

MINIREVIEW

Adult Stem Cells for Cardiac Repair: A Choice Between Skeletal Myoblasts and Bone Marrow Stem Cells

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The real promise of a stem cell-based approach for cardiac regeneration and repair lies in the promotion of myogenesis and angiogenesis at the site of the cell graft to achieve both structural and functional benefits. Despite all of the progress and promise in this field, many unanswered questions remain; the answers to these questions will provide the much-needed breakthrough to harness the real benefits of cell therapy for the heart in the clinical perspective. One of the major issues is the choice of donor cell type for transplantation. Multiple cell types with varying potentials have been assessed for their ability to repopulate the infarcted myocardium; however, only the adult stem cells, that is, skeletal myoblasts (SkM) and bone marrow-derived stem cells (BMC), have been translated from the laboratory bench to clinical use. Which of these two cell types will provide the best option for clinical application in heart cell therapy remains arguable. With results pouring in from the long-term follow-ups of previously conducted phase I clinical studies, and with the onset of phase II clinical trials involving larger population of patients, transplantation of stem cells as a sole therapy without an adjunct conventional revascularization procedure will provide a deeper insight into the effectiveness of this approach. The present article discusses the pros and

cons of using SkM and BMC individually or in combination for cardiac repair, and critically analyzes the progress made with each cell type. *Exp Biol Med* 231:8–19, 2006

Key words: cell therapy; heart failure; myocytes; stem cells; transplantation

Introduction

Myocardial infarction (MI) is associated with dysfunction and irreversible loss of cardiomyocytes. The heart, with a scarce number of resident stem cells and a limited capacity of cardiomyocytes to re-enter into the cardiac cycle, has an inadequate capacity to repair itself (1). Recent studies have elucidated the ability of cardiomyocytes to regenerate in the event of myocardial injury (2, 3). Therefore, the existence of cardiac muscle-specific resident stem cells has been revisited (4). However, how cardiomyocytes are reactivated for proliferation to compensate for the loss of functioning cardiomyocytes remains unknown. Significant progress has been made in pharmacologic and surgical approaches to the treatment of MI and in rescuing the functionally viable myocardium after coronary artery disease (5). Cardiac allograft is the gold standard treatment modality but is limited by the shortage of donors and immunosuppressive complications. There is a pressing need to develop alternative methods to manage this problem.

Cell implantation has been viewed as a potential strategy for patients with coronary artery disease and heart failure. Most of the reported studies have documented improved heart function after implantation of donor cells via regeneration of heart muscle (6). Two of the most extensively studied cell types for cardiac repair are bone

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marrow-derived stem cells (BMC) and skeletal myoblasts (SkM). Extensive preclinical and limited clinical studies have shown their safety, efficacy, and feasibility for cardiac repair. Both cell types have advantages over other cell types, as summarized in Table 1.

This review analyzes and discusses the results of recent studies using both SkM and BMC for cardiac repair in animal models as well as human clinical trials. The advantages and inherent limitations of using each of the cell types are discussed as are future challenges confronting cell transplantation.

SkM Transplantation. SkM as a Substitute for Cardiomyocytes. In the setting of skeletal muscle injury, skeletal myocytes re-enter the cell cycle, proliferate, and fuse to repair the damaged muscle (7). Skeletal myocytes are experimentally the most well-studied cells for cardiac repair. The use of autologous SkM avoids issues of immunology, ethics, tumorigenesis, and donor availability; furthermore, these cells have a high growth potential *in vitro* and a strong resistance to ischemia *in vivo*. Skeletal myocytes from various species, such as mice, rat, rabbit, sheep, and pig have been studied in different animal heart models (Table 2). Survival of the transplanted SkM has been reported for up to 7 months in ischemic porcine heart models and for up to 1 year in rat and sheep heart models (30, 34, 35).

Skeletal myocyte transplantation for cardiac repair was first studied by Marelli *et al.* in a dog heart model of cryoinjury (21), and results of the study set the pace for extensive safety and feasibility assessments of SkM transplantation in heart (33). Transplantation of autologous SkM into cryoinjured heart of rabbit and monitoring cardiac function during 2–6 weeks after treatment revealed islands of elongated striated cells that retained characteristics of both skeletal and cardiac cells inhabiting the infarct region (19). By using micromanometry and sonomicrometry, the same group of researchers later showed that SkM transplantation improved diastolic compliance before systolic performance (18). In a sheep heart model, increased regional strain, decreased dynamic stiffness, and unaffected static stiffness found in SkM-transplanted animals indicated restoration of diastolic function (18); and delimited deterioration of the ejection fraction (EF) and improved systolic function in the scar area indicated improvement in systolic performance (30). Similar findings from other research groups further confirmed that SkM engraftment improved regional and global left ventricle (LV) function after MI (32).

