Myocardial cell sheet therapy and cardiac function

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Fujita J, Itabashi Y, Seki T, Tohyama S, Tamura Y, Sano M, Fukuda K. Myocardial cell sheet therapy and cardiac function. Am J Physiol Heart Circ Physiol 303: H1169–H1182, 2012. First published September 21, 2012; doi:10.1152/ajpheart.00376.2012.—Heart failure (HF) is the leading cause of death in developed countries. Regenerative medicine has the potential to drastically improve treatment for advanced HF. Stem cell-based medicine has received attention as a promising candidate therapy over the past decade; however, it has not yet realized this potential in terms of reliability. The cell sheet is an innovative technology for constructing aligned graft cells, and several cell sources have been investigated for making a feasible cell sheet. The most representative thus far is skeletal myoblast, although such cells raise the issue of arrhythmogenicity. Regenerative cardiomyocytes (CMs) derived from pluripotent stem cells (PSCs), such as embryonic stem cells or induced PSCs, are the most promising, because a myocardial cell sheet (MCS) constructed with regenerative CMs can potentially enable contraction recovery and electromechanical coupling with host CMs. The functional outcomes of experimental MCS are reduction of ventricular wall stress and paracrine effects rather than contraction recovery. Several technical obstacles still hamper the clinical application of MCSs, with graft survival the most pivotal issue. Ischemia, apoptosis, inflammation, and immune response can all cause graft cell death, and a stable blood supply to the MCS is critical for successful engraftment. Ventricular tachycardia must also be considered in any myocardial cell therapy, and multiple layering of MCS (>3 layers) is necessary to reconstruct human myocardium. Innervation is also a potential issue. The future application of myocardial cell therapy with MCS for advanced HF depends on resolving these difficulties.

Myocardial cell sheet; cardiac function; pluripotent stem cells; skeletal myoblasts; graft cell survival

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Introduction

Heart failure (HF) is the most notorious disease in developed countries, with a prevalence reaching 1% of the population aged over 65 years (30). Survival rates and prognosis of patients with HF are as low as for cancer (3). In addition, HF patients who are resistant to conventional medical, surgical, and device therapy (cardiac resynchronization therapy) require a left ventricular assist device and/or cardiac transplantation (30). Even though left ventricular assist device success rates have improved recently, the device still gives rise to many problems including stroke, bleeding, and infection, and long-term prognosis is unknown (90). At present, cardiac transplantation is the only radical treatment for severe HF; however, only 4,000 patients worldwide are fortunate enough to receive cardiac transplantation (93). Innovative medical therapy for HF is therefore clearly needed to supplement or replace heart transplantation.

The idea of regenerative therapy became attractive in recent times as an alternative treatment for restoring lost cardiac function because of advanced HF. Various cell sources were investigated for myocardial cellular therapy (MCT) including bone marrow stem cells (BMSCs), skeletal myoblasts (SMs), and cardiac progenitor cells (CPCs). Clinical trials have been ongoing in the past decade (6, 51, 55, 72, 94), although the feasibility of MCT for severe HF remains unclear. MCT with human pluripotent stem cell-derived cardiomyocytes (hPSC-CMs), such as human embryonic stem cell-derived cardiomyocytes (hESC-CMs) or human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs), have not yet been used clinically for MCT, although they are potentially ideal to induce full functional recovery. The transplantation approach is the most critical factor in the successful clinical application of MCT, with three main methods conceived (Fig. 1).
15% of the transplanted CMs surviving (22, 103). To avoid aggregation and necrosis, some researchers have also developed cell transplantation methods using biosynthetic scaffold materials (7, 73). Moreover, it is necessary to transplant cells repeatedly to gain significant improvement in cardiac function. The current transplantation approaches with catheter and open-chest surgery (14) do not allow successful direct cell transplantation.

The application of innovative approaches such as pericardial endoscopy, which enables repeated cell deliveries if necessary (39), together with the use of effective biomaterials might improve the rate of successful transplantation of cells in host hearts (7, 73).

