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CLINICAL PERSPECTIVE

Transplantation of stem or progenitor cells has the potential to improve and restore cardiac function. To date, experimenters investigating the possible therapeutic effects of stem cells in the heart have used models of infarction, and little information is available about the therapeutic potential of cell transplantation for heart failure due to dilated cardiomyopathy. In the present study, we demonstrated that transplantation of stem cells improved cardiac function in a model of myocarditis. We found evidence that stem cells may work to improve heart function by both myogenesis and angiogenesis while inhibiting myocardial fibrosis. Based on our data, part of the mechanism for this improvement may occur through the action of stem cells as a source of growth factors and cytokines in the heart. This study supports the overall notion that mesenchymal stem cells transplanted into the failing heart have potential as a new therapeutic strategy for the treatment of dilated cardiomyopathy.

Monolayered mesenchymal stem cells repair scarred myocardium after myocardial infarction

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Mesenchymal stem cells are multipotent cells that can differentiate into cardiomyocytes and vascular endothelial cells. Here we show, using cell sheet technology, that monolayered mesenchymal stem cells have multipotent and self-propagating properties after transplantation into infarcted rat hearts. We cultured adipose tissue-derived mesenchymal stem cells characterized by flow cytometry using temperature-responsive culture dishes. Four weeks after coronary ligation, we transplanted the monolayered mesenchymal stem cells onto the scarred myocardium. After transplantation, the engrafted sheet gradually grew to form a thick stratum that included newly formed vessels, undifferentiated cells and few cardiomyocytes. The mesenchymal stem cell sheet also acted through paracrine pathways to trigger angiogenesis. Unlike a fibroblast cell sheet, the monolayered mesenchymal stem cells reversed wall thinning in the scar area and improved cardiac function in rats with myocardial infarction. Thus, transplantation of monolayered mesenchymal stem cells may be a new therapeutic strategy for cardiac tissue regeneration.

Myocardial infarction, a main cause of heart failure, leads to loss of cardiac tissue and impairment of left ventricular function. Therefore, restoring the scarred myocardium is desirable for the treatment of heart failure. Although needle injections of bone marrow cells into the myocardium have been performed for cardiac regeneration^{1–5}, it is difficult to reconstruct sufficient cardiac mass in the thinned scar area after myocardial infarction.

Recently, our colleagues have developed cell sheets using temperature-responsive culture dishes⁶. These cell sheets allow for cell-to-cell connections and maintain the presence of adhesion proteins because enzymatic digestion is not needed^{7–10}. Therefore, cell sheet transplantation may be a promising strategy for partial cardiac tissue reconstruction. Skeletal myoblasts, fetal cardiomyocytes and embryonic stem cells have been considered as candidates for an implantable cell

source^{11–13}. It is difficult, however, to produce a multilayered construct requiring a vascular network. Thus, autologous somatic stem cells with self-propagating properties that can induce angiogenesis are a desirable cell source for a transplantable sheet.

Mesenchymal stem cells (MSCs) are multipotent adult stem cells that reside within the bone marrow microenvironment^{14,15}. MSCs can differentiate not only into osteoblasts, chondrocytes, neurons and skeletal muscle cells, but also into vascular endothelial cells¹⁶ and cardiomyocytes^{17–20}. In contrast to their hematopoietic counterparts, MSCs are adherent and can expand in culture. Recently, MSCs have been isolated from adipose tissue^{21–24}, which is typically abundant in individuals with cardiovascular disease. Here, we investigated the therapeutic potency of monolayered MSCs derived from adipose tissue using cell sheet technology.

RESULTS

Characteristics of adipose tissue-derived MSCs

We isolated MSCs from subcutaneous adipose tissue of male Sprague-Dawley rats on the basis of the adherent properties of these cells. We obtained $1.7 \times 10^5 \pm 0.2 \times 10^5$ cells from 1 g adipose tissue in a 12-h culture. By day 4 of culture of the minced adipose tissue, spindle-shaped adherent cells were apparent and formed symmetric colonies. After approximately three to four passages, most adherent cells expressed CD29 and CD90 (Supplementary Fig. 1 online). In contrast, the majority of adherent cells were negative for CD34 and CD45. They were also negative for CD31, a marker for vascular endothelial cells, and negative for α smooth muscle actin (α SMA), a marker for smooth muscle cells. A small fraction of adherent cells expressed CD71, CD106 and CD117. These results were similar to those from bone marrow-derived MSCs^{15,22,25} (Supplementary Fig. 1 online). Using previously described methods^{16,22,26}, we confirmed that these adipose-derived adherent cells, like bone marrow-derived MSCs, were multipotent, as judged by their ability to differentiate into adipocytes, osteoblasts and vascular endothelial cells. Thus, we

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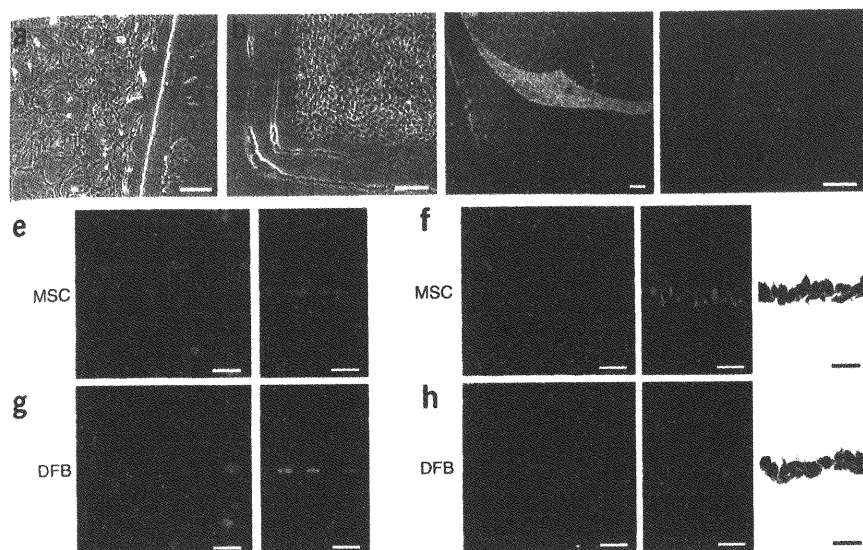


Figure 1 Preparation of monolayered MSCs.

(a) MSCs 2 d after seeding on a temperature-responsive dish. (b) Cultured MSCs expanded to confluence within the square area of the dish by day 3. (c) The monolayered MSCs detached easily from the culture dish at 20 °C. (d) The completely detached monolayered MSCs were identified as a 12 × 12 mm square sheet. (e–h) Cross-sectional analysis of GFP-expressing monolayered MSCs and DFBs before detachment (e and g, confocal images) and after detachment (f and h, left and center, confocal images; right, Masson trichrome). The thickness of both monolayers was 3.5-fold greater than the thickness before detachment, and constituent cells were compacted. Scale bars in a–c, 100 μm; in d, 5 mm; in e–h, 20 μm.

Engraftment and growth of monolayered MSCs

To identify the transplanted cells in myocardial sections, we used GFP-expressing cell

confirmed that the majority of adherent cells isolated from adipose tissue were MSCs.

Preparation and transplantation of monolayered MSCs

We cultured adipose tissue–derived MSCs (5×10^5 cells) on temperature-responsive dishes for 3 d until confluent. MSCs were attached on the poly-*N*-isopropylacrylamide (PIPAAm)-grafted area (24 × 24 mm; Fig. 1a,b). As the culture temperature was decreased from 37 °C to 20 °C, MSCs detached spontaneously and floated up into the culture medium as a monolayer of MSCs within 40 min (Fig. 1c,d). As a control, we prepared dermal fibroblasts (DFBs) by the skin explant technique²⁷. DFBs (8×10^5 cells) were cultured on the temperature-responsive dishes, and monolayered DFBs were fabricated as described above. The final cell counts for monolayered MSCs and DFBs before transplantation were $9.4 \pm 0.6 \times 10^5$ and $8.6 \pm 0.6 \times 10^5$ cells, respectively ($n = 6$ each). To identify the thickness of monolayered MSCs, we used green fluorescent protein (GFP)-expressing cell grafts derived from the GFP-transgenic Sprague-Dawley rats. Immediately after detachment, cells became compacted, possibly owing to cytoskeletal tensile reorganization, and the thickness of monolayered MSCs and DFBs was approximately 3.5-fold greater than the thickness before detachment (MSCs, 6.2 ± 0.3 to 21.5 ± 0.8 μm; DFBs, 6.5 ± 0.4 to 22.4 ± 1.1 μm; Fig. 1e–h). MSCs on the temperature-responsive dishes were positive for vimentin and slightly positive for collagen type 1, whereas DFBs were positive for both markers (Fig. 2a). We transferred detached monolayered MSCs above the myocardial scar (Fig. 2b) and then attached them to the surface of the anterior scar (Fig. 2c).