A larger number of SkM may fully replace infarcted myocardium with an increased fractional area change and a decreased LV diastolic dimension in a dose-dependent manner (20). However, this generalization may hold true only for primary SkM, which proliferate in the heart for up to 4 weeks after transplantation (11). An endless proliferating capacity of immortalized stable cell lines may lead to impaired heart function. In an interesting study, trans-

plantation of the cell lines MM14 and C2C12 showed that, unlike MM14, C2C12 had extensive proliferation after transplantation into rat heart, and subsequently showed substantial incorporation of bromodeoxyuridine. Although C2C12 transplantation gave rise to transmural graft formation, the heart function was impaired, possibly because of an altered and expanded contour of the LV wall. Immunohistochemical results revealed a lack of connexin-43 expression, and, therefore, a lack of gap junctions between the cell graft and the host cardiomyocytes (15). It was concluded that both primary SkM and immortalized SkM lines were incapable of electromechanical coupling with the host cardiomyocytes unless the cells were genetically modulated to do so.

In addition to SkM transplantation as a sole therapy, studies to assess the efficacy of SkM transplantation in comparison with other cell types have also been investigated. These studies have documented SkM transplantation for cardiac repair as efficient and efficacious as fetal cardiomyocytes and BMC (20). A fascinating approach is to combine SkM transplantation with gene therapy, using SkM as carriers of exogenous therapeutic genes (23, 30). This combination therapy approach would be superior to either of the strategies used alone as a monotherapy. We have shown that delivery of vascular endothelial growth factor (VEGF) *via* human SkM leads to significantly enhanced neovascularization at the site of the graft in a porcine heart model of chronic MI (29). Capillary density at low-power field ($\times 100$ magnification) was 57.13 ± 4.20 in an experimental group receiving SkM over expressing VEGF, which was significantly higher than the control group (Fig. 1). Regional blood flow was significantly improved 6 and 12 weeks after transplantation (2.41 ± 0.11 and 3.39 ± 0.11 $\text{ml}^{-1}\text{min}^{-1}\text{g}^{-1}$, respectively) in the experimental group. Left ventricular EF increased from $31.25 \pm 4.09\%$ to $43.0 \pm 2.68\%$ at 6 weeks in the experimental group. These results also indicate the potential of SkM as transgene carriers for myocardial delivery.

Fate of Transplanted SkM: Cardiomyocyte or Skeletal Myofiber? The fate of the donor SkM after transplantation in heart remained ambiguous until recently. The functional benefits of SkM transplantation are associated with expression of the slow isoform of the myosin heavy chain (MHC), which makes the newly formed muscle fibers suitable for heart workload (26, 27). Earlier studies suggested milieu-dependent transdifferentiation of donor SkM into cardiac-like cells (14, 18, 19, 21). Whatever their fate may be, the cells fail to couple electromechanically with the host myocardium, thus keeping the contractile activity in the scar tissue, independent of neighboring cardiomyocytes (25). Although gap junctions have been described during the early stages of muscle development, with concurrent expression of N-cadherin and connexin-43, they are absent from adult skeletal muscle, with a coincident downregulated expression of N-cadherin and connexin-43 (36). *In vitro* co-culture studies with neonatal or adult cardiomyocytes

Table 1. Comparison of SkM and BMC

	SkM	BMC
Autologous availability	Yes	Yes (for young patient)
Cell number	Sufficient	Limited
Ethical problem	No	No
Immunosuppression	No	Yes (for allograft)
Purity	Up to 99% pure	Varies according to different marker
Differentiation after transplantation	Muscle fibers	Cardiomyocytes (uncertain) and endothelial cells
Safety	Under scrutiny	Under scrutiny
Feasibility	Established	Established
Efficacy of animal studies	Yes	Yes
Efficacy of clinical trials	Limited	Limited

showed N-cadherin and connexin-43 expression between skeletal myotubes and cardiomyocytes. However, these *in vitro* experiments did not correlate well with results from *in vivo* studies. Side populations of muscle-derived stem cells develop into hematopoietic progenitor cells *in vitro* and give rise to myocytes when co-cultured with SkM (37).