Three-dimensional scaffolds. Since Langer and Vacanti (44) addressed the idea of “tissue engineering,” biological materials comprising a mixture of tissues with cells and matrix were developed in the attempt to reconstitute various organs. The three-dimensional (3-D) reconstruction of myocardium is a challenging issue for tissue engineering applications in the field of cardiovascular therapy. As with injectable materials, a lot of biomaterials to construct 3-D scaffolds are now available for regenerative therapy (7, 73). However, it is difficult to align donor CMs in scaffolds and create dense myocardial tissue. Long-term effects such as inflammation, foreign body reaction, and the arrhythmic potential of transplanted biomaterials must be evaluated before clinical applications can be fully realized (7).

Cell sheets. Cell sheet technology is an attractive alternative for treating advanced HF, especially after myocardial infarction (MI) that has progressed significantly over the past two decades. Myocardial cell sheet (MCS) technology enables construction of myocardial tissue with correctly aligned CMs. In vivo studies have shown that MCSs recover contraction, strengthen the infarcted wall, enhance vasculogenesis, and decrease fibrosis (Table 1). Even though several issues remain to be addressed before clinical applications of MCSs are feasible, it has strong potential as a novel radical treatment for advanced HF. This review focuses on current topics using MCSs for myocardial regenerative therapy.

Cell Sheet Engineering

Temperature-responsive culture dish. Okano and colleagues (37, 66, 84) developed a way to generate 3-D myocardial tissues without a scaffold by layering two-dimensional cell sheets (Fig. 2A). To do this, temperature-sensitive culture dishes were created by grafting temperature-responsive polymer (poly-N-isopropylacrylamide) onto the surface of the dish (66). At 37°C, the polymer surface is hydrophobic and cells can attach onto the dishes. When the temperature is dropped below 32°C, the surface becomes hydrophilic and the grafted polymer rapidly hydrates, making it expand and causing the cells to detach from the surface. When compared with enzymatic digestion of a scaffold, which often disrupts the cell-cell contacts, this innovative technology enables a cell sheet to be generated just by cooling the dishes to room temperature and using multiple layered cell sheets (20).

Polymerized fibrin-coated culture dish. Fibrin patches with mesenchymal stem cells (MSCs) or hESC-derived vascular cells have been successfully used in MCT (49, 106). Itabashi and colleagues (27) developed a novel and simple method for a functional MCS using thin, biodegradable, polymerized fibrin-coated dishes. Culture dishes were coated with fibrinogen monomers mixed with thrombin and stored at 4°C. Rat neonatal CMs were seeded onto the fibrin polymer, which was then degraded within 4 days by proteases secreted from the CMs (Fig. 2B). The MCS could be detached from the dish with a cell scraper. This invention also enables the generation of triple-layered MCSs with well-organized CMs. When these MCSs were overlapped, they connected to each other through connexin 43, and conducted action potentials (APs) were recorded via an electro-optical mapping system. MCSs generated using this method and transplanted onto subcutaneous areas of nude rats beat spontaneously. A fibrin-based cell sheet with SMs was reportedly also effective for myocardial repair after MI (18).

Cell Sources for Cell Sheets

Several cell sources have been tested for cell sheet construction including fibroblasts, endothelial cells, MSCs, SMs, and CMs (Table 1). For clinical applications, BMSCs, SMs, adipose tissue-derived cells, CPCs, and PSC-CMs are potential candidates for cell sheet construction (Fig. 3), with each cell type offering unique beneficial characteristics.

