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From bench to bedside, work in cell-based myocardial regeneration therapy

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ABSTRACT

In clinical cellular cardiomyoplasty, bone marrow cells and myoblasts are introduced mainly to ischemic cardiomyopathy tissue via several cell delivery systems, such as needle injection or catheter. These clinical studies have demonstrated the safety and feasibility of this technique, but its effectiveness for treating heart failure, especially in the long term, is still under discussion. Neither of these cell types can differentiate into cardiomyocytes; rather, they improve the failing heart mainly by the paracrine effects of some cytokines, such as Hepatocyte growth factor (HGF) and Vascular endothelial growth factor (VEGF). Thus, many researchers have a great interest in stem cells, which exist in bone marrow, circulating blood, atrium, and adipose tissue, and can differentiate into cardiomyocytes. Although several stem cells with the potential to differentiate into various cell types have been reported, few can differentiate into cardiomyocytes. Moreover, beating cells that can demonstrate synchronized contraction with native cardiomyocytes are critical for the complete repair of severe heart failure. Therefore, stem cells with a high differentiation capacity should be explored for the goal of completely repairing severely damaged myocardium. In this review, we summarize the clinical protocols and basic experiments for cellular cardiomyoplasty using bone marrow cells, myoblasts, and other stem cells.

KEYWORDS

Stem Cells; Heart Failure; Cell Therapy

1. INTRODUCTION

To overcome heart failure many basical studies have been done and some technologies have been introduced to the treatment of heart failure clinically based on the experimental data.

Although cell therapy was recently introduced to clinical situation in heart failure, tremendous experimental studies (Bench work) have been done before clinical trials (Bed side work). In this review we present and analyze recent achievements in the laboratory and clinic in cellular cardiomyoplasty.

2. CELLULAR CARDIOMYOPLASTY

The adult heart has no regenerative ability to repair damaged myocardium by itself. Cellular cardiomyoplasty was introduced to compensate for this lack of regenerative ability by delivering viable cells to distressed myocardium that has almost no functional cells (**Figure 1**). To develop this attractive method, many kinds of cells were implanted into small animal or large animal models through various routes. Based on the results of these experiments, several clinical trials have already been performed, and revealed the feasibility and efficacy of this technique. Although the mechanisms for the functional improvement after cellular cardiomyoplasty have not been completely elucidated, most researchers believe that its efficacy mainly depends on the paracrine effect of cytokines, because the incidence of transplanted cell differentiation to cardiomyocytes is low, there is no contractile function in most of the implanted cells [1], and the implanted cells represent only a small fraction of the LV mass [2].

2.1. Skeletal Myoblasts

2.1.1. Experimental Achievements

Unlike heart muscle, skeletal muscle has its own regenerative system. As soon as skeletal fibers are injured, skeletal myoblasts under the basal membrane of the skeletal fibers are mobilized and fuse with neighboring

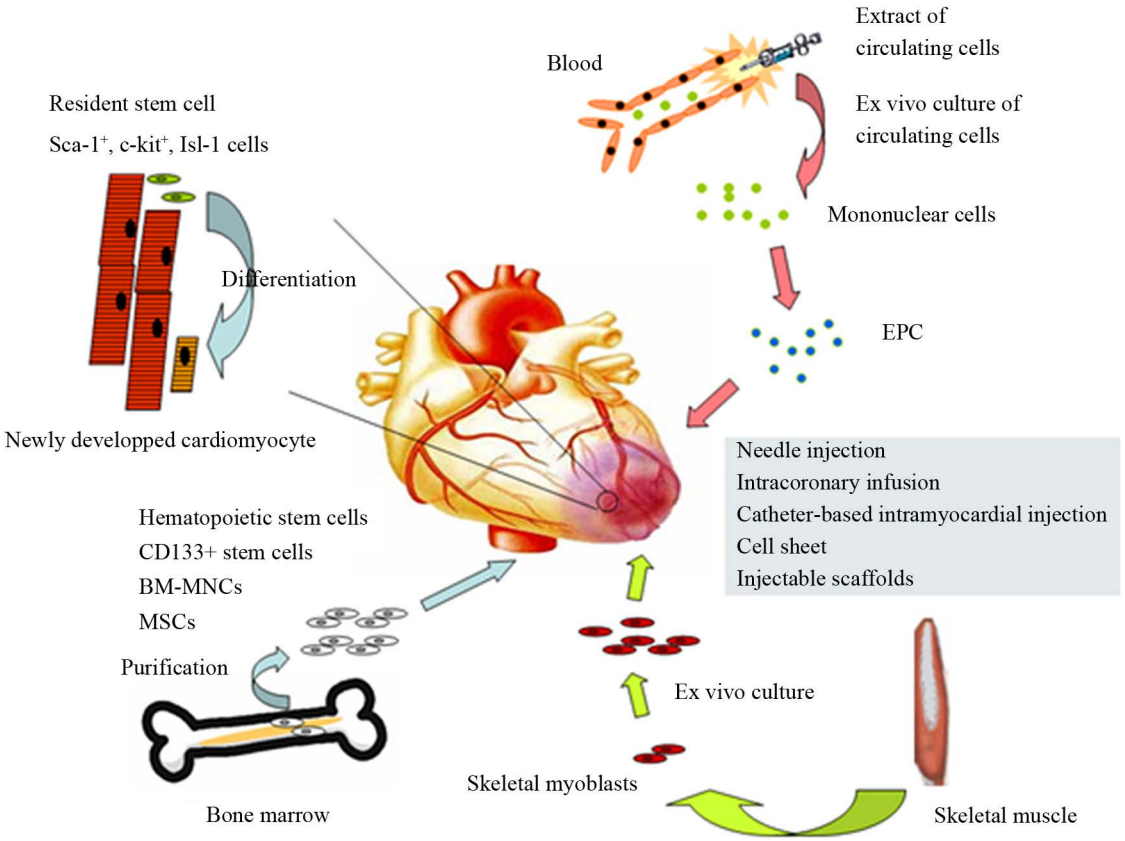

Figure 1. Cell sources for myocardial regeneration. Current clinical trials include the transplantation of skeletal myoblasts, bone marrow mononuclear cells, mesenchymal stem cells, or circulating progenitor cells. Cells used in experimental studies are resident stem cells and hematopoietic stem cells. There are various kinds of cell delivery routes, such as needle injection, intracoronary infusion, catheter-based intramyocardial injection, and cell sheet.

myoblasts, leading to regenerated, functional skeletal muscle. To exploit their self-regenerative capacity, myoblasts were implanted into the distressed myocardium, which has no regenerative system. The viability of the transferred myoblasts and their affinity for the myocardium were studied, and many experiments on the cell survival, differentiation to cardiomyocytes, and electrical coupling with recipient myocytes were performed to examine the effectiveness of myoblast implantation.