Secretion of angiogenic factors from monolayered MSCs

We measured secretion of angiogenic factors from MSCs 24 h after monolayers had formed, equivalent to day 4 after initial cell seeding. The monolayered MSCs secreted significantly larger amounts of angiogenic and antiapoptotic factors such as vascular endothelial growth factor (VEGF) and hepatocyte growth factor (HGF) than did the monolayered DFBs ($P < 0.01$; Fig. 2d). The control medium supplemented with 10% fetal calf serum contained less than 5 pg/ml of VEGF or HGF. These results suggest that the paracrine effects of monolayered MSCs on host myocardium are greater than those of monolayered DFBs.

grafts derived from the GFP-transgenic Sprague-Dawley rats. We grafted monolayered MSCs or DFBs onto the scar area of the anterior wall (Fig. 3). Fluorescence microscopy showed that GFP-expressing monolayered MSCs gradually grew *in situ* and developed into a thick stratum, up to ~600 μm thick over the native tissue at 4 weeks (Fig. 3a–f). The engrafted MSC tissue tapered off toward the healthy myocardium (Fig. 3d,e), although most of the monolayered MSCs were attached only to the scar area in the anterior wall because of the large infarct. We rarely detected TUNEL-positive MSCs in the sheet (<1%) 48 h after transplantation (Fig. 3g), implying that cell viability in the sheet was maintained. In contrast, we frequently detected TUNEL-positive cells (15% ± 2%) in the DFB sheet, which was observed as a thin layer above the scar. Subsequently, the DFB sheet was undetectable 1 week later. Masson trichrome staining showed increased thickness of the anterior wall and attenuation of left ventricle enlargement after transplantation of monolayered MSCs (Fig. 3h), although the infarct size did not differ significantly among the untreated, DFB and MSC groups (Supplementary Table 1 online).

Reconstruction of cardiac mass

After growth *in situ*, GFP-expressing MSC tissue contained a number of mature vascular structures that had positive staining for von Willebrand factor (vWF) and αSMA (Fig. 4a,b). A small fraction of the MSC tissue had positive staining for cardiac troponin T and desmin (Fig. 4c,d). On the other hand, a large proportion of the MSC tissue was positive for vimentin, a marker for mesenchymal lineage cells (Fig. 4e). The percentages of graft-derived cells that expressed endothelial (vWF), smooth muscle (αSMA), cardiac (troponin T) and mesenchymal (vimentin) markers were $12.2\% \pm 0.6\%$, $5.0\% \pm 0.3\%$, $5.3\% \pm 0.3\%$ and $57.8\% \pm 2.2\%$, respectively. Notably, based on expression of these markers, two-thirds of vascular endothelial cells, four-fifths of smooth muscle cells and one-twentieth of cardiomyocytes within the MSC tissue were GFP⁺ and hence were derived from the host. The MSC tissue stained modestly for collagen type 1 (Fig. 4f). Picosirius red staining showed that collagen deposition was found mainly in the extracellular matrix and the epicardial margin of the MSC tissue (Fig. 4g). Excluding staining in blood vessels, the MSC tissue was also negative for αSMA, a marker for myofibroblasts (Fig. 4b). This phenotype was consistent with properties of MSCs

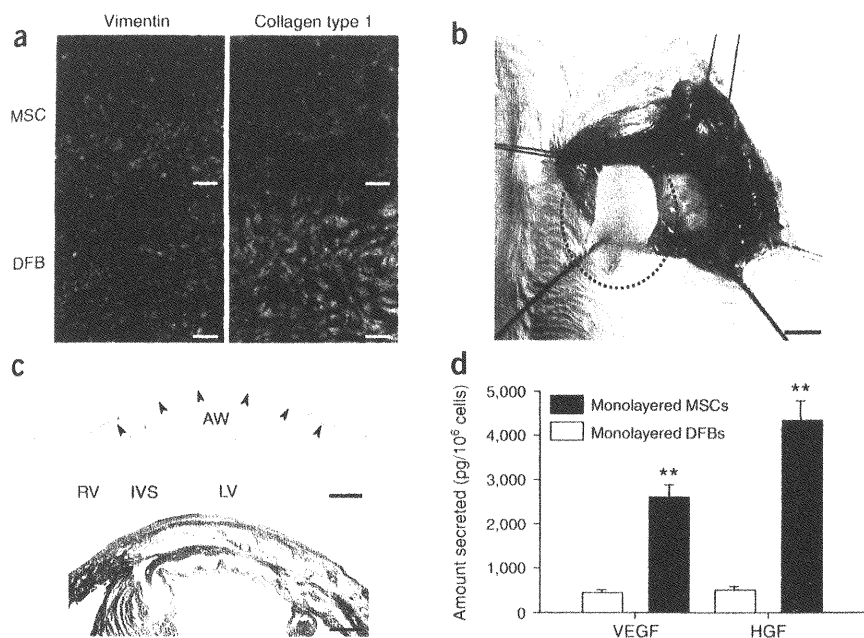


Figure 2 Characteristics of monolayered MSCs. (a) Properties of constituent cells in the monolayered grafts. Compared with DFBs (green), MSCs (green) are positive for vimentin (red) and slightly positive for collagen type 1 (red). (b) Monolayered MSCs (in the dotted circle) transferred to the infarcted heart. (c) Extent of monolayered MSCs 48 h after transplantation (arrows). AW, anterior wall; LV, left ventricle; RV, right ventricle; IVS, interventricular septum. (d) Comparison of secretion of growth factors between monolayered MSCs and DFBs. $**P < 0.01$ versus DFBs. Scale bar in a, 20 μm ; in b, 5 mm; in c, 100 μm .

before transplantation (Fig. 2a and Supplementary Fig. 1 online), suggesting that the MSC tissue includes a number of undifferentiated MSCs. Taken together, the grown MSC tissue was composed of newly formed blood vessels, undifferentiated MSCs and few cardiomyocytes.

Fluorescence *in situ* hybridization analysis

We performed fluorescence *in situ* hybridization (FISH) to detect X and Y chromosomes after sex-mismatched transplantation of monolayered MSCs. We transplanted GFP-expressing monolayered MSCs derived from male rats to female Sprague-Dawley rats that had suffered an infarct. Four weeks later, newly formed cardiomyocytes that were positive for GFP had only one set of X and Y chromosomes, whereas we detected two X chromosomes exclusively in GFP⁺ host-derived cells (Fig. 4h). We counted the X and Y chromosomes in male and female control rats and in the MSC sheet-transplanted rats (Supplementary Table 2 online), and we did not detect extra copies of the X or Y chromosome in graft-derived GFP⁺ cardiomyocytes. When we compared the frequencies of the occurrence of zero, one, two and more than two X chromosomes in the GFP⁺ cardiomyocytes with the frequencies in male control cardiomyocytes, the GFP⁺ cardiomyocytes did not show an increased proportion of X chromosomes ($0.25 > P > 0.10$, χ^2 test).

Effects of monolayered MSCs on cardiac function

Heart failure developed 8 weeks after coronary ligation, as indicated by an increase in left ventricle end-diastolic pressure (LVEDP) and attenuation of maximum and minimum rate of change in left ventricular pressure (dP/dt). Autologous transplantation of monolayered MSCs, however, resulted in decreased LVEDP (Fig. 5a). Left ventricle maximum and minimum dP/dt were significantly improved in the MSC group (Fig. 5b,c). We did not observe these hemodynamic improvements in the DFB group. The MSC group also had significantly lower right ventricular weight and lung weight than the DFB and untreated groups 4 weeks after transplantation (Supplementary Table 1 online). These results suggest that transplantation of monolayered MSCs has beneficial hemodynamic effects in rats with chronic heart failure.

in diastole was markedly lower in the MSC group than in the DFB and untreated groups (Supplementary Table 3 online). Plasma atrial natriuretic peptide (ANP) in the DFB and untreated groups was markedly elevated 8 weeks after myocardial infarction (Fig. 5g). Transplantation of the monolayered MSCs inhibited the increase in plasma ANP.