Fig. 1. Transplantation approach for myocardial cell therapy. Applications of myocardial regenerative therapy comprise 3 major transplantation methods: cell transplantation in situ with or without biopolymers (left), 3-dimensional biological scaffolds (middle), and cell sheets (right).
<table>
<thead>
<tr>
<th>Species</th>
<th>Model</th>
<th>Cell Type</th>
<th>Systolic Function</th>
<th>Diastolic Function</th>
<th>LV Diastolic Chamber Volume</th>
<th>Wall Thickness</th>
<th>Neovasculization</th>
<th>Fibrosis</th>
<th>Functional Assessment</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat MI</td>
<td>Fibroblast</td>
<td>↑</td>
<td>–</td>
<td>→</td>
<td>↑</td>
<td>↑*</td>
<td>↓*</td>
<td>Echo</td>
<td>(41)</td>
<td></td>
</tr>
<tr>
<td>Rat MI</td>
<td>Fibroblast + EPC</td>
<td>↑*</td>
<td>–</td>
<td>→</td>
<td>↑*</td>
<td>↑*</td>
<td>↓*</td>
<td>Echo</td>
<td>(54)</td>
<td></td>
</tr>
<tr>
<td>Rat MI</td>
<td>MSC</td>
<td>↑*</td>
<td>↑*</td>
<td>→</td>
<td>↑*</td>
<td>↑*</td>
<td>–</td>
<td>Hemo &amp; Echo</td>
<td>(60)</td>
<td></td>
</tr>
<tr>
<td>Rat MI</td>
<td>MSC</td>
<td>↑*</td>
<td>–</td>
<td>→</td>
<td>↑*</td>
<td>↑*</td>
<td>↓*</td>
<td>Echo</td>
<td>(109)</td>
<td></td>
</tr>
<tr>
<td>Rat MI</td>
<td>MSC</td>
<td>→</td>
<td>–</td>
<td>→</td>
<td>↑*</td>
<td>–</td>
<td>–</td>
<td>Echo</td>
<td>(67)</td>
<td></td>
</tr>
<tr>
<td>Rat MI</td>
<td>Cardiomyoblast</td>
<td>↑*</td>
<td>–</td>
<td>→</td>
<td>↑*</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Rat MI</td>
<td>Myoblast</td>
<td>↑*</td>
<td>–</td>
<td>→</td>
<td>↑*</td>
<td>↑*</td>
<td>→</td>
<td>Echo</td>
<td>(79)</td>
<td></td>
</tr>
<tr>
<td>Rat MI</td>
<td>Myoblast</td>
<td>↑*</td>
<td>–</td>
<td>→</td>
<td>↑*</td>
<td>↑*</td>
<td>↓*</td>
<td>Echo</td>
<td>(58)</td>
<td></td>
</tr>
<tr>
<td>Rat MI</td>
<td>Myoblast</td>
<td>→</td>
<td>–</td>
<td>→</td>
<td>↑*</td>
<td>↓*</td>
<td>→</td>
<td>Echo</td>
<td>(40)</td>
<td></td>
</tr>
<tr>
<td>Rat MI</td>
<td>Myoblast + Bcl-2</td>
<td>↑*</td>
<td>–</td>
<td>→</td>
<td>↑*</td>
<td>↑*</td>
<td>–</td>
<td>Echo</td>
<td>(87)</td>
<td></td>
</tr>
<tr>
<td>Swine MI</td>
<td>Myoblast</td>
<td>↑*</td>
<td>–</td>
<td>→</td>
<td>↑*</td>
<td>↑*</td>
<td>→</td>
<td>Echo</td>
<td>(85)</td>
<td></td>
</tr>
<tr>
<td>Rat MI</td>
<td>Myoblast</td>
<td>→</td>
<td>–</td>
<td>→</td>
<td>↑*</td>
<td>↑*</td>
<td>→</td>
<td>Echo</td>
<td>(86)</td>
<td></td>
</tr>
<tr>
<td>Monkey MI &amp; reperfusion</td>
<td>ESC-CPC + ADSC</td>
<td>↑*</td>
<td>–</td>
<td>→</td>
<td>↑*</td>
<td>↑*</td>
<td>→</td>
<td>Echo</td>
<td>(4)</td>
<td></td>
</tr>
<tr>
<td>Rat MI</td>
<td>Rat cardiomyocytes</td>
<td>↑*</td>
<td>–</td>
<td>→</td>
<td>↑*</td>
<td>↑*</td>
<td>→</td>
<td>Echo</td>
<td>(108)</td>
<td></td>
</tr>
<tr>
<td>Rat MI</td>
<td>Human cardiomyocytes</td>
<td>↑*</td>
<td>–</td>
<td>→</td>
<td>↑*</td>
<td>↑*</td>
<td>→</td>
<td>Echo</td>
<td>(76)</td>
<td></td>
</tr>
<tr>
<td>Rat MI</td>
<td>Rat CM</td>
<td>↑*</td>
<td>–</td>
<td>→</td>
<td>↑*</td>
<td>↑*</td>
<td>→</td>
<td>Echo</td>
<td>(53)</td>
<td></td>
</tr>
<tr>
<td>Rat MI</td>
<td>ESC-CM + EC</td>
<td>↑*</td>
<td>–</td>
<td>→</td>
<td>↑*</td>
<td>↑*</td>
<td>→</td>
<td>Echo</td>
<td>(77)</td>
<td></td>
</tr>
</tbody>
</table>