Implanted myoblasts engrafted to cryoinjured dog myocardium [3,4] prevented LV remodeling and improved cardiac performance [5,6]. The implanted myoblasts did not transdifferentiate into cardiomyocytes, showing a mature skeletal muscle phenotype [7]. Mature skeletal muscle grafts in the distressed myocardium had no connexin43 or N-cadherin, indicating they did not undergo electrical coupling with the host myocardium *in vivo* [8]. However, a low incidence of myoblast fusion with cardiomyocytes was observed [9], and a small number of these fused cells showed connexin43 expression [10]. Suzuki *et al.* reported that connexin43-overexpressing myoblasts formed functional gap junctions, suggesting the potential for synchronous contraction with host myocytes [11]. However, implanted myoblasts isolated from the recipient myocardium could not contract synchronously with host cardiomyocytes [12]. Myoblasts are thought to be the best candidate for cardiomyogenesis in the clinical setting, because cardiomyocytes cannot be cultured for clinical use, and only myoblasts can differentiate to muscle. However, implanted myoblasts can be isolated from the host myocardium electrically *in vivo*, indicating that they do not differentiate, and the cardiomyogenesis in the failing heart is quite incomplete.

2.1.2. Clinical Achievements

Despite the lack of affinity of implanted myoblasts for the host myocardium, many papers have reported functional improvement in small animal and large animal models following myoblast transplantation, leading to clinical applications. This technique is attractive because of its high resistance to ischemia and use of autologous cells [13], even though the mechanisms underlying its effects are still unclear.

Several groups reported the clinical efficacy of myoblast transplantation through needle injection [14-18] or catheter [19] (**Table 1**). These clinical studies indicated

Table 1. Clinical trials of myoblast transplantation. HD, high dose; LD, low dose; OMI, old myocardial infarction; TR, treatment; CABG, coronary artery bypass grafting; LVAD, left ventricular assist device; F.U. (mo), follow up (months); IM, intramuscular; TE, transendocardial; EF, ejection fraction; ESV, end systolic volume; EDV, end diastolic volume; PET, positron emission tomography; VT, ventricular tachycardia.

Study	Study design	Concomitant TR	Number of cells	F.U.(mo)	Route	Results	Adverse effects
Menasche, 2003	No control	CABG	8.7×10^8	10.9	IM	Symptom↓, EF↑(8%)	VT(4/10)
n = 10	OMI		(86% myoblast)			Systolic thickening↑(14/22)	
Herreros, 2003	No control	CABG	2.2×10^8	3	IM	EF↑(18%), Regional contractility↑	No
n = 12	OMI		(65.6% myoblast)			Regional Viability↑(PET),	
Smits, 2003	No control	~	2.0×10^8	6	TE	EF↑(9%)	VT(1/5)
n = 5			(55% myoblast)			Wall thickening ↑at target area	
Pagani, 2003	No control	LVAD	3.0×10^8	68 - 191 days	IM	Detection of matured myofiber	VT(3/5)
n = 5	OMI		(43% - 97% myoblast)			small vessel formation↑	
Ince, 2004	Controlled, n = 6	~	2.1×10^8	12	TE	EF↑(7.9%), Regional contractility↑	No
n = 6	OMI		(70% myoblast)			Symptom↓, EF↑(8%)	
Siminiak, 2004	No control	CABG	$0.04 - 5.0 \times 10^7$	12	IM	EF↑(6.8%)	VT(2/10)
n = 10	OMI		(65.4% myoblast)				
Dib, 2005	No control	CABG(n = 24)	$0.1 - 3.0 \times 10^8$	11 - 45	IM	EF↑(8%, 2 years), EDV, ESV↓	VT(3/30)
n = 30	Non randamised	LVAD(n = 6)	(79% myoblast)			Detection of skeletal myofiber	
	multicenter, OMI						
Siminiak, 2005	No control	~	$0.17 - 1.1 \times 10^8$	6	Transvenous	EF↑(6/9)	No
n = 10	OMI				approach		
Gavira, 2006	Controlled, n = 14	CABG	2.2×10^8	12	IM	EF↑(19.5%), Regional contactility↑	No
n = 12	OMI		(65.6% myoblast)			Myocardial viability, perfusion↑(PET)	
Hagege, 2006	No control	CABG	8.7×10^8	49.4	IM	EF↑(1mo, 6.7%), Symptom↓	VT(5/9)
n = 9	OMI		(86.6% myoblast)			EF→(long term follow up)	
Menasche, 2008	Randmised	CABG	HD; 8×10^8	6	IM	EF→, Regional wall motion→	VT(12%; LD, 17%; HD)
n = 33(HD)	Placebo-Controlled, n = 30		LD; 4×10^8			ESV↓(HD)	
n = 34(LD)	Double blind						
	Multicenter						

its feasibility by establishing human myoblast cell culture methods in Good Manufacturing Practice-approved facilities and procedures for injecting cells into the human myocardium [13]. Two papers successfully identified mature myofibers in the human myocardium after transplantation [17,20], but its efficacy needs to be further investigated because of the absence of control groups and concomitant coronary bypass surgery in many studies. Although the incidence of ventricular tachycardia after engrafting was high, occurring in 4 out of 10 patients [15] and 1 out of 5 patients [19], a causal relationship between the myoblasts and ventricular tachycardia remains unknown. To evaluate its efficacy and arrhythmogenicity, Menasche *et al.* performed a multicenter, randomized, placebo-controlled, double-blind study and revealed a high incidence of early postoperative arrhythmic events and prevention of LV remodeling with a high-dose cell injection, although no improvement in global systolic function was seen [21].