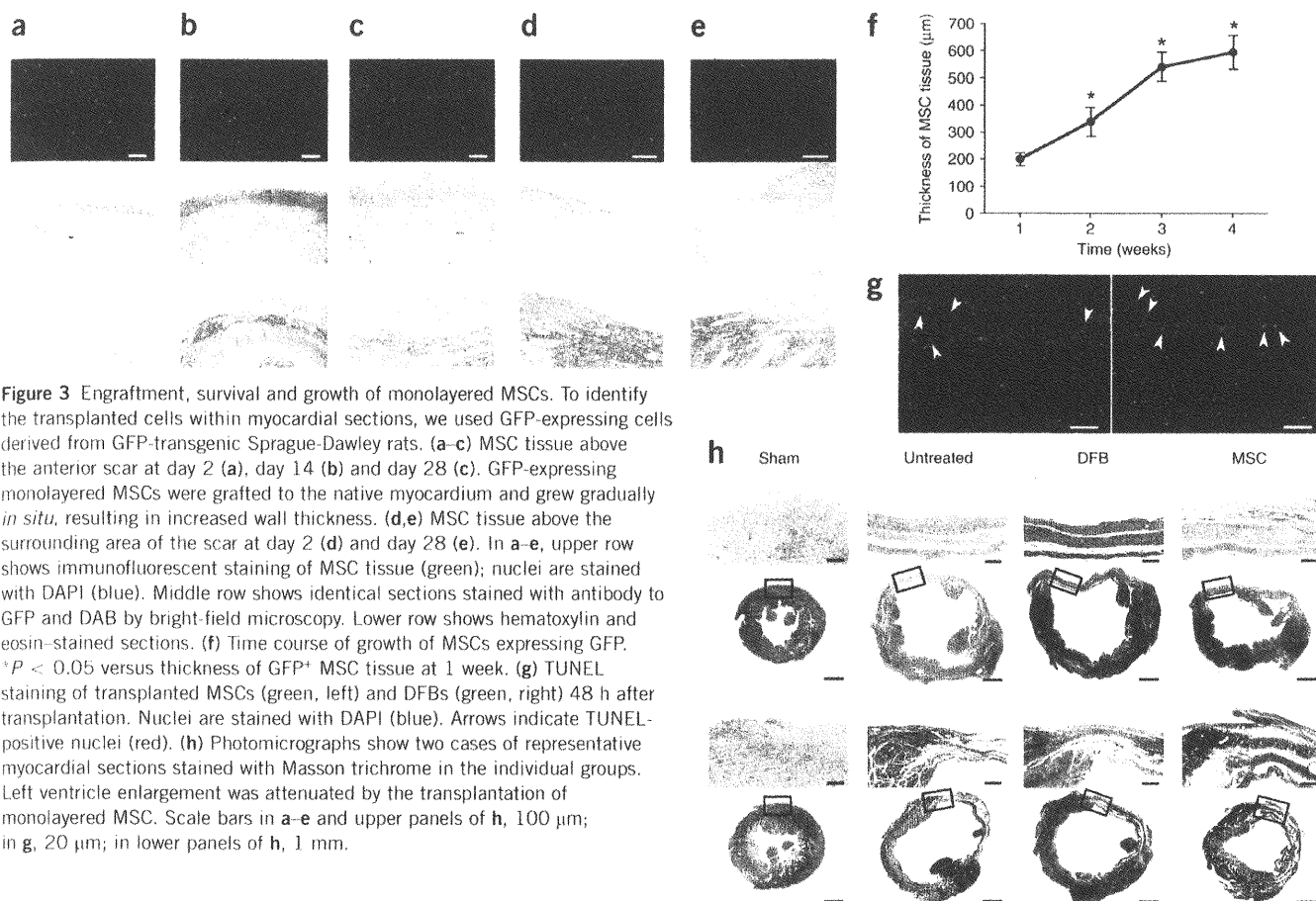
Survival analysis

The Kaplan-Meier survival curve showed that 4-week survival after coronary ligation did not differ significantly between the untreated and MSC groups before transplantation (Fig. 5h). Notably, however, no rats died after transplantation of monolayered MSCs. Therefore, the survival rate after transplantation was markedly higher in the MSC group than in the untreated group (4-week survival after transplantation was 100% for the MSC group versus 71% for the untreated group, log-rank test, $P < 0.05$).

DISCUSSION

There are several advantages to monolayered MSC transplantation. First, the self-propagating property of MSCs *in situ* leads to the formation of a thick stratum on the surface of the scarred myocardium. Second, the multipotency of MSCs and their ability to supply angiogenic cytokines allows neovascularization in the MSC tissue. Third, the reconstruction of thick myocardial tissue reduces left ventricle wall stress and results in improvement of cardiac function after myocardial infarction. Finally, a substantial part of the transplanted tissue is composed of undifferentiated MSCs, and it is tempting to speculate that such cells may act against future progressive left ventricle remodeling.

Cellular cardiomyoplasty using needle injections is emerging as a treatment option for individuals with chronic heart failure, but it may be limited by failure to regenerate cardiac mass. The cell sheet allows for cell-to-cell connections owing to the lack of a need for enzymatic digestion^{6–10}. Thus, the cell sheet has attracted considerable interest as a tool for tissue engineering²⁸. Here, we used adipose tissue-derived MSCs as a cellular source for the cell sheet, which resulted in successful autologous transplantation in heterogenic rats without immunological



rejection. Using flow cytometry, we did not find any substantial differences between adipose tissue-derived MSCs and bone marrow-derived MSCs, consistent with results from previous studies^{22,25}. Adipose-derived MSCs readily attached to and propagated on the temperature-responsive dish. Abdominal subcutaneous adipose tissue is clinically redundant and easily accessible by rapid and minimally invasive surgery such as liposuction. Thus, adipose tissue may serve as a source of stem cells for therapeutic cell sheets.

Here, monolayered MSCs could readily be transferred and grafted to the scarred myocardium without additives or suturing. This may be attributable to cell-to-cell connections as well as extracellular matrix deposits on the basal surface of the monolayered MSCs. Regeneration of myocardial mass is thought to require multilayered constructs of the cell sheet. Unfortunately, however, the lack of a vascular network has limited the formation of a thick construct^{10,29}. The transplanted monolayered MSCs thickened gradually, developing into a stratum of up to 600 μm in thickness over the native tissue 4 weeks after transplantation, suggesting that monolayered MSCs have an ability to grow *in situ*. As a result, the transplanted MSC tissue reversed wall thinning of the infarcted myocardium. On the other hand, the fibroblast sheet did not grow *in situ*. It should be noted that the MSC tissue included a large number of newly formed blood vessels. These vessels were composed of graft-derived cells, host-derived cells or both. The MSC sheet secreted a large amount of angiogenic and antiapoptotic cytokines, including VEGF and HGF, as compared with the fibroblast sheet. These results suggest that MSCs induce neovascularization within the sheet not only through their ability to differentiate into vascular cells but also through growth factor-mediated paracrine

regulation. Thus, we believe that the angiogenic action of MSCs is important for reconstruction of cardiac mass by the MSC tissue.

Four weeks after transplantation, a small fraction of the engrafted MSCs were positive for cardiac proteins such as cardiac troponin T and desmin, suggesting the presence of cardiomyocytes within the MSC tissue. FISH analysis suggested that the most cardiomyocytes within the MSC tissue were not derived from cell fusion, but we are unable to exclude the possibility that some were. Further studies are necessary to investigate the mechanisms by which MSCs within the MSC tissue regenerate cardiomyocytes. The majority of the MSC tissue was positive for vimentin, a marker for undifferentiated MSCs and fibroblasts. In addition, the majority of MSCs within the graft were negative for collagen type 1 and αSMA, a marker for myofibroblasts. These results suggest that the grown-up MSC tissue is composed of newly formed blood vessels, undifferentiated MSCs and few cardiomyocytes.

We have also shown that transplantation of the monolayered MSCs significantly increased left ventricle maximum dP/dt , decreased LVEDP and inhibited the development of left ventricle enlargement in rats with chronic heart failure secondary to myocardial infarction. These results suggest that transplantation of monolayered MSCs improves cardiac function. But the presence of cardiomyocytes within the MSC tissue seemed to be rare. Thus, this improvement may be explained mainly by growth factor-mediated paracrine effects of the MSC sheet and a decrease in left ventricle wall stress resulting from the thick MSC tissue. Furthermore, no rats treated with the monolayered MSCs died during the study period, although untreated rats died frequently. These results indicate that fatal arrhythmogenic problems were not caused by integration of the MSC tissue.

