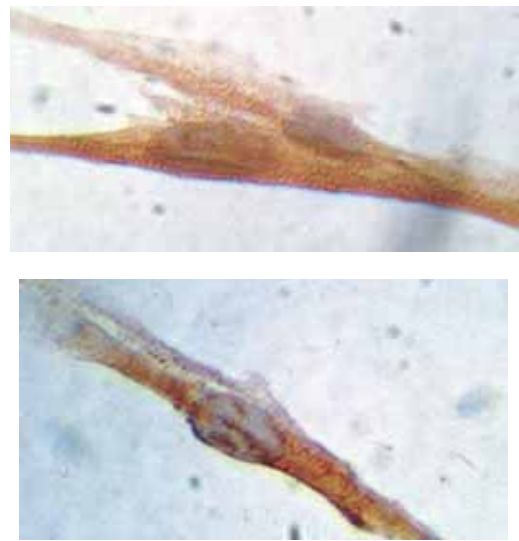


Fig. 1. Striated cardiomyogenic human mesenchymal stem cells express sarcomeric α -actinin (top) and cardiac titin (bottom) proteins.

though these compounds were reported to enhance differentiation of mouse ES and EC P19 cells toward cardiomyocytes.^{94,95}

A Case for Restoration of Systolic Contraction or Diastolic Compliance?

Clinical evidence of the beneficial effect of cell therapy has been shown despite ambiguity in the mechanisms of improved cardiac performance. Observed improvements in systolic contractile function following BMSC cardiomyoplasty attributed to myocardial cell regeneration should be viewed with caution. Greater systolic contraction was observed following transplantation with foetal cardiomyocyte¹⁷ or skeletal myoblasts¹⁸ than smooth muscle or fibroblasts. This may be due to the contractile properties of cardiomyocytes and skeletal muscle. Interestingly, only 5-aza-induced myogenic BMSCs but not untreated BMSCs supported systolic improvement despite both showing similar levels of enhanced angiogenesis following transplantation into infarcted myocardium.²⁸ These studies may indicate that cell transfer confers passive support for diastolic compliance through prevention of LV dilatation and angiogenesis, but only certain cell types are capable of actively contributing to the contractile improvement through systolic force development. More significant outcomes may be expected, should transplanted cells contribute to direct contractility by forming new functional cardiomyocytes, rather than imparting upon surviving cardiomyocytes the benefits of angiogenesis and scar restriction.⁹⁶ Understanding whether angiogenesis, direct contractility or ventricular remodelling is the mechanism for functional improvement after cell therapy would allow us to maximise the potential of this therapy.

Building on Consensus

Clinical cellular cardiomyoplasty is in its infancy and optimal protocols to reap the maximum benefits from this therapy remain unknown. Critical issues, such as types of bone marrow cells (surface marker sorted versus unsorted cells, adherent versus non-adherent, peripheral blood cells versus bone marrow cells, haematopoietic stem cells or side population cells versus mesenchymal stem cells), ideal number/concentration of cells, route of delivery (intracoronary versus transmyocardial or transendocardial), timing of delivery (acute versus chronic infarction) and targets of delivery (infarcted versus peri-infarcted regions, ischaemic versus hibernating regions), are still being investigated.^{96,97} Studies will be needed to differentiate between the most therapeutically beneficial types of bone marrow cells, the most appropriate animal models for their study, the most efficient route of cell delivery, the most accurate imaging techniques for assessment of successful transplant and to determine the issue of long-term safety. Whether specifically selected subfractions like CD34⁺, CD34⁻ or CD117⁺, CD133⁺ have any advantage over the whole bone marrow and MNCs will need to be determined. It is crucial to ascertain if the same therapeutic approach or the same type of cells will be suitable for all the different facets of cardiac dysfunctions such as myocardial infarction, myocarditis, non-ischaemic or dilated cardiomyopathy or pressure/volume overload induced cardiac remodelling.⁹⁸ The dose response relationship between myocardial engraftment and physiological effects that are verifiable by imaging modalities in clinic will need to be determined. The challenge will be to refine the current imaging modalities, such as echocardiography, single photon emission computed tomography (SPECT), positron emission tomography (PET) and magnetic resonance imaging (MRI) so as to provide scientific validation for efficiency of myocardial regeneration from a functional and structural standpoint.⁹⁹ In addition, more innovative technologies and devices will be needed for non-invasive tracking of stem cells in vivo.