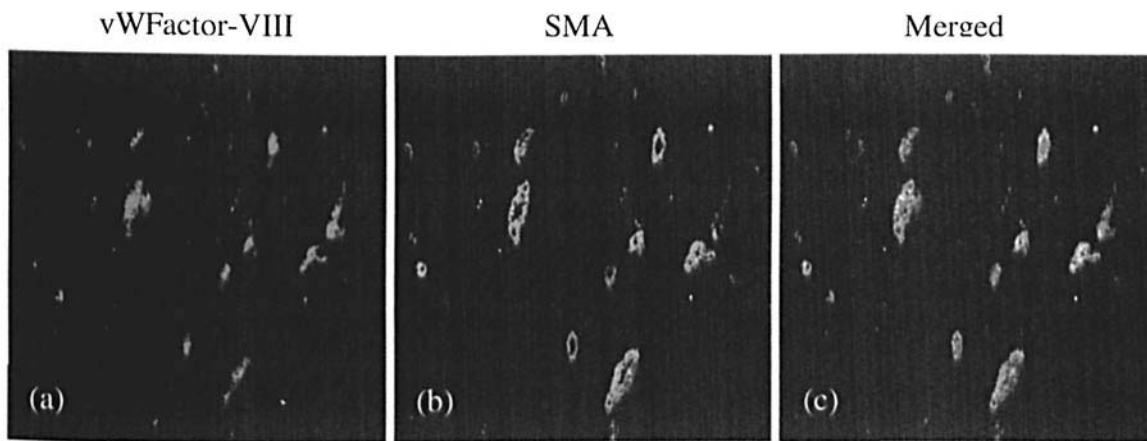
Human SkM fused to form myotubes that remained viable within the fibrosis area for up to 1–5 years after SkM transplantation into a patient (38). Immunofluorescence studies showed that a mean of 35% of the myotubes expressed only the fast isoform of MHC; 32% expressed the slow isoform of MHC; and 33% co-expressed both of the isoforms. These results support the hypothesis that functional myotubes are formed in the heart, with a phenotypic switch toward slow-twitch fibers that allowed the fibers to sustain cardiac workload. Similar results have been reported by Pagani *et al.* (39).

Survival of Transplanted SkM. Extensive early cell death, poor survival of the cell graft, and limited translocation from the site of injection are some of the limiting factors that interfere with the success of SkM transplantation (40–42). Massive numbers of the transplanted SkM are rapidly lost in the 48 hrs after implantation (43). The accelerated death of SkM is multifactorial, and, in part, is related to host immune and inflammatory reactions in the infarcted myocardium (44, 45). The other major players include mechanical injury, maladaptation, anoxic conditions, and the origin and quality of the SkM preparation (22, 46, 47). A significant role of immune cells in the early death of donor SkM, using outbred mice as hosts for transplantation of primary cultured muscle cells and T-antigen-immortalized SkM, has also been reported (42). There was a brief neutrophil invasion in the first 48 hrs after trans-

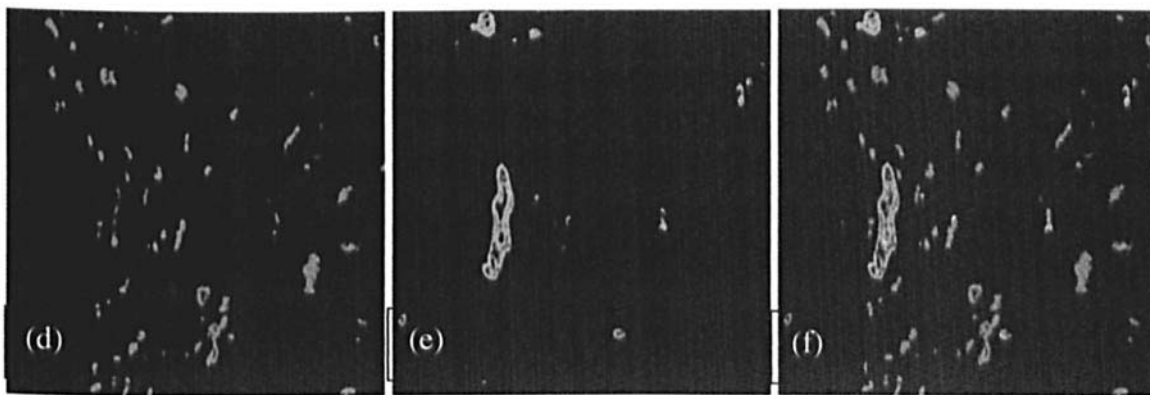
Table 2. Animal Studies Using SkM Transplantation for Cardiac Repair^a

Model	Animal	References	SkM Source	ROA	Pretreatment/gene modulation
Normal	Mouse	(8)	C2C12	IMI	Genetically modified with TGF- β 1
		(9)	Primary culture	IMI	Genetically modified with VEGF
	Rat	(10)	L6	IMI	Heat-shock pretreatment
		(11)	Syngenic	IMI	No
	Pig	(12)	Autologous	IMI	No
		(13)	Autologous	IMI	No
Cryoinjury	Rat	(14)	Neonatal	IMI	No
		(15)	C2C12	IMI	No
		(16)	H9C2	IMI	Genetically modified with VEGF
Cardiomyopathy	Hamster	(17)	Syngenic	IMI	Cryopreserved myoblast
		(18)	Autologous	IMI	No
	Rabbit	(19)	Autologous	IMI	No
		(20)	Autologous	IMI	No
		(21)	Autologous	IMI	No
Ligation	Dog	(22)	Autologous	IMI	No
		(23)	Syngenic	IMI	No
	Rat	(24)	Autologous	IMI	Genetically modified with VEGF
		(25)	Neonatal	IMI	Combining with ACE inhibitor
		(26)	Autologous	IMI	No
		(27)	Autologous	IMI	No
		(28)	Synergic	IMI	No
		(29)	Xenogenic	IMI	Genetically modified with human VEGF ₁₆₅
	Sheep	(30)	Autologous	IMI	No
		(31)	Syngenic	ICI	No
Toxin	Rat	(32)	Autologous	IMI	No
	Sheep	(33)	Autologous	IMI	No
Cryoinjury	Dog	(33)	Autologous	IMI	No

^a RAO, route of administration; IMI, intramyocardial injection; TGF, transforming growth factor; ACE, angiotensin-converting enzyme; ICI, intracoronary injection.