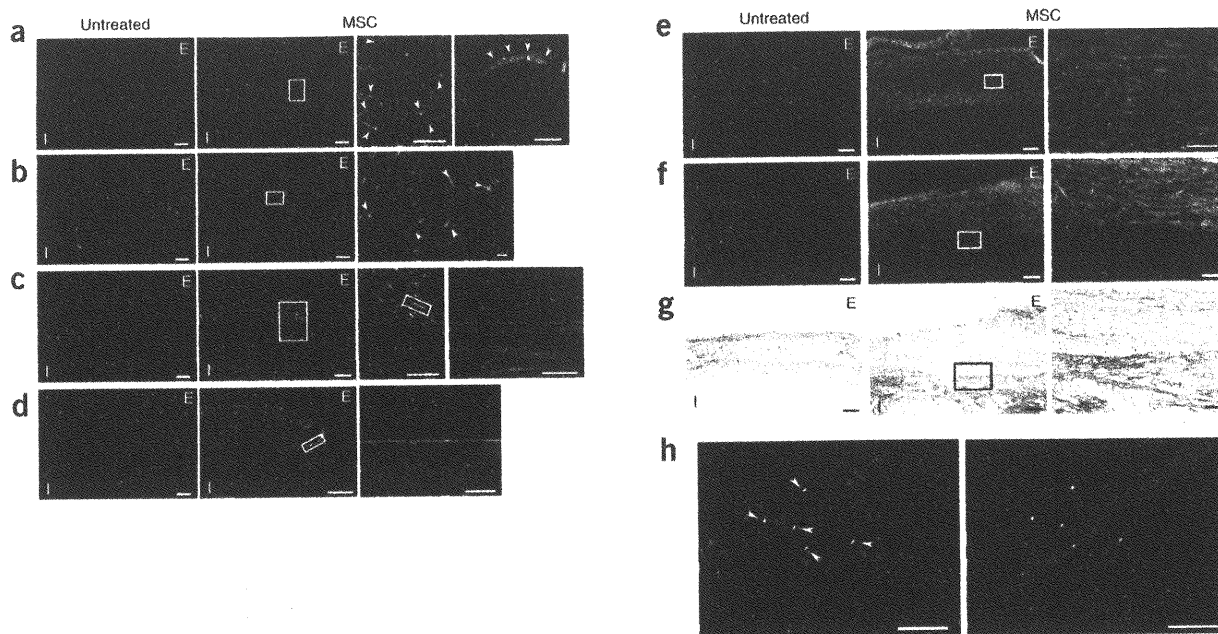


Figure 4 Differentiation of MSCs within the MSC tissue after growth *in situ*. (a,b) GFP-expressing MSCs (green) were identified as a thick stratum at the epicardial side of the myocardium. The MSC tissue contained a number of vascular structures positive for vWF (red, a) and α SMA (red, b). MSCs that did not participate in blood vessel formation were only rarely positive for α SMA, a marker for myofibroblasts. Arrows indicate transplanted MSCs positive for vWF or α SMA. (c,d) Some MSCs within the MSC tissue were positive for cardiac markers cardiac troponin T (red, c) and desmin (red, d). (e) Most of the MSC tissue was positive for vimentin (red). (f) The MSC tissue modestly stained for collagen type 1 (red). (g) Collagen deposition was also detected by picrosirius red staining. (h) FISH analysis. Newly formed cardiomyocytes (desmin, red) that were positive for GFP (green) had only one set of X (purple) and Y chromosomes (white), whereas two X chromosomes were detected exclusively in GFP host-derived cells. Nuclei are stained with DAPI (blue, a–f and h). Scale bars in left three panels of a and c and in two left panels of b and d–g, 100 μ m; in h and far right panels of a–g, 20 μ m. E, epicardial side; I, intimal side.

In summary, adipose tissue–derived monolayered MSCs can be readily engrafted to the scarred myocardium, grow gradually *in situ* and become a thick stratum that includes newly formed vessels, cardiomyocytes and undifferentiated MSCs. The engrafted MSCs reversed wall thinning in the scar area and improved cardiac function and survival in rats with myocardial infarction. Thus, transplantation of monolayered MSCs may be a new therapeutic strategy for cardiac tissue regeneration.

METHODS

Model of heart failure. All protocols were performed in accordance with the guidelines of the Animal Care Ethics Committee of the Japanese National Cardiovascular Center Research Institute. We used male Sprague-Dawley rats (Japan SLC) weighing 187–215 g. A myocardial infarction model was produced by ligation of the left coronary artery, as described previously³⁰. Briefly, we anesthetized rats with sodium pentobarbital (30 mg/kg) and ventilated them with a volume-regulated respirator. We exposed hearts by left thoracotomy, and ligated the left coronary artery 2–3 mm from its origin between the pulmonary artery conus and the left atrium with a 6-0 Prolene suture. The sham group underwent thoracotomy and cardiac exposure without coronary ligation. The surviving rats were maintained on standard rat chow.

Study protocol. We randomly placed rats into four groups: rats with chronic heart failure that underwent transplantation of monolayered MSCs (MSC group; $n = 12$), rats with chronic heart failure given monolayered DFBs (DFB group; $n = 12$), rats with chronic heart failure without transplantation (untreated group; $n = 12$) and sham-operated rats without transplantation (sham group; $n = 10$). Four weeks after coronary ligation, the MSC and DFB groups underwent autologous transplantation of each monolayered cell graft onto the anterior wall, including the scar area (**Supplementary Methods** online). The other two groups underwent the same operative procedures

without transplantation. We performed hemodynamic studies, echocardiography and histological assessments 4 and 8 weeks after coronary ligation (**Supplementary Methods**). Upon killing at 8 weeks after coronary ligation, only those rats with infarct size > 25% of the left ventricle area were included in this study. Therefore, the variation in infarct size between the experimental rats was relatively low (28–41%, average $33.9\% \pm 1.9\%$).

Isolation and culture of MSCs from adipose tissue. Immediately after coronary ligation, we acquired subcutaneous adipose tissue (1.1 ± 0.1 g) from the right inguinal region of each rat. We minced adipose tissue with scissors and digested it with 10 ml of type I collagenase solution (0.1 mg/ml, Worthington Biochemical) for 1 h in a 37 °C water bath shaker. After filtration with mesh filter (Costar 3480, Corning) and centrifugation at 780g for 8 min, we suspended isolated cells in α -MEM supplemented with 10% FCS and antibiotics, plated them onto a 100-mm dish and incubated them at 37 °C with 5% CO₂. A small number of spindle-shaped cells were apparent in visible symmetric colonies by days 5–7.

Preparation of temperature-responsive dishes. Specific procedures for preparation of square-designed PIPAAm-grafted dishes have been previously described⁹. Briefly, we spread IPAAm monomer (Kohjin) in 2-propanol solution onto 60-mm polystyrene culture dishes (Corning). We then subjected the dishes to irradiation (0.25-MGy electron beam dose) using an Area Beam Electron Processing system (Nisshin High-Voltage) to immobilize IPAAm on the dish surface; we then rinsed dishes with cold distilled water and dried them in nitrogen gas. In the second step, we masked the PIPAAm-grafted surface with a square glass coverslip (24 × 24 mm, Matsunami Glass). We spread acrylamide (AAm) monomer solution in 2-propanol onto the masked dish surface. We then irradiated the dish surface with an electron beam and washed it. As a result, the central square area of each dish was PIPAAm grafted (temperature responsive), and the surrounding border was poly-AAm grafted (non-cell adhesive). This PIPAAm-grafted surface is hydrophobic under culture

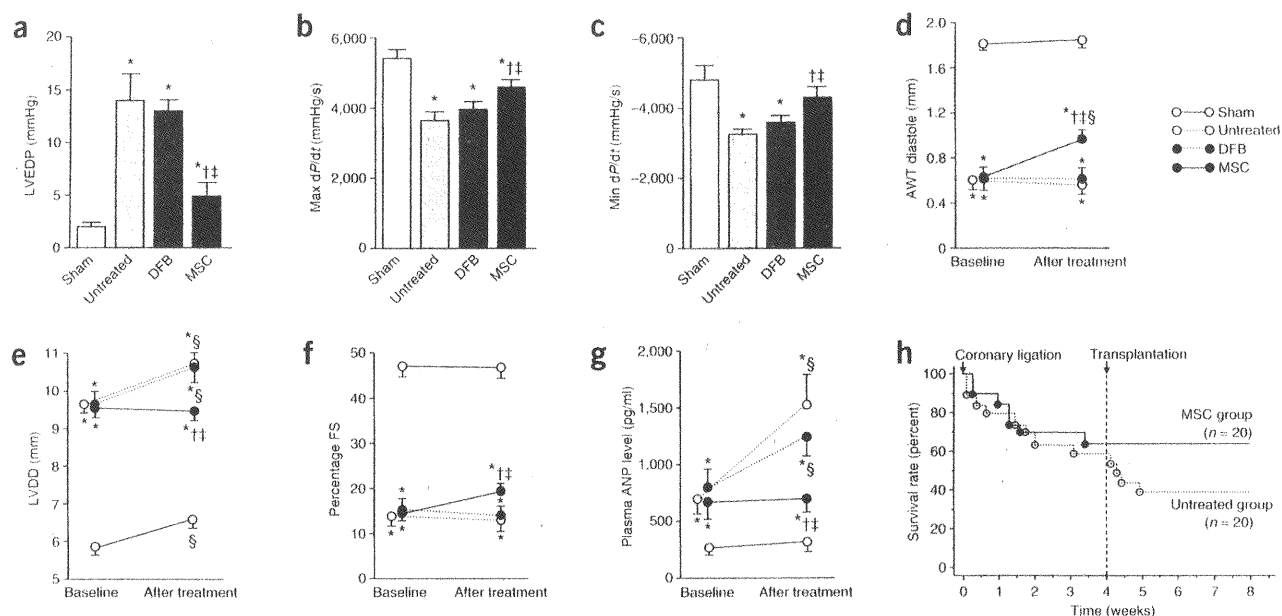


Figure 5 Cardiac structure and function after transplantation of monolayered MSCs. (a–c) Hemodynamic parameters obtained by catheterization. LVEDP, left ventricle end-diastolic pressure. (d–f) Echocardiographic findings. AWT, anterior wall thickness; LVDD, left ventricle end-diastolic dimension; FS, fractional shortening. (g) Plasma atrial natriuretic peptide (ANP) level. Baseline represents measurements 4 weeks after coronary ligation; 'after treatment' represents measurements taken 4 weeks after transplantation (8 weeks after coronary ligation). Data are mean \pm s.e.m. * $P < 0.05$ versus sham group; ⁱ $P < 0.05$ versus untreated group; ^j $P < 0.05$ versus DFB group; ^k $P < 0.05$ versus baseline. (h) Survival of rats with chronic heart failure with or without monolayered MSC transplantation. The Kaplan-Meier survival curve demonstrates an 8-week survival rate of 65% for the MSC group versus 45% for the untreated group. Survival rate after transplantation was significantly higher in the MSC group than in the untreated group (100% versus 71% 4-week survival rate after transplantation, log-rank test, $P < 0.05$).

conditions at 37 °C and becomes reversibly hydrophilic below 32 °C. Therefore, cultured cells that adhere to the dish surface spontaneously detach from the grafted surface without enzymatic digestion.