The window of cell transplantation is likely to be crucial for technical as well as therapeutic considerations. If improved LV contractility were the direct consequence of the transplanted cells, then it would be expected to be equally effective in improving cardiac performance of both acute and chronic infarcts. However, should diastolic compliance via LV remodelling be the principal mechanism in cardiac improvement, timely cell transplant may be more efficacious, since premature cell transplant may lead to massive cell death by infarct-associated inflammatory reaction while late transplantation may be ineffective once remodeling has completed.¹⁰⁰⁻¹⁰³ If such constraints prove valid, off-the-shelf allogeneic MSCs might offer an

advantage over the autologous isolation of skeletal myoblasts and MSCs, which may be time-consuming and incompatible with acute therapy. Cryopreserved MSCs have been shown to be viable and retain their multipotency, therefore allogeneic MSCs may be frozen and thawed when needed.^{104,105} This coupled with the recent findings that MSCs may have an immuno-privileged status¹⁰⁶⁻¹⁰⁹ augurs well for the universal allogeneic use of these cells. This is especially relevant since elderly cardiac patients, which form the bulk of CHF patients, may have insufficient cells for autologous application because of age and other morbidities that influence the yield and quality of skeletal myoblasts,^{110,111} EPCs¹¹² or BMSCs.^{34,113-115}

The Future of Stem Cell Cardiomyoplasty

Human bone marrow is estimated to contain only limited quantity of haematopoietic (1% to 2%) and mesenchymal stem cells (<0.05%).⁹⁸ Further enrichment is hindered by the fact that no specific markers of true stem cells have been identified. Despite their 'plasticity' and great potential for proliferation and differentiation, there is a wide variation in the characteristics, expandability and multipotentiality of the cells isolated from different bone marrow aspirates. There are subtle but perhaps important differences in the expression of surface markers despite similar isolation and growth condition in vitro.¹¹⁶⁻¹¹⁹ There is need for a universally acceptable characterisation and classification system for stem cells and progenitor cells to facilitate comparison of results from various studies.¹¹⁷

It may not be unreasonable to expect diminished quantity and quality of these cells in elderly patients with comorbidities such as diabetes, hypertension and hypercholesterolaemia. Therefore, one of the biggest hurdles in stem cell research is the identification of culture conditions that enable propagation of undifferentiated stem cells. This could be achieved by influencing the microenvironment of the cultured cells. Some studies have suggested the definitive role of extracellular matrix (ECM) in the lineage development of stem cells, whereby cellular differentiation was significantly influenced by interaction with fibronectin.¹²⁰⁻¹²⁴ Our group is currently investigating the interaction of MSCs with various ECM, including fibronectin, gelatin, laminin, collagen I, III, IV and V, vitronectin and polylysine, and preliminary results suggest that collagen-based matrices are effective in promoting undifferentiated proliferation of MSCs.

Another approach to improve the outcome of cell transplant is to 'tissue engineer' these cells in biodegradable 3-dimensional scaffolds to enhance tissue growth and biocompatibility and to confer mechanical strength to the grafted constructs. Akhyari et al¹²⁵ grafted foetal cardiac cells in a gelatinous mesh and mechanical stretching was

applied to improve the graft strength. Zimmermann et al¹²⁶ seeded neonatal cardiac cells in a collagen/matrigel matrix and applied cyclical stretching to help the cells develop into mature adult cardiac tissues. Similar work on MSCs has been performed by using fibrin gel,¹²⁷ hyaluronan polymers,¹²⁸ collagen gel¹²⁹ and gelatin¹³⁰ for bone tissue engineering which may be equally applicable to cardiovascular tissue engineering. Furthermore, there may be additive effect in combining cellular cardiomyoplasty and gene delivery with respect to cardiac perfusion and apoptosis as demonstrated by VEGF transfected skeletal myoblasts¹³¹ and Akt transfected MSCs.¹³²

Cell therapy for heart failure has the potential to offer hope to many patients but numerous questions remain to be answered. What will the life span of the cells be after engraftment? Will donor cells survive as well in the chronically infarcted area of the heart as the viable peri-infarct areas demonstrated in most studies? What are the optimal angiogenic milieu for the survival of the transplanted cells in the hypoperfused tissue? Will there be age-/morbidity-dependent changes in the ability of recipients to support milieu-dependent differentiation of stem cells? Can the nascent cardiomyocytes beat synchronously within the infarcted myocardium or will they provide a fertile proarrhythmia substrate instead? Is the observed plasticity of stem cells the result of cell fusion? How transferable are the impressive animal data to humans?

Despite these challenges, there is a cautious optimism that a new era of cardiac therapy is on the horizon. We envisage that percutaneous, minimally invasive methods of cellular cardiomyoplasty will be used to tide over the 'no option' cardiac failure patient till whole organ transplant is available. We believe adult BMSCs will become a core aspect of this therapy since they circumvent moral, ethical and perhaps more importantly immunological complications associated with the use of cardiomyocytes derived from foetal tissues or ES cells. BMSCs have a significant advantage over skeletal myoblasts since they are able to differentiate into the 3 main cell types that are structurally and electrically compatible with the regenerating myocardium. Whether derived from peripheral blood or bone marrow, BMSCs are readily accessible and are already part of the current clinical arsenal for management of haematological diseases. With further technical refinements in standardisation of protocols and rigorous scientific validation of clinical efficacy, we believe BMSC-based cell therapy will be an option for the many 'no option' CHF patients we have today.

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