Ad-Null skeletal myoblast group



Ad-human VEGF₁₆₅ skeletal myoblast transplanted group

Figure 1. Transplantation of human SkM transduced with adenoviral vectors (Ad) carrying no therapeutic gene (Ad-Null) and human VEGF₁₆₅ (Ad-hVEGF₁₆₅) in a porcine heart model of MI at 6 weeks after cell transplantation. Transplantation of hVEGF₁₆₅-transduced SkM resulted in extensive neovascularization at the site of the graft as compared with the Ad-Null-transduced SkM.

plantation, followed by macrophage infiltration from Days 1 to 7. CD4-positive cell infiltration also started at Day 3. In contrast, donor-immortalized SkM completely disappeared during the 7-day follow-up. Starting on Day 2, there was an intense infiltration of cytolytic T lymphocytes and macrophages, with moderate CD4-positive cell infiltration and lower amounts of natural killer cells.

Our own experience with xenomyoblast transplantation in a porcine heart model of MI revealed the importance of transient immunosuppression in achieving a successful cross-species engraftment of human SkM (48). Based on our experience of working with human SkM, we hypothesized that human SkM enjoyed a conditionally immunoprivileged status that may be exploited by using transient immunosuppression during the earlier phase after SkM transplantation to achieve longer-term survival of the cell graft. We observed extensive survival of the xenomyoblast in pig heart until 30 weeks after transplantation. Discontinuation of immunosuppression therapy after 6 weeks resulted in no adverse effects toward the survival of human SkM.

These results implied the role of immunosuppression in reducing the early phase of SkM death after transplantation.

Adoption of anti-inflammatory strategies improves the survival of SkM after transplantation. Genetic modulation of SkM to express the interleukin-1 receptor antagonist (49), depletion of host C3 complement (50), and prevention of the humoral immune response against donor SkM by treating the host with anti-CD154 before and after SkM transplantation resulted in prolonged SkM survival (51). Similarly, insulin-like growth factor I (52), VEGF (53), pretreatment of SkM with basic fibroblast growth factor (54), and heat shock (10) have been shown to stimulate proliferation and to enhance survival of SkM.

Clinical Trials Using SkM for Cardiac Repair. Clinical studies in human patients involving SkM transplantation have mostly been performed in patients with severe heart failure. Menasche *et al.* reported the first autologous SkM transplantation adjunct to coronary artery bypass graft (CABG) (55), followed by a report of nine more patients (56). The average New York Heart Associ-

ation (NYHA) class improved from 2.7 to 1.6, and the average EF increased from 23.8% to 32.1%. However, ventricular tachycardia was observed in four patients, and one of those patients died from a stroke caused by acute occlusion of the left subclavian artery, 17.5 months after SkM transplantation (38). Immunohistochemistry studies on that patient's heart showed that the transplanted SkM had fused to form myotubes that remained viable within fibrosis, with the presence of a normal contractile apparatus. The newly formed myotubes were immunohistochemically negative for cardiac-specific connexin-43, desmosomes, and pan-cadherin expression.

Encouraged by these results, many other groups reported SkM transplantation in humans, essentially as an adjunct to a routinely used coronary artery revascularization procedure (Table 3). The first off-pump CABG performed together with SkM transplantation was reported by the authors at the National University Hospital, Singapore (57). Pagani *et al.* adopted an interesting approach that allowed them to study the *in vivo* fate of the transplanted SkM. The end-stage heart failure patients included in the study were candidates for heart transplantation, and received SkM transplantation concurrent with LV assist device (LVAD) implantation as a bridge to orthotopic heart transplantation (39). Hearts removed from the patients were later subjected to immunohistochemical studies. Newly formed skeletal muscle fibers expressing slow-twitch myosin isoforms and mostly aligned in parallel with the host myocardial fibers were observed in and around the area of infarct. There was no difference in morphology or survival of the transplanted cells between the cells in the infarct and the peri-infarct myocardium.

Use of autologous SkM is advantageous in many aspects. However, the delay of more than 3–4 weeks between the harvest of skeletal muscle biopsy from each patient and each transplantation procedure (because of processing to achieve the required number of the cells) is time consuming and less cost effective. These logistic considerations led to the first human SkM allograft transplantation, reported by Law *et al.* (2003) (63). Two patients received 110 and 120×10^6 allogenic SkM, respectively, and were maintained on transient immunosuppression. The results of the study showed 14.6% and 10.5% increases in EF in the patients with reduced perfusion defects, as measured by single-photon emission computed tomography.