Preparation of monolayered cell grafts. We suspended MSCs at the third or fourth passage from adipose tissue or DFBs at the second passage by trypsinization, and plated the cell suspension containing 3 ml of complete medium onto a 60-mm temperature-responsive dish at 5×10^5 cells per dish (MSCs) or 8×10^5 cells per dish (DFBs) and cultured cells at 37 °C. After 3 d of culture, confluent MSCs or DFBs on the temperature-responsive dishes were incubated at 20 °C. By 40 min, both MSCs and DFBs detached spontaneously and floated up into the medium as monolayered cell grafts. Immediately after detachment, we gently aspirated the monolayered cell grafts using a 1,000 μ l pipette tip and transferred them onto an elastic plastic sheet.

Statistical analysis. Numerical values are expressed as mean \pm s.e.m. There are four groups of continuous variables in this study. Therefore, for multiple comparisons of more than two groups, we performed one-way analysis of variance (ANOVA). If the ANOVA was significant, we used the Newman-Keul procedure as a *post hoc* test. For repeated measurement such as echocardiographic parameters, we performed two-way repeated ANOVA with the Newman-Keul test. Comparisons of parameters between two groups were made by unpaired Student *t*-test. A value of $P < 0.05$ was considered significant.

Note: Supplementary information is available on the Nature Medicine website.

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COMPETING INTERESTS STATEMENT

The authors declare competing financial interests (see the *Nature Medicine* website for details).

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心筋再生

Cardiomyogenesis and myocardial angiogenesis

特集

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 NAKATANI Takeshi NAGAYA Noritoshi TOMITA Shinji

再生医学—クローン・幹細胞から医療へ—

Key words 細胞移植 骨髄単核球細胞 間葉系幹細胞 血管新生 環境因子

各種治療法の限界を越えた重症心不全に対し、確実な治療効果が期待できる治療手段は心臓移植である。国際レジストリーではこれまでに6.6万例以上実施され、1年生存率は82%である。わが国においても、臓器移植法施行後22例に施行され、生存率は100%で最長5年を越え、社会復帰率は70%以上と良好な成績を示している。しかし、心臓移植を必要とする症例数に対し、移植施行数が少なく、待機期間が長期に及んでいる。このため、補助人工心臓(VAS)装着数が増加し、補助期間も延長し、長期間の入院が必要となっている。

重症心不全に対する新たな治療法として、自己の広背筋を心臓周囲に巻き付け電氣的トレーニングにより骨格筋の心筋化を計り心補助を行う心筋形成術が開発され、臨床応用もなされた¹⁾。しかし、大きな手術侵襲にもかかわらず心補助効果を得るのに時間がかかり、またその補助効果も限られているため、施行されなくなった。しかし、心筋形成術を研究していたChiuらのグループは、骨格筋細胞を直接心筋へ移植する新たな心補助手段の検討を行い、その後、心臓への細胞移植の研究が本格的に行われるようになった²⁾。その後、用いる細胞源について各種の研究がなされてきた。しかし、非自己の細胞を用いる場合には拒絶反応への対応が必要であり、倫理的な問題がある。これらの問題を回避し得るものとして自己細胞を用いる方法が検討され、その採取が臨床的に広く行われている骨髄細胞が注目されている。本稿では、骨髄細胞による心筋再生を中心に述べる。

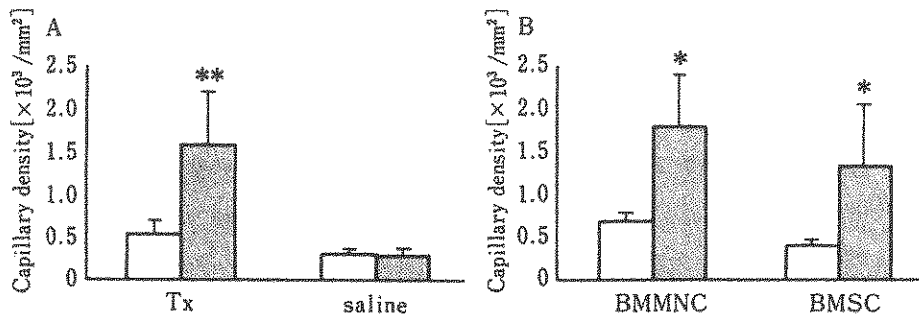


図1 梗塞作成非移植部(白柱)と梗塞作成移植部(黒柱)における capillary density
梗塞作成移植部は梗塞作成非移植部よりCDが有意に高かった。しかし、mononuclear cell (BMMNC)と stromal cell (BMSC)では差を認めなかった。
(文献7より)

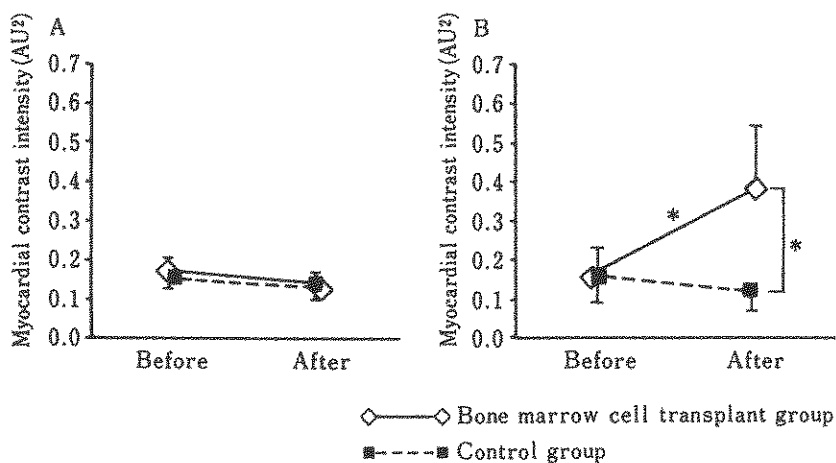


図2 移植前後における myocardial contrast intensity の変化
実線：移植群(BMMNC および BMSC)，破線：非移植群
A：梗塞作成非移植部，B：梗塞作成移植部
Bにおいて移植群で有意にMCIの増加を認めた。
(文献7より)

I. 骨髄単核球細胞(MNC)による心筋および血管組織の再生

骨髄細胞は、造血幹細胞が多数をしめているが、間葉系幹細胞(Mesenchymal stem cell: MSC)が存在し、骨、軟骨、脂肪細胞へ分化誘導が可能である。また、1997年にAsaharaらは、末梢血に骨髄由来の血管内皮前駆細胞(endothelial progenitor cells: EPC)が存在し、このEPCが虚血心筋での脈管新生(vasculogenesis)や血管新生(angiogenesis)に貢献していると報告した³⁾。さらに、Shintaniらは、骨髄単核球細胞(bone marrow mononuclear cell: BMMNC)が虚血肢での血管新生をもたらすことを報告した⁴⁾。このBMMNC

を用いた血管再生療法は、わが国において臨床応用が行われ、その安全性と有効性ととともに、骨髄細胞からのサイトカインが血管新生に大きな役割を果たしていると考えられることが報告されている⁵⁾。さらに、虚血心筋に対しても外科的に注入する方法が試みられている⁶⁾。

しかし、この骨髄単核球細胞移植における血管新生効果の評価が困難であり、問題であった。そこで、われわれは、心筋コントラストエコー法を用いる方法をブタ心筋梗塞モデルに対する骨髄細胞移植により検討した⁷⁾。NIBSブタの左前下降枝を結紮し、心筋梗塞を作成した。1ヵ月後、骨髄細胞を梗塞部へ直接注入し、myocardial contrast intensity (MCI)を測定した。また、移植1ヵ月後に犠牲死させ、組織学的に毛細血管密度

(capillary density(CD))を測定した。その結果、MCIとCDの間に正の相関を認め、多くの毛細管の直径は10 μ m以下であった。さらに移植梗塞部のMCIとCDは、非移植梗塞部のものよりも有意に増加した(図1, 図2)。したがって、骨髄細胞移植により、心筋梗塞部位での血流は改善し、この血流改善効果はコントラストエコーにより非侵襲的にベッドサイドでの評価を行い得ることが示された。

これまでに述べた血管再生に加え、1999年には骨髄細胞に5-azacytidine 処理を行うことにより、心筋細胞が分化誘導されることが1999年に報告された⁸⁾⁹⁾。