MI, myocardial infarction; HF, heart failure; DCM, dilated cardiomyopathy; HGF, hepatocyte growth factor; PH, pulmonary hypertension; EPC, endothelial progenitor cell; MSC, mesenchymal stem cell; CM, cardiomyocyte; EC, endothelial cell; MC, mural cell; LV, left ventricle; RV, right ventricle; CPC, cardiac progenitor cell; ADSC, adipose-derived stromal cell; OP, omentopexy; ESC-, embryonic stem cell derived; Echo, echocardiography; Hemo, hemodynamic study. *Statistically significant.
Bone marrow stem cells and adipose tissue-derived cells. Clinical trials have shown that the direct injection of BMSCs is safe but not highly effective because only a small fraction of cells contributed to myocardial repair (94) (Table 2). Adipose-derived stem cells (ADSCs) and adipocytes can be used to construct cell sheets that improve left ventricular function (19, 26). Adipose tissue-derived cells therefore have potential as a cell source for the construction of cell sheets designed to ameliorate cardiac dysfunction. MSCs are found as a population of cells among BMSCs or ADSCs and have the potential to differentiate into CMs (17, 32, 50). Therefore, MSCs are an ideal candidate for the generation of cell sheets (60, 109).

Cardiac progenitor cells. It has been generally thought that heart cells do not proliferate or regenerate after birth; however, recent studies provided evidence for the existence of CPCs (5, 65, 91, 102). Such cells could be used to make MCS (108), particularly if they divide and grow following transplantation. A disadvantage of this approach is the need for open-chest

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**Fig. 3.** Cell sources for cell sheets. The main candidate cell sources of cell sheets are bone marrow stem cells, adipose tissue-derived cells, cardiac progenitor cells, skeletal myoblasts (SMs), and pluripotent stem cell-derived CMs.
### Table 2. Experimental studies with direct cell injection

<table>
<thead>
<tr>
<th>Species</th>
<th>Model</th>
<th>Cell Type</th>
<th>Cell Number $\times 10^5$ (CM)</th>
<th>Systolic Function</th>
<th>Diastolic Function</th>
<th>LV Diastolic Chamber Function</th>
<th>Functional Assessment</th>
<th>Newvasculization</th>
<th>Fibrosis</th>
<th>Graft Survival, %</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>MI</td>
<td>BMC</td>
<td>0.015–0.1</td>
<td>*</td>
<td>–</td>
<td>–</td>
<td>Hemo (68)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>(8)</td>
</tr>
<tr>
<td>Rat</td>
<td>MI &amp; reperfusion</td>
<td>Myoblast</td>
<td>1</td>
<td>*–</td>
<td>1</td>
<td>1</td>
<td>–</td>
<td>Echo (29)</td>
<td>–</td>
<td>–</td>
<td>(8)</td>
</tr>
<tr>
<td>Sheep</td>
<td>MI</td>
<td>Myoblast</td>
<td>–</td>
<td>*</td>
<td>–</td>
<td>2</td>
<td>Echo (15)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>(10)</td>
</tr>
<tr>
<td>Mouse</td>
<td>MI</td>
<td>hESC-CM</td>
<td>20–25</td>
<td>–</td>
<td>–</td>
<td>2.3 (total), 10.6 (CM)</td>
<td>MRI</td>
<td>Echo (103)</td>
<td>–</td>
<td>–</td>
<td>(103)</td>
</tr>
<tr>
<td>Rat</td>
<td>MI</td>
<td>Myoblast</td>
<td>2.3</td>
<td>*</td>
<td>–</td>
<td>–</td>
<td>Echo (71)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>(8)</td>
</tr>
<tr>
<td>Rat</td>
<td>MI</td>
<td>BMC</td>
<td>10</td>
<td>*</td>
<td>1</td>
<td>0.07</td>
<td>Echo (11)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>(8)</td>
</tr>
<tr>
<td>Mouse</td>
<td>MI</td>
<td>hESC-CM</td>
<td>1 (20–25)</td>
<td>*</td>
<td>–</td>
<td>0.08</td>
<td>Echo (106)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>(8)</td>
</tr>
</tbody>
</table>