BMC Transplantation. *BMC: Regenerating Cardiomyocytes and Blood Vessels.* Bone marrow-derived stem cells have multilineage potential (66). Treatment of BMC with the demethylating agent, 5-azacytidine, yielded spontaneously beating cells that had cardiomyogenic potential (66). More interestingly, the cells expressed atrial natriuretic and brain natriuretic factors together with MHC, myosin light chain, and α -actin, phenotypes of fetal ventricle cardiomyocytes. Electron microscopy revealed a cardiomyocyte-like ultrastructure. All cells had a long action-potential duration, a resting membrane potential,

and a pacemaker-like late diastolic slow depolarization. These landmark findings paved the way for the preprogramming of BMC as a substitute strategy before transplantation (67, 68). As an alternative to this approach, to achieve directed differentiation of the donor BMC, Min *et al.* ascertained the effectiveness of cotransplantation of human mesenchymal stem cells plus fetal cardiomyocytes into infarcted pig hearts (69). This approach led to a greater degree of neovascularization at the site of the cell graft.

Bone marrow-derived stem cells implanted for cardiac repair in infarcted-heart animal models have shown the feasibility, safety, and efficacy of the approach. They have also been reported to differentiate into cardiomyocytes in intact, nonischemic hearts (70). Similar results have also been reported in a hypoperfused rat heart model, with concomitant increases in the microvessel density (71). These results reveal the influence of the cardiac micro-environment as a trigger for milieu-dependent differentiation of BMC into cardiac-like cells, whether they are implanted into a normal or an ischemic heart.

In addition to entire BMC populations, subpopulations of BMC have also been investigated to assess their myogenic differentiation potential. Bone marrow mononuclear cells (BMMNC) (72), mesenchymal stem cells (73), c-kit+ cells (74), Sca+1 cells (75), and endothelial progenitor cells (76) have been investigated, with promising results. BMMNC contain approximately 16% cells of endothelial lineage and express basic fibroblast growth factor, VEGF, and angiopoietin-1. Transplantation of BMMNC into infarcted myocardium results in significantly upregulated expression of these growth factors, leading to markedly increased capillary density and regional blood flow (77). The results of small-animal models have been further validated in physiologically relevant large-animal models of human cardiac disease (Table 4). Using a canine heart model of MI, autologous BMC were injected into normal, marginal, and infarction areas at 30 days after coronary artery ligation (84). Longer-term follow-up after BMC transplantation revealed no significant changes in systemic biochemistry indices, including alanine aminotransferase, aspartate aminotransferase, creatinine, blood urea nitrogen, lactate dehydrogenase, or creatinine kinase and its isoform, creatinine kinase MB subunit, in comparison with the control non-BMC transplanted animals (83). Transplantation of BMC preprogrammed to adopt cardiomyogenic lineage via 5-azacytidine treatment has also been performed in a porcine heart model. The large-animal models have been developed using different approaches to achieve myocardial injury, such as left anterior descending artery occlusion, using a coil and Gelfoam sponge (87); ameroid ring placement around the left circumflex coronary artery (85); and coronary artery ligation (Table 4).

The Mechanism of BMC-Mediated Cardiac Repair. The mainstay of mechanism of BMC-mediated myocardial repair is generally reported as the outcome of transdifferentiation of BMC into cardiomyocytes. Fusion of

Table 3. Clinical Trials Based on SKM Transplantation for Cardiac Repair^a

Refer-ences	Approach	Patients	Cells	Marker/purity	Results
(55)	CABG, autograft	1	800×10^6	CD56/65%	EF increased from 25% to 35% function improved
(56)	CABG, autograft	10	871×10^6	CD56+/86%	Improved NYHA class (2.7 to 1.6); EF increased from 23.8% to 32.1%, with systolic thickening; 4 patients with ventricular tachycardia
(57)	CABG, autograft	1	374×10^6	desmin/98%	EF increased from 30% to 37% on beating heart; reduced perfusion defect
(58)	CABG, LVAD	18	$10\text{--}300 \times 10^6$	CD56/43–98%	EF increased from 21% to 29% (2 years; 12+ CABG, one patient had unsustained VT 6+ LVAD)
(59)	CABG, autograft	12	221×10^6	CD56/65.6%	EF increased from 35.5% to 53.5% improved regional contractility; no cardiac arrhythmias
(39)	LVAD, autograft	5	300×10^6	CD56+/43%–97%	Myofibers parallel to host myocardium; increased blood vessel; two patients AF, two patients VT
(60)	Transcatheter	13	275×10^6	Desmin/71%	No serious adverse events occurred, one autograft patient had nonsustained VT; EF increased from 36% to 41% (3 months) and to 45% (6 months); increased wall thickening
(61)	CABG autograft	18	300×10^6	CD56/82 \pm 9%	EF increased from 32% to 51% reduced infarct scar size; improved wall motion; NYHA class increased from 2.6 to 1.3
(62)	CABG, autograft	10	2×10^7	—	Improved segment contractility; 2 patients sustained VT; one death unrelated to cell transplantation
(63)	CABG, allograft	2	110 and 120×10^6	>98%	EF increased 14.6% and 10.5% reduced perfusion defect
(64)	CABG, autologous	3	—	—	Decreased LV diastolic diameter; improved EF by 1–11% improved wall thickness and perfusion
(65)	Transcatheter	12	$210 \times 10^6 \pm 150 \times 10^6$		EF increased from $24.3\% \pm 6.7\%$ to $32.2\% \pm 10.2\%$ at autologous transplant 12 months after myoblast implantation; NYHA class significantly improved at 1 year ($P = 0.02$ and $P = 0.001$ vs. baseline, respectively) as compared with controls