Recently myoblast sheet implantation technique in human DCM and ICM has already been introduced to clinical application. However, to prove its therapeutic effectiveness, a multicenter, randomized, placebo-controlled, double-blind study should be performed.

2.2. Bone Marrow Cells

2.2.1. Experimental Achievements

Progenitor cells within bone marrow cells (BMCs) play a large role in the regeneration of damaged skeletal muscle [22], and this exciting finding encouraged researchers to explore the regeneration ability of BMCs in the failing myocardium. The potential ability of BMCs to differentiate to cardiomyocytes *in vivo* has been shown in several reports. Orlic *et al.* sorted lineage-negative (Lin-) and c-kit+ BMCs by fluorescence-activated cell sorting from mice constitutively expressing enhanced green fluorescent protein (EGFP), and transplanted these cells into an infarcted area. Surprisingly, newly formed cardiomyocyte tissue was regenerated in approximately 68% of the infarcted area, and these transdifferentiated EGFP-positive cardiomyocytes expressed cardiomyocyte-specific markers, such as cardiac myosin and several transcription factors, and improved cardiac performance [23,24]. This discovery supported the idea of myocardial regeneration by multipotential BM stem cells. The work of Jackson *et al.* suggested that hematopoietic stem cells (HS cells; a component of BM stem cells that is CD34+, the so-called side population cells) can differentiate into cardiomyocytes. These researchers implanted HS cells from Rosa26 mice that constitutively expressed beta-galactosidase into lethally irradiated mice and made an infarction model of these mice. The transplanted beta-galactosidase-positive HS cells migrated to the periinfarcted region, and were identified as newly differentiated cardiomyocytes at a prevalence of approximately 0.02%, and as endothelial cells at 3.3%, of all myocytes [25]. Although this report is exciting, the incidence of differentiation to cardiomyocytes was revealed to be quite low, unlike the above-mentioned study by Orlic *et al*. Another exciting study appeared to show that BMCs can differentiate into cardiomyocytes in human samples. Deb *et al.* examined female uninjured hearts from patients who had undergone male bone marrow transplantation, and detected approximately 0.23% Y chromosome-positive cardiomyocytes by the FISH method. This study also indicated that the transdifferentiation to cardiomyocytes was very rare. Several groups have since examined the reproducibility of BMC differentiation to cardiomyocytes, motivated by these promising results.

However, Murry *et al.* introduced a new system for determining cell transdifferentiation to cardiomyocytes using genetic methods without a histological detection system, and clearly showed that no HS cells differentiated into cardiomyocytes after their transplantation to infarcted myocardium [1]. Moreover, Balsam *et al.* failed to reproduce the transdifferentiation of Lin- and c-kit+ bone marrow cells to cardiomyocytes *in vivo* [26]. Several groups clearly revealed that BMCs can spontaneously fuse with other cells, such as liver cells [27], cardiomyocytes, and Purkinje neurons [28], and that the BMCs can obtain the phenotype of the cells with which they have fused [29]. This special ability of BMCs to undergo cell fusion can explain the apparent transdifferentiation of BMCs to cardiomyocytes [30]. In addition, Laflamme *et al.* pointed out that the apparent transdifferentiation to cardiomyocytes evaluated by histological detection systems was owing to misleading artifacts in confocal microscopy. in his review [31]. However, Kajstura *et al.* [32] and Yoon *et al*. [33] offered counterarguments to the lack of HS cell transdifferentiation to cardiomyocytes. Thus, there is presently no consensus about whether HS cells transdifferentiate to cardiomyocytes *in vivo*.

BMCs contain two components, HS cells and mesenchymal stem cells (CD 34−, present in the bone marrow stroma). Several reports revealed that mesenchymal stem cells (MSCs) can differentiate into cardiomyocytes *in vitro* under certain conditions [34-36], while the incidence of their transdifferentiation to cardiomyocytes *in vivo* is quite low [37,38]. In spite of the low incidence of differentiation to cardiomyocytes and small amount of newly developed cardiomyocyte tissue, MSC [39] implantation induces a marked improvement in cardiac function.

2.2.2. Clinical Achievements

In spite of the low evidence for BMC differentiation to cardiomyocytes and unclear mechanism, intracoronary

bone marrow mononuclear cell (BMMC) implantation was quickly introduced to the clinical setting, mainly for acute myocardial infarction, because methods were already well-established for BMC aspiration in hematological treatments, and there was no need to perform cell culture. Generally speaking, although the trials for BMMC implantation revealed its safety and feasibility, its effectiveness varied. This variability may have been owing to differences in the heterogeneous population of BMCs in each study, the number of cells transferred, and the time after acute myocardial infarction (AMI) that treatment was performed.

Several clinical trials of BMC implantation for AMI revealed its safety, feasibility, and efficacy. All of the studies used the intracoronary injection method (**Table 2**). Five randomized, controlled studies have been reported. The BOOST randomized controlled clinical trial for 60 AMI patients (BMCs, 30 patients; Control, 30 patients) was done in 2004 and showed that the global systolic function measured by magnetic resonance imaging (MRI) with BMC treatment was significantly improved at the 6-month follow-up, compared with the sham-injected controls [40]. In 2006, the same group checked the long-term results at 18 months after implantation in a randomized, controlled study, and observed no significant improvement in the left ventricular ejection fraction (LVEF), despite the significant improvement seen at 6 months [41]. However The Balance study revealed that BMC implantation induce significant improvement of cardiac performance and prolong survival rate 60 months after implantation [42]. Although this is very exciting study reporting the long-term results after BMC implantation in AMI, studies which comment about long term results are not so many and these studies are controversial. So further studies may be needed to elucidate long term results after BMC implantation. In 2006, the first randomized, double-blind, placebo-controlled study was reported in 67 AMI patients (BMCs, 33 patients; Placebo, 34 patients) and demonstrated a significant reduction in myocardial infarction size and a better recovery of regional systolic function, but no improvement in global function, at 4 months after implantation, with no complications [43]. Recently, the largest, randomized, placebo-controlled, multicenter study was performed using 204 patients (BMCs, 101 patients; Placebo, 103 patients) with AMI. This encouraging study revealed that the LV systolic function was significantly improved at 4 months after implantation compared with its pre-treatment value, and this improvement was better in the BMC group than in the placebo-control group, and significantly fewer adverse events were seen in the BMC group [44]. In contrast to the positive results obtained in other studies, ASTAMI, a randomized controlled study using 100 AMI patients (BMCs, 50 patients; Control, 50 patients) revealed no significant difference in the LVEF, end-diastolic volume, or infarct size at 6 months between the BMC implantation group and the control group, as measured by electrocardiogram-gated single-photon-emission computed tomography (SPECT), MRI, and echocardiography [45]. In summary, although there are some discrepancies in the efficacy in BMC implantation for the short-term results, its safety and feasibility were clearly established. Further study is needed to before we can assess the results of BMC implantation in the long term.