この心筋への分化において環境因子(cardiac milieu)の重要性が指摘されていた¹⁰⁾¹¹⁾が、その詳細は不明であった。そこで環境因子のひとつとしての細胞同士の直接接触について検討を行った¹²⁾。ホスト心筋(CM)としてラット新生児心筋細胞を、移植細胞としてGFP 遺伝子組み換えマウス由来骨髄細胞(GFP-BMC)を各々用い、共培養実験系を作成した。隔壁をGFP-BMCとCMとの間においたdouble chamber 培養では、GFP-BMCに特に変化を認めなかった。これに対し、GFP-BMCとCMを混合した共培養系では、2日後からCMと同期収縮を開始するGFP-BMCが現れた。また、免疫組織染色では、1日後からmyosin heavy chain-slow が、2日後からコネキシン43と心房性ナトリウム利尿ペプチド(ANP)が、4日後からはトロポニンIが各々経時的に発現し漸増した。5日後にはmyosin heavy chain-slow陽性細胞は約2.5%になった。この結果、骨髄細胞の心筋への分化において、ホストの心筋細胞との直接接触が重要な役割を果たしていることが判明した。

この共培養における問題点として、2002年に細胞融合(cell fusion)が報告された¹³⁾。この報告では、ES細胞とGFPマウス由来骨髄細胞との共培養において、GFPを発現した細胞が分化増殖するようにみえるが、その細胞の核内にはES細胞

由来のDNAも含まれていたとしている。しかし、細胞融合の割合が低く、十分には解明されていない。また、前述のわれわれの実験では経時的に心筋細胞の特性を獲得しており、融合のみとは考えられない。今後さらに検討が必要である。

骨髄細胞の心筋への分化は確認されているが、心臓移植対象者の多数をしめる拡張型心筋症に対して、骨髄細胞による心筋再生療法が有効であるかの研究は少ない。そこで、ラットにおけるドキシソルピシン投与不全心に対して骨髄単核球移植を行い、心機能改善効果を検討した¹⁴⁾。ラットドキシソルピシン不全心モデルを用い、骨髄単核球移植群、生理食塩水注入群、sham手術群を作成し、移植4週間後に心エコー・Langendorff灌流装置にて心機能を測定し、さらに心重量・腹水量測定を行った。その結果、骨髄単核球移植群は、心エコーにおいて心筋壁厚が有意に維持され、心筋壁厚/内腔比は高値であった。さらに、Langendorff灌流装置による検討においても、他群に比しDeveloped pressureが有意に高値(図3)で、end-diastolic pressureが低値であった。さらに、心重量においても有意に大きく(図4)、腹水量は有意に少量であった。したがって、ラットドキシソルピシン心筋症モデルに対して骨髄単核球移植は有用であり、臨床応用への可能性が示された。

これまで外因性の細胞移植が検討されてきたが、2001年にOlicらは、マウス急性心筋梗塞モデルに対し、顆粒球コロニー刺激因子(G-CSF)と幹細胞因子(SCF)を投与したところ、心機能の改善、生存率の改善を得られたと報告し、G-CSFとSCFによる幹細胞の賦活化を示した¹⁵⁾。しかし、再生された心筋がホストの心筋由来か骨髄由来かについては不明であった。そこで、われわれは、この内因性幹細胞の由来を検討した。まず、放射線照射後のC57b6マウスにGFP遺伝子組み換えマウス由来骨髄細胞(GFP-BMC)を移植し、キメラマウスを作成した。心筋梗塞モデルを作成したところ、心筋梗塞1ヵ月後には、G-CSF投与群で生存率の改善傾向を認めた¹⁶⁾。また、G-CSF

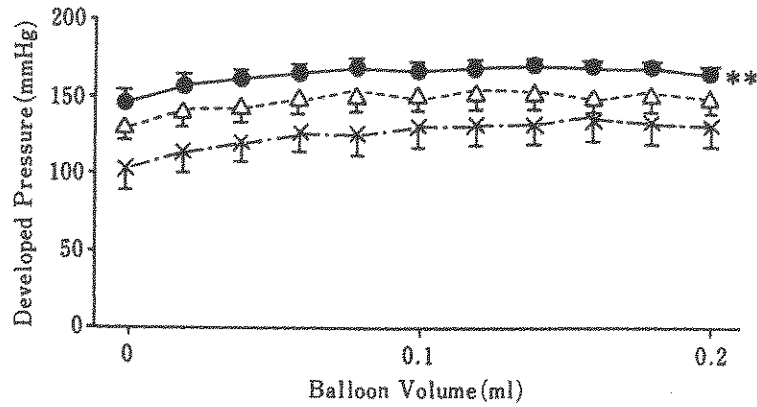


図3 Developed pressure の差
 (●:移植群, △:コントロール群, ×: sham 群)
 移植群において有意に高かった。
 (文献14より)

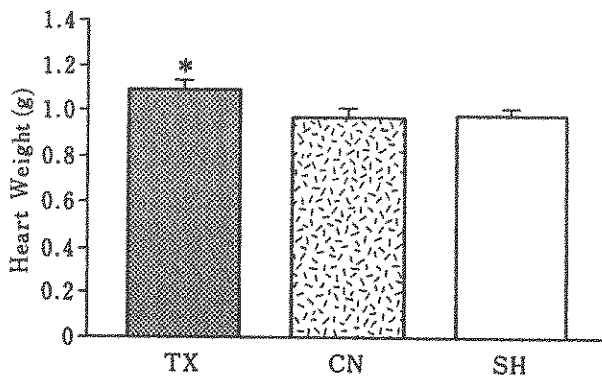


図4 心重量の差
 (TX:移植群, CN:コントロール群, SH: sham 群)
 移植群において有意に重かった。
 (文献14より)

群において、心筋梗塞境界部における GFP-BMC 数がコントロール群より有意に増加した。また、その GFP-BMC のうち約20%がトロポニン I 陽性細胞で、ネスチン陽性細胞も多数認めた。ドキシソルピシン投与心不全モデルにおいても同様の結果が得られた¹⁷⁾。したがって、再生心筋の細胞源のひとつは骨髄で、G-CSF によりその効果が増強されることが示唆された。また、G-CSF が病的な心筋に直接働き、G-CSF レセプターを介してトロポニン I 陽性細胞の増殖を増強することを確認している。しかし、骨髄由来の心筋細胞数は少なく、心臓ポンプ機能の改善効果には限界があることが示されている。

最近 Kang らが、急性心筋梗塞後に冠動脈内ステント挿入術を行った患者に G-CSF 治療を併用

すると高率でステント内再狭窄を認めたと報告している¹⁸⁾。内因性幹細胞は、障害された心筋とともに動脈硬化巣にも遊走する可能性があり、注意が必要である。今後、内因性幹細胞の遊走に関する生理学的メカニズムが解明できれば、心筋障害に対する効果的な内因性幹細胞を用いた治療が可能となると考えられる。

II. 骨髄間葉系幹細胞(MSC)による心筋および血管組織の再生

骨髄単核球細胞(MNC)の利用は、培養する必要がなく用いやすい。しかし、十分な細胞数の摂取には全身麻酔が必要であり侵襲が大きい。これに対し、増殖能力の高い間葉系幹細胞(MSC)を用いると、少量の骨髄細胞の採取を採取し、生体外で大量に培養し、必要量が得られてから移植することが可能となる。

そこで、まず MSC 移植の血管再生効果を、骨髄単核球細胞(MNC)移植と比較検討した。ラットの左総腸骨動脈を結紮・切除し下肢虚血を作成した後に同数の MSC あるいは MNC を移植した。移植3週間後には両群とも未治療群と比較し有意な血流増加を認めたが、MSC 群はより高度な血流改善を示した(図5)。また、毛細血管数の定量評価においても、MSC 群は MNC 群より増加していた。両群とも移植局所において移植細胞由来

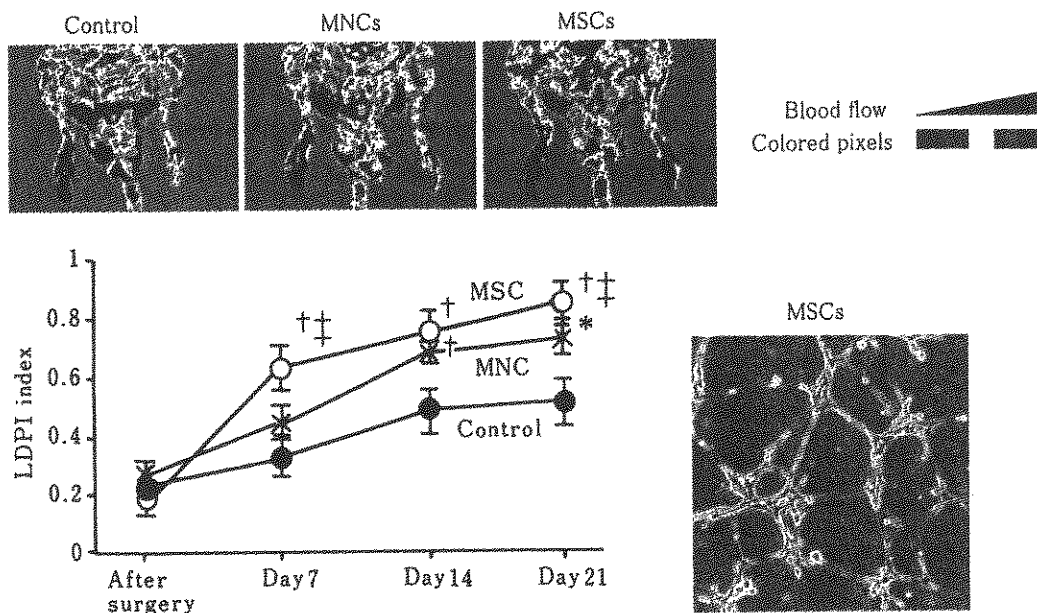


図5 レーザードブラを用いたラット下肢血流評価と *in vitro* での血管形成
MSC 移植は MNC 移植に比べ、有意に血流を改善させた。また MSCs は低酸素マトリゲル上で管腔を形成した。