| BMC, bone marrow cell | hESC-CM, human embryonic stem-derived cardiomyocyte | *Statistically significant. |

BMC, bone marrow cell; hESC-CM: human embryonic stem cell-derived cardiomyocyte. *Statistically significant.

Many studies on rat neonatal CMs have shown that CMs are the most ideal cell source for the construction of MCS (Table 1). The MCS generated with CMs improve cardiac contraction, connect with host CMs via gap junction, and show low arrhythmic potential in vivo and in vitro (12, 28, 95). The critical issue for the therapeutic application of this approach is being able to isolate or derive human CMs for clinical applications. In 1998, Thompson and colleagues (101) revealed that hESCs were true PSCs. In 2007, hiPSCs were generated by transfecting fibroblasts with pluripotency-associated genes (97, 107). These hiPSCs have the potential to develop into functional CMs (34, 98, 110, 113). hiPSC-derived CMs could be the most feasible cell source for MCSs with the added advantage that immunosuppressive therapy and ethical issues are not limiting factors. A disadvantage of hiPSC technology is the possibility of introducing genetic and epigenetic mutations during their culture (16, 25, 48). Therefore, we need to be cautious about the quality of hiPSC populations for clinical use. hiPSC-CMs appear to be a good candidate for MCS construction; however, for the successful transplantation of MCSs made with PSC-CMs, some critical issues need to be overcome, such as teratoma formation, sufficient cell number to restore cardiac function, and effective engraftment. If these issues are addressed, regenerative hiPSC-CMs could be an ideal cell source for the generation of MCSs.

### Function of MCSs

MCS and other such cell sheets can strengthen the infarcted wall in the acute phase of MI and can contribute to cardiac function in the chronic phase, especially systolic function and possibly also diastolic function (Fig. 5). However, most previous studies have been done using small animals with observation periods of <3 mo (Table 1). The functional
The relevance of cell sheets and the direct injection approach is summarized in Tables 1–3. Based on results from a number of studies (Table 2), clinical trials focusing on the direct injection approach have been conducted (Table 3). These clinical trials found that myoblasts can trigger fatal arrhythmia, that BMSCs were safe, and that the overall improvement of cardiac function with BMSCs was not great. CPCs were revealed to be effective in phase 1 clinical trials, and further larger trials employing CPCs are currently underway.

For effective clinical application of cell sheets, it is necessary to provide evidence of significant functional improvement.
with large animal models (canine or swine) over the long term. It is also essential to clarify how the transplanted cells improve cardiac function (70, 72).