In several studies, BMMCs were transplanted via transendocardial injection into patients with chronic myocardial infarction (**Table 3**). In one prospective, nonrandomized, open-label study, BMMCs were delivered via NOGA catheter into patients with chronic ischemic heart disease (treatment, 14 patients; control, 7 patients). This study showed improvement of the ejection fraction (EF) and myocardial blood flow compared with the control [46], and at the 12-month follow-up, the BMMC-treated patients demonstrated better myocardial perfusion and exercise capacity [47]. Fuchs *et al.* implanted BMMCs into 10 patients with chronic ischemic heart disease without controls, and showed improvements in the coronary flow and angina score compared with the pre-treatment value, but no improvement of EF [48]. Tse *et al.* showed the improvement of symptoms, myocardial perfusion, and regional function, but no improvement in global systolic function at 3 months compared with the pre-treatment value in 8 patients after BMMC implantation. Recently, a randomized, single-center study using 92 patients with mild heart failure caused by chronic myocardial infarction (at least 3 months previously) was performed. Improved regional and global systolic function was seen in the BMC-implantation group after 3 months compared with controls treated with circulating blood cells, and with the pre-treatment values [49]. A previous report [50] demonstrated that circulating blood cells have the same potency as BMCs for treating AMI, but for chronic-phase myocardial infarction, the circulating blood cells have no effect on myocardial regeneration. A precise evaluation of the effect of BMMC implantation for chronic ischemic heart disease still awaits a multicenter, randomized, placebo-controlled, and double-blind study.

Abdel-Latif *et al.* performed a meta-analysis, which included eighteen studies and 999 patients with ischemic heart disease. They concluded that BMC transplantation has stable effects that include improving the EF, reducing the infarct scar size, and reducing the LV end-systolic volume in patients with AMI and chronic MI compared with controls [51]. In addition, a recent review by Kloner and colleagues demonstrated that the benefits of cell therapy were as good as the currently recommended therapies: reperfusion, beta blockers and ACE inhibitors [52].

Table 2. Clinical trials of bone marrow cell transplantation for acute myocardial infarction. PCI, percutaneous coronary intervention; BM-MNC, bone marrow mononuclear cell; IC, intracoronary; SVI, systolic volume index; SPECT, single photon emission computed tomography; FDG-PET, F-18-Fluorodeoxyglucose-Positron emission tomography.

Study	Study design	Concomitant TR	Number of cells	F.U.(mo)	Route	Results	Adverse effects
Strauer, 2002	Controlled, n = 10	PTCA	1.5 to 4 × 10^6	3	IC	infarct region↓, myocardial perfusion↑	
n = 10			BM-MNC			SVI, LVEDV↑, EF→	
						Regional systolic function↑	
TOPCARE-AMI	No control	PCI	2.1 × 10^8	12	IC	EF↑(8%; 4, 12mo), ESV↓(4mo),	No
2004, n = 29			BM-MNC			Infarct size↓(12mo), Regional function↑	
BOOST, 2004	Randomised	PCI	2.4 × 10^9	6	IC	EF↑(6.7%)	No
n = 30	Controlled, n = 30		BM-MNC				
Ruan, 2005	Randomised	PCI	~	6	IC	EF↑(6%), Regional contractility↑	
n = 9	Controlled, n = 11		BMC			ESV, EDV↓(vs control)	
Janssens, 2006	Randomised	PCI	4.8 × 10^8	4	IC	EF, Regional systolic function→	
n = 33	Placebo-control, n = 34		BM-MNC			Infarct size↓	
	Double-blind						
BOOST, 2006	Randomised	PCI	2.4 × 10^9	18	IC	EF, LV volume, Wall thickening→,	No
n = 30	Controlled, n = 30		BM-MNC			Regional function→, Faster recovery	
Schachinger, 2006	Randomised	PCI	2.4 × 10^8	4	IC	EF↑(5.5%), LV volume→	No
n = 101	Placebo-control, n = 103		BM-MNC			Regional function↑	
	multicenter						
Ge, 2006	Randomised	PCI	4.0 × 10^7	6	IC	EF↑(4.8%), LV volume→	
n = 10	Controlled, n = 10		BM-MNC			Myocardial perfusion defect↓(SPECT)	
Meluzin, 2006	Nonrandmized	PCI	HD; 10^8	3	IC	EF↑(HD; 5%, LD; 3%)	
n = 22(HD), 22(LD)	Controlled, n = 10		LD; 10^7			Myocardial perfusion defect↓(SPECT)	
			BM-MNC			Regional function↑	
Lunde, 2006	Randomised	PCI	8.7 × 107	6	IC	EF→(SPECT, US, MRI)	
n = 47	Controlled, n = 50		BM-MNC			LVEDV, infarct size→	
Meluzin, 2008	Nonrandmized	PCI	HD; 10^8	12	IC	EF↑(HD; 7%, LD; 4%)	
n = 20(HD), 20(LD)	Controlled, n = 20		LD; 10^7			ESV↓(HD vs Cntrol)	
			BM-MNC				
Bartunek, 2005	Controlled, n = 16	PCI	1.3 × 10^7	4	IC	EF↑(7.1%), Glucose uptake↑(FDG-PET)	Coronary events↑
n = 19			$CD133^+$ cells			LV regional chordae shortening↑	
						Myocardial perfusion defect↓(SPECT)	
Chen, 2004	Controlled, n = 35	PCI	6.0 × 10^{10}	6	IC	EF↑(18%), ESV, EDV↓	
n = 34			MSCs			Perfusion defect↓(PET)	

Table 3. Clinical trials of bone marrow cell transplantation for chronic myocardial infarction. BMC, bone marrow cell; MSCs, mesenchymal stem cells; EPCs, endothelial progenitor cells; IC, intracoronary; WMSI, wall motion score index; DSE, dobutamine stress echocardiography.