^a AF, atrial fibrillation; CABG, coronary artery bypass grafting; EF, ejection fraction; LVAD, left ventricular assist device; NYHA, New York Heart Association; VT, ventricular tachycardia.

donor cells with host cardiomyocytes, BMC-forming mesodermal progenitor cells that differentiate to endothelial cells, and angiogenesis *via* expression of angiogenic growth factors have been proposed as additional possible contributing factors (93, 94). The newly formed heart tissue after BMC implantation occupied as much as 68% of the damaged portion of the ventricle, along with extensive neovascularization (79). Both CD34-negative and CD34-positive BMC have the ability to effectively stimulate angiogenesis and arteriogenesis (95). A hematopoietic CD34-negative “side population” of BMC can differentiate into endothelial cells and cardiomyocytes. The transplanted cells remained confined near arterioles and survived in the

fibrous tissue. An increasing number of vessels and BMC-derived myocytes were observed.

The lingering controversy regarding the ability of BMC to transdifferentiate into cardiomyocytes and their capability to fuse with host cardiomyocytes has again been brought up for discussion by recently published reports that show an inability of the BMC to cross lineage restrictions to become cardiomyocytes (96, 97). Using genetic techniques to follow the donor-cell fates of stem cells in stem cell-engrafted hearts, Murry *et al.* failed to find any evidence of transdifferentiation of the transplanted hematopoietic stem cells into cardiomyocytes in 145 transplants. They did not find any significant increase in cardiomyocyte numbers in

Table 4. Animal Studies of BMC Transplantation for Cardiac Repair^a

Cell source	Animal	Model	ROA	References
BMC	Mouse	Toxin	IMI	(70)
		Infarction	IMI	(78)
		Infarction	IMI	(79)
		Infarction	IMI	(80)
	Rat	Normal	IMI	(81)
		Ischemia	IMI	(82)
		Cryoinjury	IMI	(67)
		Cryoinjury	IMI	(68)
		Infarction	IMI	(71)
	Rabbit	Cryoinjury	IMI	(20)
		Normal	IMI	(83)
	Canine	Infarction	IMI	(84)
	Pig	Ischemia	IMI	(85)
		Ischemia	ICI	(86)
		Infarction	IMI	(87)
Infarction		IMI	(88)	
Infarction		IMI	(89)	
Infarction		IMI	(72)	
Endothelial cells	Rat	Infarction	IMI	(90)
EPC	Mouse	Normal	IMI	(91)
Human BMC	Nude rat	Infarction	IVI	(92)
Human MSC	Pig	Infarction	IMI	(69)

^a ROA, route of administration; IMI, intramyocardial injection; ICI, intracoronary injection; EPC, endothelial progenitor cells; IVI, intravenous injection; MSC, mesenchymal stem cells.

the experimental group of animals as compared with the sham-operated animals (96). In line with these findings, Balsam *et al.* suggested that even the cytokine- and growth factor-rich microenvironment of the injured heart failed to trigger c-kit⁺ enriched BMC; Lin⁻ c-kit⁺ BMC; and c-kit⁺ Thy1.1^{lo} Lin⁻ Sca-1⁺ hematopoietic stem cells to adopt anything but traditional hematopoietic fates (97). This is an important issue because transdifferentiated donor cells that adopt cardiac phenotypes, and the cells originating from fusion between donor cells and the host cardiomyocytes will have different functional characteristics, with significant therapeutic implications on the outcome of cell transplantation therapy (85).