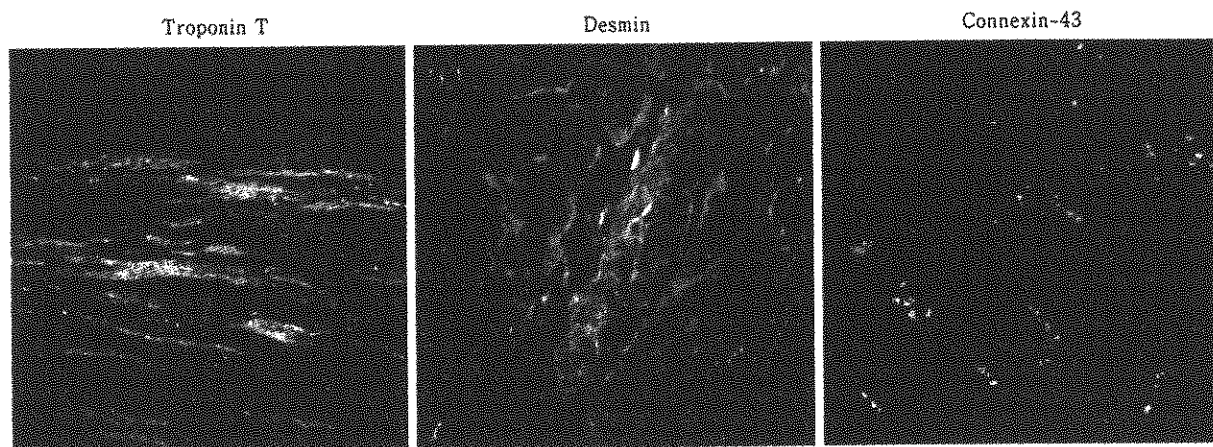


図 6
心筋内へ移植した MSC は Desmin や Troponin T 陽性を示し心筋細胞へ分化した。また移植細胞は Connexin43陽性を示し、移植細胞と既存の心筋細胞間にギャップジャンクションを形成した。

と考えられる血管内皮細胞を認めたが、その数は MSC 群において有意に多かった。また、MSC 群において移植細胞からの血管平滑筋と壁細胞への分化が確認された。さらに、MSC は MNC と比較し Vascular endothelial growth factor (VEGF), Hepatocyte growth factor (HGF), Adrenomedullin (AM) などの血管新生因子を多量に分泌していた¹⁹⁾。また *in vitro* で MSC は低酸素下で管腔を形成した。さらに、無血清培地培養下にて低酸素の状態にしたところ、MNC は MSC に比して高

率にアポトーシスを来した。以上より、MSC が低栄養および低酸素状態である移植環境下においてより高率に生存することから、MSC 移植は MNC 移植と比較して、同等もしくはそれ以上の血管再生作用を有すると考えられた。

次に、拡張型心筋症への臨床応用を目指して、MSC 細胞移植の効果をラット心筋症モデルで検討した。近交系ラットの大腿骨より骨髓組織を取り出し、培養皿底面に付着する MSC を分離・培養した。この MSC 細胞を、ミオシン投与拡張型

心筋症モデルラットの心筋壁内に心外膜より直接注入した。4週間後における心エコーおよび心臓カテーテル検査において、MSC 移植群は未治療群と比較し左室拡張末期圧の有意にな低下および左室収縮能の有意に改善を認めた。さらに、病理学的検討において MSC 移植群は心筋コラーゲン含量の減少を認め、さらに心筋壁内で血管内皮細胞や平滑筋細胞に分化し管腔構造を形成した。また、心筋内に注入した MSC の一部は免疫組織染色にて心筋組織の指標である Troponin T, Desmin, および Connexin43 が陽性であった (図 6)。さらに MSC は多くの血管新生因子やアポトーシス抑制因子を分泌した。以上より MSC は心筋細胞、血管細胞へ分化するのみならずパラクライン因子として心筋および血管再生に関与する可能性が示された。

また、MSC の心筋梗塞モデルに対する経静脈投与の効果を検討した²⁰⁾。左冠動脈結紮により作製した心筋梗塞モデルラットの頸静脈から MSC をカテーテルにより移植した。その結果、MSC の一部は梗塞巣周囲に集積し、さらに心筋細胞および血管内皮細胞に分化し、心機能を改善させた。

これらの小動物実験の結果をふまえ、ブタを用いた前臨床研究を行い、骨、軟骨、脂肪などに分化しないことや、不整脈が出現しないことなど、MSC 移植の有効性と安全性を確認した。

以上の結果より、虚血性心疾患や拡張型心筋症等による心不全を有し、利尿剤、ACE 阻害薬、 β 遮断薬などの既存の治療に抵抗性を示す症例を

対象に臨床試験は計画し、「間葉系幹細胞移植による難治性心不全治療の臨床評価」の実施を国立循環器病センター倫理委員会に申請し、承認された。MSC 移植は、患者の骨髓液 15mL を採取し、体外で培養増殖させ、カテーテルを用いて心内膜側より心筋内へ注入する。数例の難治性心不全症例に対して自己 MSC 移植を行ったが、重度の不整脈やその他の副作用はみられず、患者は順調に経過している。今後、さらに症例を積み重ねることにより、安全性および有効性を検討していく予定である。また、補助人工心臓装着例への応用なども検討中である。

おわりに

心筋再生に関する細胞移植に関して、多くの研究が行われている。その中で骨髓細胞を用いる方法は、自己の細胞を用いるため拒絶反応や副作用を避けることができる。さらに MSC を用いる場合には、MNC 移植に比べ、無菌培養が必要ではあるが、少量の骨髓液で治療に十分な細胞を確保できる。また、従来からの外因性の細胞移植に加え、内因性細胞移植による心筋再生も注目されている。骨髓細胞は、倫理的側面を含み臨床応用が行いやすく、さらに心筋内に移植することで心筋と血管が同時に再生され得るため、拡張型心筋症などの難治性重症心不全に対する治療として期待される。

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補助人工心臓

中谷 武嗣 花谷 彰久

要約：補助人工心臓は心臓ポンプ機能を代行しえる循環補助手段で、体外設置型と植え込み型がある。従来の治療限界を超えた重症心不全に対し、強力な補助効果を示すが、長期に補助を行う症例が多いため、適応に際しては十分な検討が重要である。装着例の管理においては、抗凝固、抗血栓療法とともに感染予防が重要である。わが国においても、心筋症に基づく心不全例において離脱した症例や、最長3年以上の補助後に移植された症例の報告があり、心機能の回復とともに、心臓移植へのブリッジとして有力な治療手段である。また、欧米では destination therapy としての使用も開始されている。今後、さらに小型で長期使用可能な VAS の臨床導入が期待される。

はじめに

治療抵抗性の重症心不全に対し、薬物治療に加え手術療法を含む新たな治療法が開発されてきた。しかし、広範囲におよぶ高度心筋障害例においては心臓ポンプ機能の代行が必要となり、臓器置換あるいは代替手段として、心臓移植や人工心臓がある。心臓移植は確実な治療成績が期待できるが、適当なドナー心が必要であり、施行時期や施行数に限界がある。人工心臓には、自己心を切除し植え込む全置換型人工心臓 (total artificial heart: TAH) と、自己心臓を温存し自己心の近傍に設置される補助人工心臓 (VAS) の2種があるが、最近 VAS の成績が向上し、広く用いられるようになってきた。本稿においては、この補助人工心臓 (VAS) について、わが国の現状を概説する。

補助人工心臓 (Ventricular Assist System: VAS) の概要

VAS には、図1に示すように血液ポンプを体

外に設置するタイプと、体内に植込むタイプがある。

1. 体外設置型 VAS

開心術後や急性心筋梗塞後の急性重症心不全に対し、自己心の回復を図るために1ヵ月程度の循環補助を行うことを目指して開発が行われた。わが国で開発された国立循環器病センター型 (国循環型) および東京大学型の2種のシステムは、1980年代始めから臨床応用が開始された。その後、世界に先駆けて1994年に施設の限定はあるが健康保険に採用された¹⁾。また、近年米国で開発された tube 型の Abiomed 社製 BVS-5000 が、2週間程度の補助用として用いられている。