Study	Study design	Concomitant TR	Number of cells	F.U.(mo)	Route	Results	Adverse effects
Fuchs, 2003	No control	~	7.8×10^7	3	TE	Angina score↑	No
n = 10			BMC			EF→, Coronary flow after adenosine↑	
Tse, 2003	No control	PCI, CABG	40ml BM aspirated	3	TE	Regional wall motion↑, Wall thickness↑	No
n = 8			BM-MNC			EF→, myocardial perfusion↑	
Perin, 2003	Controlled, n = 7	~	2.5×10^7	4	TE	Symptoms↓, ESV↓, EF↑(5.5%),	1 sudden death
n = 14	Nonrandmized		BM-MNC			electromechanical function (NOGA)↑	
						Treadmill Vo2max→	
Perin, 2004	Controlled, n = 9	~	2.5×10^7	12	TE	Total reversible defect(SPECT)↓	
n = 11	Nonrandmized		BM-MNC			Treadmill Vo2max↑, EF→, Symptoms↓	
IACT study	Controlled, n = 18	PCI	9.0×10^7	3	IC	EF↑(8%), Infarction area↓,	
2005	Nonrandmized		BM-MNC			Treadmill Vo2max↑,	
n = 18						Glucose uptake↑, Regional contractility↑	
Assmus, 2006	Controlled, n = 23	~	2.0×10^8	3	IC	EF↑(2.9%)	
n = 28	Randmised		BM-MNC				
Hendrikx, 2006	Controlled, n = 10	CABG	6.0×10^7	4	IC	EF, LV volume→, Wall Thickening↑	
n = 10	Randmised		BM-MNC				
Mocini, 2006	Controlled, n = 18	CABG	2.9×10^8	3	IM	EF↑(5%), WMSI↓	
n = 18	Nonrandmized		BM-MNC				
Stamm, 2003	No control	CABG	$1.2\text{-}15.7 \times 10^5$	3 - 10	IM	EF↑(4/6)	No
n = 6			$CD133^+$ cells			Infarct tissue perfusion↑(5/6, SPECT)	
Ahmadi, 2007	Controlled, n = 9	CABG	~	6	IM	WMSI↓	
n = 18	Nonrandmized		$CD133^+$ cells			Infarct tissue perfusion and viability↑(SPECT)	
Stamm, 2007	Controlled, n = 21	CABG	6.0×10^6	6	IM	EF↑(9.7%)	No
n = 37			$CD133^+$ cells			Infarct tissue perfusion↑(11/20, SPECT)	
Chen, 2006	Controlled, n = 23	PCI	5.0×10^6	3	IC	EF↑(11%)	
n = 22			MSCs			Exercise tolerance↑, Symptoms↓	
Katritsis, 2005	Controlled, n = 11	PCI	3.0×10^6	4	IC	EF→, WMSI↓	
n = 11			MSCs + EPCs			No. of improved nonviable segments↑(DSE)	
						No. of segments with reversible iscemia	
						and viability↑(SPECT)	

CD133+ cells, which include hematopoietic stem cells, are reported to have angiogenic potential [53]. Because of this potential angiogenic capacity, CD133+ cell transplantation was tested clinically to treat ischemic myocardium. These cells were introduced into six patients who had old myocardial infarction (OMI) concomitant with coronary artery bypass grafting (CABG), and caused improved global systolic function and infarct tissue perfusion compared with the pre-treatment values [54]. However, this study had no control group. In their next clinical CD133+ cell transplantation study, these researchers reported that the combination of CABG and CD133+ cell transplantation showed significant improvement in LVEF and better perfusion of the infarcted myocardium compared with CABG alone, and that the procedure was safe [55]. For AMI, Bartunek *et al.* performed intracoronary CD133+ cell implantation in nineteen AMI patients and revealed a significant improvement in LVEF and reduction in the perfusion defect at 4 months, with an increased incidence of coronary events [56].

Regarding clinical MSC transplantation, two randomized and controlled studies have been reported. MSC transplantation was found to have efficacy for cardiac performance and exercise tolerance in both AMI [57] and OMI patients [58]. However, these analyses were incomplete, and a large study of appropriate design is still needed to clarify its effectiveness.

2.3. Other Clinical Studies in Cellular Cardiomyoplasty

While clinical applications using BMCs and myoblasts are still being developed, a second wave of clinical applications is underway using circulating progenitor cells (CPCs), CD34+ cells, MSCs, the combination of MSCs and endothelial progenitor cells, and the combination of BM-MNCs and myoblasts (**Table 4**).

Recently, several reports showed that bone-marrow derived CPCs, which were demonstrated to differentiate into endothelial progenitor cells (EPCs) [59], can promote neoangiogenesis in animal ischemic myocardium models [60]. These findings encouraged clinicians to apply CPC transplantation clinically. CPC transplantation has the great advantage of being less invasive in clinical settings, requiring just peripheral blood apheresis, in contrast to BMC and myoblast transplantation. Several protocols for CPC transplantation through intracoronary infusion have been reported, and they can be divided into treatments with granulocyte colony-stimulating factor (G-CSF) and those without. The TOPCARE-AMI Trial in 2004 for AMI patients without a control showed significant improvement in the LVEF, an attenuation of LV dilatation, and reduced infarct size at the one-year follow-up [50]. Tatsumi *et al.* performed an open-label, nonrandomized, controlled clinical trial for AMI patients treated with CPC implantation without using G-CSF and cell expansion, and revealed a significant increase in the LVEF and regional systolic performance at 6 months without any adverse clinical events [61]. The first randomized, double blind, and placebo-controlled study on CPC transplantation demonstrated an improvement in coronary flow reserve, decline in the number of hibernating segments, and an increase in LVEF at 3 months after transplantation, indicating that CPC transplantation was effective for OMI patients [62]. Contrary to this finding, The MAGIC cell-3DES randomized and controlled trial revealed that, although CPC transplantation had a positive impact on cardiac performance in AMI patients, it had no effect on OMI patients [63]. Assumus *et al.* supported these results in his paper [49]. Enriched EPCs (CD34+ stem cells) collected from the peripheral blood were transplanted into patients with intractable angina through transendocardial injection in a double-blind, randomized, placebo-controlled, and dose-escalating study, and revealed its safety and feasibility [57]. Although several clinical studies of CPC transplantation confirmed its safety and feasibility, a multicenter, randomized, placebo-controlled, and double-blind study is still needed for an accurate evaluation of its efficacy and long-term results.