Clinical Studies Using BMC. In the light of the promising data pertaining to the safety and feasibility of BMC transplantation in both small- and large-animal models, human phase I clinical trials have been initiated by various research groups (Table 5). Unlike SkM transplantation, most of the clinical studies using BMC were conducted primarily in patients early after MI or in patients with retractable angina. Similar to SkM transplantation studies, most of the studies for BMC transplantation have been performed as an adjunct to routine revascularization approaches, including CABG and coronary angioplasty (101). BMC transplantation as a sole therapy has also been reported (99, 100, 102, 104). For the delivery strategies, in addition to direct intramyocardial injection, safety and feasibility of intracoronary injection and catheter-based

delivery methods have also been investigated (99, 102). The use of an electromechanical mapping system is gaining support from researchers for delivery of BMC in patients with severe, symptomatic, chronic myocardial ischemia who are not suitable for conventional revascularization (104). Cells are successfully delivered into ischemic noninfarcted myocardium without serious adverse effects, especially arrhythmia, evidence of infection, myocardial inflammation, or increased scar formation. Strauer first assessed the efficacy of autologous BMMNC transplantation after MI in a group of 20 patients (100). Ten patients received intracoronary delivery of cells as an adjunct to percutaneous transluminal coronary angioplasty, whereas the remaining 10 patients were treated by the standard therapy alone. The patients were followed for 3 months. The results showed a significantly reduced infarct region (from 30 ± 13% to 12 ± 7% $P = 0.005$). Similarly, the stroke-volume index and EF showed improved cardiac function in the cell-transplanted group of patients. Perin *et al.* first performed a prospective, nonrandomized, open-label study assessing the efficacy of BMMNC in 21 patients (treatment = 14 patients; control = 7 patients) (103). After electromechanical mapping for the identification of viable myocardium, 15 injections of 0.2 ml each were performed using NOGA catheter. At 2 weeks and 4 weeks of follow-up, there was a significant reduction in the reversible defect ($P = 0.02$) and a significantly improved EF from 20% of baseline to 29% of baseline, respectively. The most-significant finding was that the cell-injected segments of LV showed an improved mechanical function by 4 weeks of observation.

These studies showed the safety and effectiveness of BMC transplantation. None of these studies has reported problems of arrhythmia, peri-operative ischemic episodes, or postoperative complications during follow-up. Despite encouraging results, the nonuniform criterion of patient selection for inclusion in the studies, the smaller sample sizes, the variation in the selection of the injected cell type, and the variation in the assessment methodologies make it difficult to reach a clear conclusion regarding the effectiveness of the procedure. Moreover, the adjunct procedural approach and lack of proper blinded placebo controls hampers the evaluation of the outcomes.

Comparison of SkM and BMC. During the last decade, the successful engraftment of autologous SkM into various animal heart models has clearly demonstrated that autologous SkM transplantation can be performed without complications and with favorable outcome. On the other hand, BMC transplantation for cardiac repair is more recent, with the first animal report in 2001, by Orlic, who transplanted BMC from transgenic mice expressing enhanced green fluorescent protein (79). This study was followed by a vast repertoire of publications documenting success stories of BMC transplantation in experimental animals as well as clinical studies.

Both of the cell types have comparable characteristics, such as their availability from an autologous source without

Table 5. Clinical Studies Using Bone Marrow Cell for Cardiac Repair^a

Cells	Adjunct procedure	Study phase	No. of patients	ROA	Cell number	Outcome measurements	Reference
BMC	CABG	I	5	IMI	5×10^7 – 10^8	Chest radiography, electrocardiography	(98)
BMDPC or PBDPC	Sole therapy	I	20 (BMDPC = 9; PBDPC = 11)	IMI		Echo, PET, angiogram	(99)
BMMNC	Sole therapy	II	20 (Treatment = 10; control = 10)	ICI	3×10^6 – 10^7	Ventriculography, angiogram	(100)
AC133+	CABG	I	6	IMI	1.5×10^6	SPECT	(101)
BMC	via NOGA	I	8	IMI	1×10^7	MRI	(102)
BMMNC	NOGA	II	21 (Treatment = 14; control = 7)	NOGA injection	$25.5 \pm 6.3 \times 10^6$	Echo, SPECT, angiogram	(103)
BMC	Sole therapy	I	10	IMI	78.24×10^6	SPECT, treadmill, exercise	(104)
BMMNC	Sole therapy	I	1	NOGA injection	2×10^9	NOGA, SPECT	(105)
BMC	Angioplasty	II	BMC = 30; control = 30	ICI	24.6×10^8	MRI	(106)

^a ROA, route of administration; IMI, intramyocardial injection; BMDPC, bone marrow–derived progenitor cells; PBDPC, peripheral blood–derived progenitor cells; PET, positron emission tomography; ICI, intracoronary injection; SPECT, single-photon emission tomography; MRI, magnetic resonance imaging.

involvement of ethical issues and the ease of their expansion in *in vitro* culture conditions to achieve the number of cells required for transplantation. Nevertheless, each cell type enjoys superiority over the other in certain aspects. For example, SkM are more resistant to ischemia and, therefore, may be ideal for transplantation into the ischemic myocardium. One major advantage of BMC over SkM is the plastic nature of the BMC, resulting in their ability to undergo transdifferentiation into cardiomyocytes. Although previous studies showed milieu-dependent differentiation of SkM into cardiac-like cells after transplantation, these observations failed to gather much support from later studies. However, some researchers consider the plastic nature of the BMC to be a disadvantage, and have recommend caution in using BMC for cardiac transplantation (107). In this regard, the unipotential nature of SkM is advantageous because they are intrinsically myogenic, and, therefore, the possibility of their forming anything other than muscle fibers is remote. Similarly, there have been reports of cardiac arrhythmia after SkM transplantation; cardiac arrhythmia has not been observed with BMC transplantation. However, these reports also showed that the arrhythmias were pharmacologically treatable.