**Myocardial contraction and electromechanical coupling.** It is hoped that transplanted cell sheets function to restore cardiac function by aiding contraction recovery. MCSs contract synchronously in subcutaneous tissue as well as in the heart (27, 84). Electromechanical conduction contributes to functional improvement. The importance of synchronous contraction is well known from clinical experience of cardiac resynchroni-
Review

CARDIAC REGENERATIVE THERAPY WITH MYOCARDIAL CELL SHEETS

Table 3. Clinical studies with direct cell injection

<table>
<thead>
<tr>
<th>Disease</th>
<th>Cell Type</th>
<th>Cell Number × 10^6</th>
<th>Systolic Function</th>
<th>LV Diastolic Chamber Volume</th>
<th>Functional Assessment</th>
<th>Other Remarks</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICM</td>
<td>Myoblast</td>
<td>25–405</td>
<td>→</td>
<td>→</td>
<td>Echo</td>
<td>N = 86, Arrhythmogenicity (104)</td>
<td></td>
</tr>
<tr>
<td>ICM</td>
<td>Myoblast</td>
<td>400 or 800</td>
<td>→</td>
<td>↑ *(−12.6 ml/m²)</td>
<td>Echo</td>
<td>N = 42, Arrhythmogenicity (55)</td>
<td></td>
</tr>
<tr>
<td>AMI or ICM</td>
<td>BMC or PMC</td>
<td>2–292</td>
<td>↑ *(3.67%)</td>
<td>↑ *(−1.92 ml)</td>
<td>MRI, SPECT, Ang</td>
<td>Meta analysis, 18 studies, N = 999 (1)</td>
<td></td>
</tr>
<tr>
<td>Recent AMI</td>
<td>BMC or PMC</td>
<td>46–2460</td>
<td>↑ *(2.97%)</td>
<td>↑ *(−4.62 ml)</td>
<td>MRI, SPECT, Ang</td>
<td>Meta analysis, 10 studies, N = 698 (47)</td>
<td></td>
</tr>
<tr>
<td>ICM</td>
<td>CPC</td>
<td>0.5 or 1</td>
<td>↑ *(12.3 units)</td>
<td>→</td>
<td>Echo, MRI</td>
<td>N = 23</td>
<td>(6)</td>
</tr>
<tr>
<td>ICM</td>
<td>Cardiosphere</td>
<td>12.5 or 17.3 or 25</td>
<td>↑ *(−7.2 ml)</td>
<td>→</td>
<td>MRI</td>
<td>N = 25</td>
<td>(51)</td>
</tr>
</tbody>
</table>

ICM, ischemic cardiomyopathy; AMI, acute myocardial infarction; PMC, peripheral mononuclear cell; Ang, Angiography. *Statistically significant.

zation therapy (61). In addition, Sekine and colleagues (78) reported that transdifferentiated mesothelial cells in the epicardium formed a bridge between a host heart and a layer of MCSs, suggesting that synchronized beating could occur between the grafted CMs and the host CMs. For effective cardiac output, electromechanical coupling between the donor CMs and host heart is a key factor in effective regenerative therapy (12). The recovery of cardiac function might also involve preventing remodeling, new vessel formation, and paracrine effects (99, 105).

*VENTRICAL WALL THICKNESS.* Even though direct myocardial contraction was not gained, reinforcement of infarcted areas showed a functional advantage, especially in preventing ventricular remodeling (36). MCSs can strengthen the ventricular wall compared with the thinner fibrotic scar after MI, so that wall stress will be reduced and ventricular remodeling will be suppressed (Fig. 5A). However, affecting only thickness of the ventricular wall without other effects may not be helpful in restoring pump function in the chronic phase.

*Paracrine effects and neovascularization.* Numerous reports suggested that paracrine effects have a major influence on the functional success of cell sheets (Table 1). The inflammation and secreted cytokines induced by the transplanted cells stimulate host tissues to promote neovascularization and the differentiation of cardiac progenitors. The transplantation of fibrin patch with hESCs-vascular cells was reported to recruit endogenous CPCs effectively (106). Endogenous CPCs can contribute to the improvement of cardiac function in MCS therapy, especially with fibrin-based MCSs. The coculture of endothelial cells with MCSs also improved cardiac function of ischemic cardiomyopathy (77), and MCSs could also contribute to vascular formation (80). Masumoto and colleagues (53) reported that 4 wk after MCS transplantation, there were few CMs remaining but numerous newly formed vascular networks derived from the host tissue and promoted by cytokine effects. This observation suggests that the induction of new vascular networks could be a positive side effect of MCS therapy. The combination of functional CMs and effective cytokines to promote angiogenesis may be an attractive method in cardiac regenerative therapy (86).