Clinical cellular cardiomyoplasty as mentioned above is mainly used to cause angiogenesis rather than myogenesis, because the implanted cells have little capacity to differentiate into cardiomyocytes. Two studies reported the induction of both angiogenesis and myogenesis in the failing heart by the combined transplantation of angiogenesis- and myogenesis-inducing cells. A combination cell therapy using MSCs capable of differentiating into cardiomyocytes and EPCs that induce angiogenesis applied through intracoronary transplantation improved the regional systolic performance and regional blood perfusion but did not improve the global systolic function [64]. This result was disappointing, because this study aimed to induce both myogenesis and angiogenesis. The results may have been limited because MSCs have little ability to differentiate into cardiomyocytes, which was well supported by basic experiments, so the combined cell transplantation may induce only angiogenesis. Miyagawa *et al.* implanted both BMMCs and skeletal myoblasts into patients with severe heart failure caused by chronic ischemic heart disease under a left ventricular assist device (LVAD), and observed improvements in the global systolic and diastolic function in a series of LVAD-off tests [65]. This clinical therapy in which these two cell types were transplanted together into the ischemic myocardium probably enhanced the secretion of growth factors such as hepatocyte growth factor, rather than inducing angiogenesis and myogenesis [66].

Table 4. Clinical trials of circulating progenitor cell transplantation for heart failure. CPC, circulating progenitor cell; PBMNCs, peripheral bone marrow mononuclear cells; CFR, coronary flow reserve; LVEDP, left ventricle end diastolic pressure.

Study	Study design	Concomitant TR	Number of cells	F.U.(mo)	Route	Results
TOPCARE-AMI, 2002 n = 9(MNC), n = 11(CPC)	Randomised Controlled, n = 11	PCI	2.4×10^8(BM-MNC) 7.4×10^6($CD34^+$)	4	IC	EF↑(8.5%), ESV↓ Regional wall motion↑ CFR↑, myocardial viability↑(PET)
TOPCARE-AMI, 2004 n = 30	No control	PCI	5.5×10^6 ($CD34/CD45^+$)	4	IC	EF↑(8%), ESV↓, Infarct size↓
Erbs, 2005 n = 13	Randomised Double-blind placebo-controlled, n = 13 OMI	PCI	7.0×10^7 ($CD34^+$; 55%)	3	IC	EF↑(7.2%), CFR↑(PET) No. of hibernating segments↓(PET) Infarct size↓
Li, 2007 n = 35	Controlled, n = 35 AMI	PCI	7.3×10^7 CPC	6	IC	EF↑(7.1%), WMSI↓, ESV↓, EDV↓
Assmus, 2006 n = 24	Randomised Controlled, n = 23 OMI	~	2.2×10^7 CPC	3	IC	EF, Regional contactility→, ESV, EDV→, LVEDP→
Kang, 2006 n = 27(AMI), n = 20(OMI)	Controlled n = 29(AMI), n = 29(OMI)	PCI	1.4×10^9($CD34^+$; 9.3%)	6	IC	AMI; EF↑(5.1%), ESV↓ OMI; EF→, CFR↑
Losordo, 2007 n = 18	Randomised Double-blind placebo-controlled, n = 6 OMI	~	3.5×10^7($CD34^+$ Purified)	6	TE	not available
Tatsumi, 2007 n = 18	non Randamized Controlled, n = 36 AMI	PCI	5.0×10^9($CD34^+$; 0.12%) PBMNCs	6	IC	EF↑(13.4%), Regional EF↑ Perfusion defect ↓
Choi, 2007 n = 10	non Randamized Controlled, n = 32 AMI	PCI	2.0×10^9($CD34^+$; 3.1×10^6)	6	IC	EF→

2.4. Paracrine Effect of Cytokines after Cellular Cardiomyoplasty

Given that the incidence of stem-cell differentiation to cardiomyocytes is quite low, as described above, why does stem-cell transplantation show beneficial effects on heart failure? This discrepancy can be explained by the fact that these cells provide paracrine growth factors. Takahashi *et al.* reported that various cytokines (VEGF, IL-1beta, PDGF, and IGF-1) are highly detected in the supernatant of BM-MNCs under hypoxic conditions, and injection of the cytokine-rich supernatant into an AMI rat model increased the microvessel density, significantly improved in cardiac function, and inhibited cardiomyocyte apoptosis [67]. Uemura *et al.* reported that preconditioned BM stem cells that can secrete high levels of cell survival factors such as Akt and eNOS could prevent apoptosis in cardiomyocytes around the site of infarction [68]. Moreover, Kamihata *et al.* revealed that transplanted BM-MNCs can improve angiogenesis by secreting cytokines with angiogenic potential and by being incorporated into neocapillaries [69]. However, the angiogenesis promoted through the release of several cytokines, such as VEGF and bFGF, has a greater impact on the formation of neocapillaries than did the direct incorporation of cells into new vessels [70]. The bone marrow stromal cell conditioned medium, which includes arteriogenic cytokines, promoted the proliferation and migration of endothelial cells and smooth muscle cells [71]. Although cytokine release from transplanted cells is one of the mechanisms for myocardial regeneration in cellular cardiomyoplasty, the degree of their contribution to myocardial regeneration is not clearly known, and it may depend on the severity of the diseased myocardium.

Many researchers then sought to exploit this cytokine effect, and introduced the transplantation of cytokine-overexpressing cells to enhance the therapeutic effects of the original cells used in myocardial regeneration strategies. Askari *et al.* transplanted Stromal cell derived factor 1 (SDF-1)-overexpressing fibroblasts into an ischemic cardiomyopathy model, which enhanced stem-cell homing to the injured myocardium [72]. SDF-1 released from a myoblast sheet could mobilize CXCR4+ cells to the injured myocardium [2]. Moreover, stem-cell mobilization and homing therapy by G-CSF was introduced to another myocardial regeneration treatment as an alternative to cellular cardiomyoplasty [73,74].