Although comparison of BMC and cardiomyocytes in a porcine MI model improved preload-recruitable stroke work and prevented thinning and expansion of the infarct region, some BMC differentiated into endothelial cells in newly formed blood vessels in infarcted myocardium (88). On the other hand, comparing the efficiency of BMC with SkM showed that intracardiac transplantation of an equal number of autologous BMC or SkM progenitor cells resulted in a similar degree of improvement in a derivative of stroke work in both of the transplantation groups (20). Interestingly, with both cell types, no expression of gap-junction proteins was observed, demonstrating a lack of integration of both of the donor cell types into the host cardiac tissue after transplantation.

Because of the divergent mechanisms after transplantation of the cells, which impart different beneficial effects on the injured myocardium, the choice of the donor cell between BMC and SkM depends on the required outcome of the procedure. Transplantation of SkM, which later differentiate into skeletal muscle fibers, may provide tenacious support for the weakened myocardium, prevent LV dilation, and enhance diastolic cardiac function more significantly; whereas BMC transplantation may more importantly improve systolic function. Considering these deviating, but significant, outcomes, a more relevant approach would be to combine both of the cell types for simultaneous transplantation to achieve the benefits associated with the use of each cell type (108). The synergistic effect of the two cell types will be clinically more appropriate.

The Challenges of SkM and BMC Transplantation for Heart Repair Preclinical data has shown that SkM transplantation is able to improve the regional and

global heart function of a damaged myocardium. Some fundamental issues remain that need to be fully resolved to exploit the benefits of SkM transplantation. For optimal prognosis, donor cells must be electrically integrated with and beat synchronously with host cardiomyocytes. Such functional association of SkM with host cardiomyocytes has not been reported. Additionally, the optimal cell number required to replace the infarcted tissue remains undefined. The problem is further accentuated by rapid and extensive donor SkM death after transplantation, which undermines the success of the procedure. Clinical data suggests that surgical delivery of SkM may cause electrical abnormalities because of the distinctly different electrophysiologic properties of the skeletal and cardiac muscles. Electrical coupling of the transplanted SkM with host cardiomyocytes may lead to a reduced arrhythmic threshold (109). Thus, gap junctions are important to form electromechanical communication between cardiomyocytes and the SkM. Transplantation of L6 SkM that overexpress connexin-43 resulted in a better integration of the transplanted cells with the surrounding host cardiomyocytes (109). Additionally, SkM showed an improved and more-rapid differentiation potential, along with synchronous contractility with the host myocardium. However, because significant improvement of damaged heart function has been well documented without the expression of connexin-43, the feasibility of overexpressing connexin-43 in SkM remains questionable (109).

As with SkM, fundamental issues regarding BMC transplantation regarding the source of the cells, the number of cells required, the identification of subpopulations of BMC with cardiomyogenic potential, etc., need to be optimized. Wang *et al.* showed that bone marrow stromal cells can be obtained repeatedly by bone marrow aspiration and expanded *in vitro* before implantation (110). In its practicality, however, it is difficult to aspirate sufficient BMC from older patients to perform autologous transplantation. The cardiomyogenic and angiogenic potentials of individual subpopulations of BMC taken from older patients also remain uncertain (111). The age-related impairment of angiogenic activity is associated with altered expression of VEGF, VEGF receptors (Flt-1 and Flk-1), and angiopoietin-1 and -2 (111). In certain pathologic conditions, such as type II diabetes, insulin resistance is associated with impaired endothelial proliferation and angiogenesis (112). Likewise, injection of whole BMC populations may result in stronger inflammation because of the presence of inflammatory cells in the bone marrow. Some of the relevant complications and risks associated with BMC implantation are calcifications, microinfarctions, inflammation, and restenosis (113–115).

From the clinical standpoint, the long-term fate of the cell graft, the safety, and the implications of both SkM- and BMC-based treatment strategies of severe ischemic heart disease need to be investigated in double-blinded, randomized studies with comparison groups, in larger patient

cohorts. An ideal scenario would be to test the effectiveness of the approach as a sole therapy.

Conclusion

Although cellular cardiomyoplasty using SkM or BMC has shown great promise in both preclinical and clinical settings, further studies are required for deeper insight to judge the outcome of the transplantation, in general, and on a longer-term basis, in particular. A combined therapy based on simultaneous administration of both of the cell types may help to supplement the shortcomings of each cell type. Further studies to understand the mechanism of their synergistic interaction may help to exploit their therapeutic potential.

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