**Survival of Transplanted Cardiomyocytes.** A low rate of cell survival is a potential bottleneck for MCS therapy. The survival rate of graft cells transplanted by direct injection is generally low (Table 2). It is reported that grafted CMs in MCSs survive longer than directly injected CMs (76). Shimizu and colleagues (82) reported survival of MCSs up to 24 mo after transplantation to subcutaneous tissues. However, it was also reported that most of the grafted cells in cell sheets do not survive for long in host hearts after cell sheet transplantation (53, 75, 95) (Fig. 6). Many graft cells die within 8 wk of transplantation, supposedly because of ischemia, apoptosis, inflammation, and immunological rejection (Fig. 6). Several issues remain unclear in terms of improving graft survival, and studies into avoiding cell death are necessary to achieve improvements in MCS therapy.

**Blood supply.** It is essential to supply enough blood to MCSs to prevent graft ischemia. The adult heart ventricle consists of multilayered myocardium. In general, when there are more than three layers of cell sheets, they become resistant to new blood supplies and thus susceptible to necrosis (21). Repeated transplantation of triple-layered MCSs in subcutaneous tissue is reported to produce thick and vascularized myocardial tissues 2 wk after transplantation (83). Furthermore, polysurgery for a heart is difficult, and epicardium is an obstacle to vascularization. The development of newly formed vessels from coronary arteries or myocardial tissue in situ was limited after transplantation of MCSs, subsequently hindering the functional survival of thick MCSs. A possible solution is cytokine overexpression with MCSs (86). Alternatively, graft-derived endothelial cells created vessels in the fusion with host endothelial cells at the border between the host dorsal subcutaneous tissue and graft cells (77). Nevertheless, it is highly possible that epicardium prevents vascular network formation between the host CMs and MCSs. Furthermore, arteriogenesis may be necessary to use newly formed vessels effectively (52). Coronary artery bypass graft may be the answer. However, sometimes patients with advanced HF due to ischemic cardiomyopathy have both damaged coronary arteries and damaged graft vessels, suggesting that the blood supply from the graft vessels could not penetrate the MCSs. Omentum (OM) is an ideal vascular source to supply oxygen and nutrition that is sometimes resected in general abdominal surgery without causing major problems. The OM could also act as a source of angiogenetic factors in MCSs (112). Kanamori and colleagues (31) used omentopexy (OP) with bone marrow-derived mononuclear cells for MCT. OP also played a role as a source of capillary vessels and large arterioles, while Suzuki and colleagues (95) proved that this method could supply enough blood to transplanted MCSs in vivo (Fig. 7). The combination of MCSs with OP drastically increased the blood supply through enhanced angiogenesis and migration of small arteries into the MCS and also significantly improved graft survival, resulting in improved cardiac function. The effectiveness of OP was also indicated in a large animal model (85), whereas a combination of OP and growth factors effectively induced arteriogenesis and increased collateral arteries (96). The combination of MCSs with OP and cytokines may be the best choice to improve blood supply for future MCS therapy.
Apoptosis and prosurvival factors. CM grafting is limited by apoptosis, which can be caused by ischemia, acute inflammation, and loss of matrix (62). The Murry group (111) cited the first 4 days as the most critical period for CM death after graft transplantation by direct injection. They found that activating the Akt pathway and heat shock before transplantation reduced the graft cell death. They also reported that using a prosurvival cocktail (Matrigel, a peptide from Bcl-XL, cyclosporine A, ATP-dependent K⁺ channel opener, insulin-like growth factor-1, and caspase inhibitor) prevented apoptosis of transplanted cells and restored cardiac function (43). Bcl-2 overexpression was also reported to prevent graft apoptosis in SM sheets (40, 87). It is possible that a prosurvival cocktail together with antiapoptotic agents could improve graft survival.