If stem cells play only act as growth factor suppliers, allogenic stem cells that can supply growth factors to the diseased myocardium may regenerate the failing heart. Based on this theory, Imanishi *et al.* introduced allogenic MSCs, which have low immunogenicity [75], to treat acute myocardial infarction and observed that they triggered the secretion of vascular endothelial growth factor (VEGF) and preserved cardiac performance, accompanied by angiogenesis [76]. This strategy has the advantage of being applicable to emergent cases in clinical settings, but the possible disadvantage of having no effect on chronic heart failure. Some unique approaches using both myoblasts and BMCs to regenerate distressed myocardium have been reported. Memon *et al.* demonstrated that the implantation of both myoblasts and BMCs enhanced the secretion of growth factors and improved cardiac performance compared with each single-cell therapy [66].

2.5. Mechanisms of Myocardial Regeneration in Cellular Cardiomyoplasty in the Failing Heart

We can speculate that the following mechanisms underlie the myocardial regeneration induced by cellular cardiomyoplasty in the failing heart, but these pathways also affect each other, causing the mechanisms to be complicated (**Figure 2**).

1) Transplanted stem cells differentiate into cardiomyocytes, leading to improved regional systolic and diastolic function. However, this pathway is not crucial, because the incidence of transdifferentiation to cardiomyocytes is quite low.

2) Cytokines released from the transplanted stem cells have effects that promote healing.

- Angiogenic cytokines (VEGF, HGF) induce angiogenesis, which can supply blood and nutrition to the transplanted cells and ischemic host cardiomyocytes.
- Anti-cell-death cytokines (Akt, eNOS etc.) prevent the apoptosis of cardiomyocytes in infarcted and peri-infarcted regions.
- Stem-cell mobilizing cytokines (SDF-1 etc.) can induce stem-cell homing from the BM, and the migrated stem cells can become incorporated into capillaries.
- Antifibrotic cytokines may regulate the progression of fibrosis in the failing heart.

3) Transplanted stem cells are incorporated into capillaries by differentiating into endothelial cells and smooth muscle cells.

4) The thickening of the thin LV wall by newly supplied of elastic cells reduces wall tension, preserves LV geometry, and improves the elasticity in rigid scar tissue.

3. FUTURE PROSPECTS

3.1. Pluripotent Stem Cells

Although recently many reports depicted that stem cells such as c-kit positive [23], sca-1 positive cells [77], Human Amniotic Fluid Stem Cells (hAFSC) [78], Adipocyte Derived Stem Cells [79], Cardiospheres [80], or

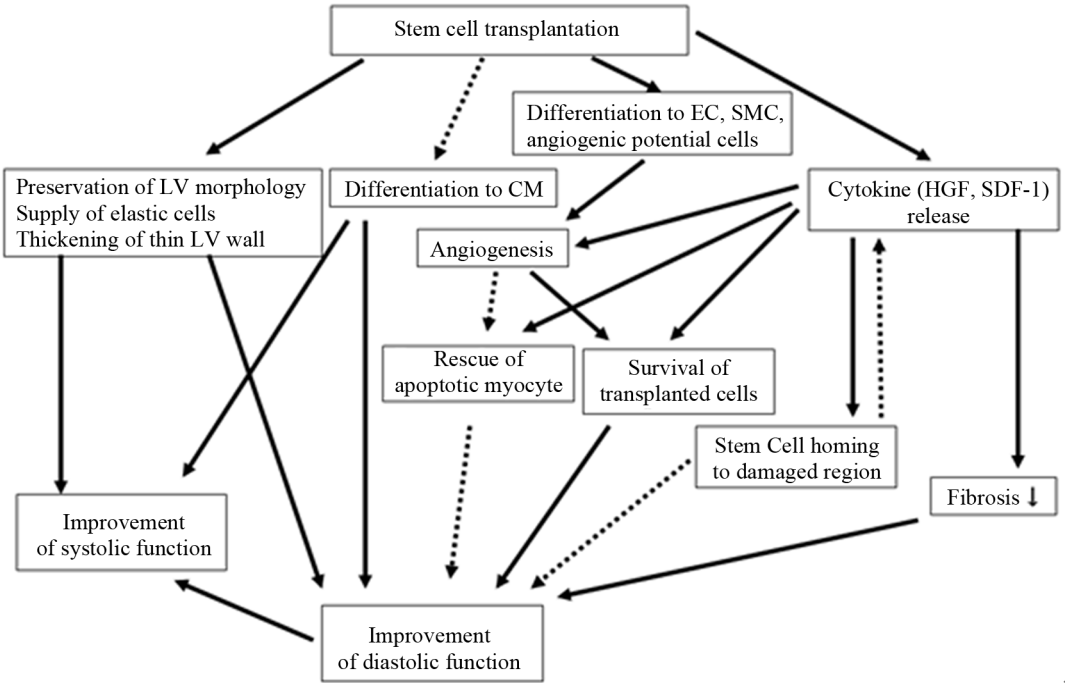

Figure 2. Mechanisms of stem cell therapy for heart failure. The differentiation of transplanted stem cells into cardiomyocytes may provoke the improvement of regional systolic and diastolic function. However, this mechanism has little impact on cardiac repair, because the incidence of transdifferentiation to cardiomyocytes is quite low. Cytokines released from the transplanted stem cells induce angiogenesis, which can support survival of the transplanted cells and reverse the ischemia in the failing heart, prevent cell death, and mobilize stem cells to induce cardiac repair. Some of the transplanted stem cells differentiate into smooth muscle cells and endothelial cells and become incorporated into capillaries. Moreover, the transplanted stem cells can increase elasticity and thickening in the rigid thin scar tissue, leading to improved diastolic function and the preservation of LV geometry. These pathways affect each other, causing the mechanisms to be complicated.

mecenchymal stem cells [81] has a capability of differentiation to cardiomyocyte and can express some structural proteins such as actin or troponin, these cells cannot contract spontaneously *in vitro* and it is unknown whether these cells have same micro structures as cardiomyocytes. If these cells are implanted to damaged myocardium, these cells may not offer contractile force directly to impaired heart but affect the cardiac performance probably by cytokine paracrine effect. Almost all reports concerning about cellular cardiomyoplasty showed improvement of cardiac performance and symptoms mainly via angiogenesis induced by angiogenic cytokines. But some groups introduced myogenic progenitor cells such as c-kit positive cells or spheroid cells and these cells are introduced to clinical trials. One exciting clinical protocol was reported that c-kit positive cells are transplanted to chronic ischemic myocardium via trans-catheter concomitant with bypass surgery and this cellular cardiomyoplasty significantly improved cardiac performance after cellular therapy. These c-kit positive cells demonstrated myogenic differentiation but differentiated cells only expressed some myogenic structural proteins and are unknown about synchronous contraction ability *in vitro* and *vivo*. Further studies are needed to elucidate whether these cardiomyogenic cells may impact on cardiac performance via paracrine effect or cardiomyogenesis.

So in regenerative medicine, stem cells which can contract spontaneously and have a capability of integration with myocardium via connexin 43 or cell-cell adhesive system. One of candidates in contractile cells may be Embryonic stem cells (ES cells) or Induced puluripotent stem (iPS) cells derived cardiomyocytes.

60 billion cells, which can differentiate over two hundred kind of cells, consist of Human body and these cells are components of tissues (skin, bone, and muscle) and organs (stomach, liver, and pancreas). Although, generally speaking, differentiation of cells in higher forms of life is irreversible, only a fertilized egg has a capability of puluripotency. ES cells are isolated from inner cell clusters which were recognized when a fertilized egg grows to germinal vesicle by repeated proliferation and this ES cells have capability of puluripotency [82].

The methods of differentiation to cardiomyocytes in ES cells have been already established and some papers reported its therapeutic effectiveness for heart failure [83]. But in the consideration of clinical application, many

drawbacks exists in this field. One of the problems is how to culture a large amount of cells in cell processing center without DNA damage. We speculate that over 10^8 cells are needed to repair the human damaged myocardium, but culture methods have not been examined in world wide. Second problem is how to avoid teratoma formation after implantation. There are no answers to elucidate these problems.

Although ES cells have ethical problem for clinical application, iPS cells have been developed to avoid ethical problems which ES cells have. Yamanaka reported that somatic stem cells which are transfected with four factors (Oct3/4, Sox2, Klf4, c-Myc) have a character of ES like pluripotent stem cells [84-86]. Same as ES cells, methods of differentiation to cardiomocytes have been already established and its therapeutic efficacy for heart failure has been reported [83]. But some problems are exist for clinical application as mentioned above in ES cells. As for iPS cells, some papers reported that cardiomyocytes differentiated from iPS cells are systematically selected form undifferentiated iPS cells by specific marker of mitochondria [87]. And another paper reported that T cell derived iPS cells can differentiate to cardiomyocytes and these cells might not change to teratoma after vivo implantation [88]. Many challenges have already been tried to avoid teratoma formation, but culture method of a large amount of cells has not been established. But special cell delivery methods such as cell sheet or combination with some angiogenic or antiapoptotic drugs might help to solve drawbacks of cell numbers which fail to improve cardiac function in the damaged heart [89]. Moreover retrovirus, trasnfection tool of 4 factors, may not be safe for clinical application. So safe transfection method of 4 factors may be needed for clinical application. To aim of clinical application, several papers reported that iPS cell derived cardiomyocyte sheet induced functional recovery in large animal [90] or small animal heart failure model [91] and these implanted cells were survived in myocardium. Proof of concept has already been proven in pre-clinical study, so clinical application of iPS cell derived cardiomyocytes will be started after verification of safety.

Recently Ieda *et al.* reported that fibroblasts can directly differentiate to cardiomyocytes with combination of several factors and with direct reprogramming [92]. This technology is innovated, but some issues (how about differentiation rate to cardiomyocytes? Do differentiated cardiomyocytes poses microstructure of native cardiomyocyte? etc.) should be elucidated.

3.2. Cell Delivery Methods

It is natural to discuss which cell source is best for the regeneration of damaged myocardium. However cell delivery method is also crucial for the cell therapy to enhance its therapeutic effects. Many papers reported that various kinds of cell delivery method, such as needle injection, trans coronary artery injection, trans coronary sinus injection, scaffolds containing cells, and cell sheet technique, have been reported to be effective to repair the damaged heart. Especially cell sheet implantation is reported to be superior to needle injection method in experimental study. In concerning of cell delivery methods, combination of some angiogenic drugs [93] or vascular rich tissue such as omentum with cells may be next step to enhance therapeutic effectiveness in cell based myocardial regeneration therapy [89].

Moreover repeated implantation of skeletal myoblast has a capability of the improvement of cardiac performance in a swine model of chronic myocardial infarction [94]. So in clinical setting repeated cell implantation may have some impacts on the enhancement of therapeutic effect in cell transplantation, but coronary artery embolism may be happen in this treatment.

4. CONCLUSIONS

We have summarized the recent advances in cellular cardiomyoplasty in both the laboratory and the clinic. Although many kinds of cells with the capacity to differentiate to cardiomyocytes have been reported, few stem cells can probably actually differentiate to cardiomyocytes. However, a cardiomyocyte cell source that is applicable to clinical settings is critical for treating severe heart failure, so researchers should continue to look for stem cells that can differentiate into cardiomyocytes.

We surveyed many encouraging clinical studies, which used many different kinds of cells, and found that almost all of the cell types had some effects on cardiac performance in the damaged heart. The important questions to ask when applying these cell-based therapies to clinical situations are:

1) Which cells have the greatest impact on myogenesis, angiogenesis, and cardiac performance?

2) Is a heterogeneous cell population or purified cell type better to regenerate the failing heart?

3) Which stage of heart failure can cellular cardiomyoplasty repair?

Genuine myocardial regeneration involves the induction of cardiomyogenesis and angiogenesis by smooth muscle cells and endothelial cells in the completely distressed myocardium. To reach this goal will require the discovery of new cells that can differentiate into cardiomyocytes, better cell delivery methods that enable as many functional cells as possible to reach the failing heart, and new ways to promote functional vasculogenesis.

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