Acute inflammation. In the first few days (inflammatory phase) after MI, many cytokines/chemokines including interleukin-1, tumor necrosis factor-α, are released from dead CMs and neutrophils. The healing process subsequently progresses to the proliferative phase with multiple inhibitory pathways, including transforming growth factor-β and interleukin-10, and then the healing phase (9, 46). It may therefore not be feasible to transplant MCSs into an infarcted area during the acute period, when the inflammation is very strong, and, in turn, anti-inflammation strategies may contribute to the graft survival. However, some inflammation and cytokines promote neovascularization and may give a benefit to the engraftment (23, 88). On the other hand, the effect of MCS therapy must be reduced at the chronic phase after ventricular remodeling progresses. Therefore, the proper timing of transplantation is important for achieving successful MCS therapy.

Immunosuppressive therapy. Immunological rejection is also an important factor affecting the success of MCT. Using patient-derived iPSCs as cell sources should remove the need to consider this issue. However, it takes some time to establish patient-derived iPSCs, and their quality might not always be sufficiently high or reliable for cell therapy. In such cases, human leukocyte antigen-matched iPSCs or ESCs cell banks would be of value (64). In other cases, immunosuppressive therapy will be needed to avoid tissue rejection. Now only a few immunosuppressive drugs are available clinically (93), and such an approach would necessitate better immunosuppressive therapy or the development of new types of immunosuppressive drugs.

Further Considerations for MCS Therapy

Arrhythmia. Fatal arrhythmias such as ventricular tachycardia/ventricular fibrillation can lead to sudden cardiac death. In the current medical setting, an implantable cardioverter defibrillator is applied to high-risk patients to prevent sudden cardiac death. It remains controversial whether MCSs induce arrhythmia. It has been reported that MCS connect with the host heart and propagate APs without arrhythmia ex vivo (12). hESC-CMs could connect with each other via connexin 43-positive gap junctions to beat synchronously and contract together with the host heart (13, 33, 35). It was also reported that hESC-CMs could also repair ventricular tachycardia if they were transplanted in slow conduction areas of reentrant circuit (10, 81, 100). These data support the safety of MCSs, and using PSC-CMs would further reduce the risk of fatal arrhythmia. However, native cardiac tissues consist of 3-D-thick myocardium, and careful monitoring for such complications would still be necessary for clinical application.

Innervation. The heart is innervated via the autonomic nervous system, and cardiac innervation density is changed after MI. This causes sympathetic nerve sprouting and unbalanced innervation, which results in lethal arrhythmia (38). Regional denervation of MCSs could cause arrhythmia and graft damage even if the transplanted CM has comparable...
Fig. 7. Omentopexy to supply oxygen and nutrition to MCSs. A: Omental flap is applied to MCSs over the infarcted area to support sufficient blood delivery to the grafted MCSs. B: Proportion of α-actinin-positive CMs (alive CMs) in green fluorescent protein-positive cells (grafted CMs) increased to the additional 15% with omentopexy (OP) at 8 wk. Scale bars are 100 μm. OM, omentum. Modified figures are from Suzuki et al. (95).
functional and electrophysiological properties to the host heart. To fully understand these issues, it is necessary to monitor the patients closely in the clinical setting.

Concluding Remarks
Replacing lost myocardium with regenerative CMs and supportive materials would be the ideal regenerative therapy following HF. This century has already seen numerous studies into regenerative cardiac medicine; however, regenerative therapies are yet to become a standard treatment for HF. We have strong evidence that hPSC-CMs are more suitable than other sources for cellular therapy and that MCS generation is a useful transplantation method of regenerative CMs in advanced HF, especially after MI. In vivo survival of donor CMs in multilayered MCSs and functional engraftment to a host heart are key factors for the success of MCS therapy. The realization of regenerative medicine depends on breakthroughs that overcome these bottlenecks, but we believe that MCS remains a realistic future tool for cardiac cellular therapy.

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AUTHOR CONTRIBUTIONS

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CARDIAC REGENERATIVE THERAPY WITH MYOCARDIAL CELL SHEETS

Review

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