現在多く用いられている東洋紡製国循環型 VAS の血液ポンプはセグメント化ポリウレタン製空気圧駆動ダイアフラム型で、1回拍出量 70 ml、最大拍出量 7 l/min である (図2左)。制御駆動装置 VCT-50 (図2右) は、固有レートモードあるいは心電図同期モードを用い、内蔵するバッテリーと空圧ポンプにより30分間駆動可能で、病院内の移動が行える。左心補助 (LVAS) として

Ventricular Assist System

国立循環器病センター臓器移植部 (〒565-8565 吹田市藤白台5-7-1)
ICU と CCU 29(4):265~273, 2005

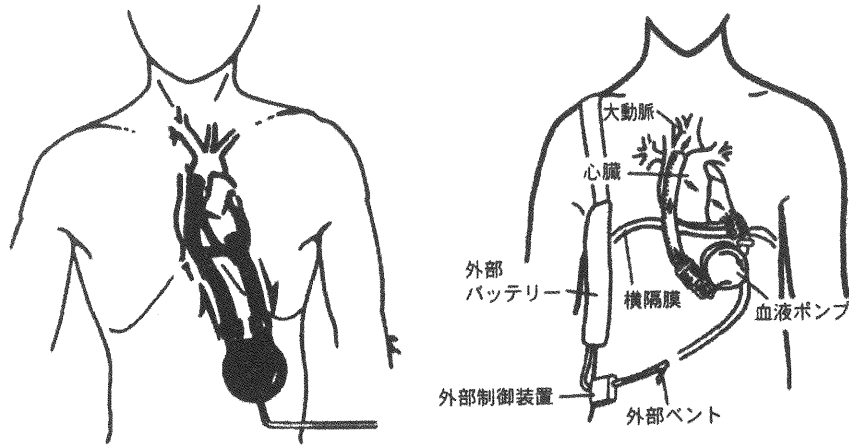


図1 LVAS 装着図
 左：左室脱血方式体外設置型
 右：体内植込み携帯型

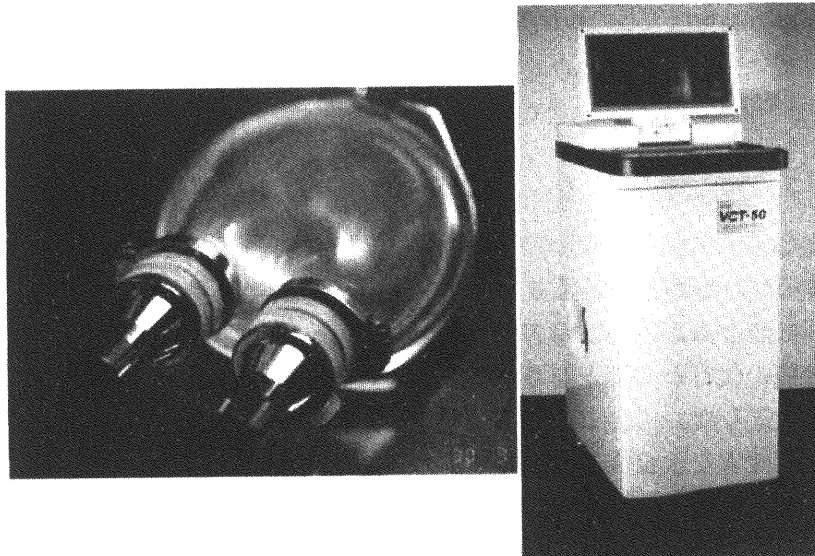


図2 東洋紡製国立循環器病センター型 VAS
 左：血液ポンプ
 右：制御駆動装置 (VCT-50)

の装着は、当初左房脱血—上行大動脈送血方式であったが、自己左室内での血栓形成や体位による脱血量の変動などが問題であった。このため、左室から直接脱血し左室内血栓形成の危険性も減少する左室心尖脱血方式が1999年に導入され、最近では主に後者が用いられる(図1左)。右心補助(RVAS)は、右房脱血—主肺動脈送血で行われる。送・脱血管は上腹部で体外へ出し、上腹部に設置された血液ポンプに接続される(図1左)。

2. 体内植え込み型左心補助人工心臓

わが国では、米国で開発された左室心尖脱血—上行大動脈送血の Novacor LVAS (図3右) および Thoratec 社製 HeartMate-VE LVAD (図3左) が臨床応用されている。Novacor は電磁力駆動プッシュプレート型で、耐久性に優れている。HeartMate-VE (Vented Electric) は、モータ駆動プッシュプレート型で、血液接触面は他のシステムでの smooth surface と異なり rough surface とすることで抗血小板剤の投与の

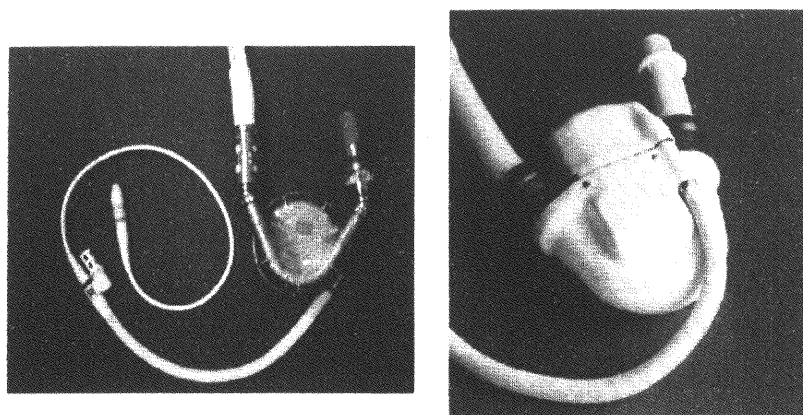


図3 体内植込み携帯型LVAS

左：Thoratec社製HeartMate Vented Electric (VE) LVAS
 右：Worldheart社製Novacor LVAS

表1 急性重症心不全の判定基準

主徴	
左心不全	
心係数	< 2.0 l/min/m ²
収縮期動脈圧	< 80~90 mmHg
左房圧	> 18 mmHg
右心不全	
心係数	< 2.0 l/min/m ²
収縮期動脈圧	< 80~90 mmHg
右房圧	> 18 mmHg
左房圧	< 5 mmHg
副徴	
尿量	< 0.5 ml/kg/h
SvO ₂	< 65 %
A-VDO ₂	> 7.0 vol %
臨床的印象	

みで良好な抗血栓性が得られている。Heart-Mateには空気圧駆動 (Implantable pneumatic type (IP)) 方式もあり、VE typeにおいて駆動系に問題が生じた場合には空気圧で駆動を続けることが可能である。血液ポンプ部は、左腹壁内か腹腔内に収納され、細いチューブにより体外の制御装置およびエネルギー源と接続される。小型制御装置およびバッテリーは体側への装着ができ、良好な活動性が得られ、退院が可能である (図1右)。しかし、両者とも開発においては体重

70~80 kg 前後の成人男性が想定されており、対象とされる患者の体表面積は1.5 m²以上で、体格の小さい人は対象外となる。

Novacorは、2004年4月に心臓移植へのブリッジ使用として、高度先進医療として心臓移植を経験している施設において、日本臓器移植ネットワーク登録している拡張型心筋症および拡張相肥大型心筋症患者への適応が健康保険で認められた。なお、HeartMate-VEは治験が終了した段階である。

補助人工心臓の適応とシステムの選択^{2)~4)}

循環補助手段としてはIABP、経皮的心肺補助法的 (PCPS) およびVASが一般的である。IABPは、簡便に用いることができるが、補助能力は自己心機能に依存しており限界がある。PCPSは、大腿動・静脈からアプローチでき、70%程度の循環補助が可能で呼吸補助も行える。しかし、左室の直接的な減負荷は得られず、左室機能高度低下例では肺水腫を来しやすい。また、長期施行が困難である。これに対し、VASは装着には開胸手術が必要であるが、心機能の100%代行が可能である。

急性心不全に対する補助循環の適応基準を表1に示すが、各種治療にもかかわらずこの基準から改善しない場合、上記に述べた各補助法の特徴を考慮してIABP、PCPSあるいはVASの適応を検討する。

表 2 慢性心不全急性増悪例に対する補助人工心臓の適応基準

1) 左心補助人工心臓	
内科的治療および/あるいは IABP に反応しない心不全	
1) 血行動態	PCWP ≥ 20 mmHg および 収縮期血圧 ≤ 80 mmHg あるいは心係数 ≤ 2.0
2) 副徴	1 時間排尿 ≤ 0.5 mL/kg SvO ₂ ≤ 60 % 臨床経過 急激な血行動態の変化 進行する腎機能障害* 進行する肝機能障害**
2) 右心補助人工心臓	
左心補助人工心臓駆動下において内科的治療および NO (一酸化窒素) 吸入に反応しない右心不全 (中等度以上の三尖弁逆流を伴う場合には三尖弁形成術を併用) CVP < 18 mmHg では、収縮期血圧 ≤ 80 mmHg あるいは心係数 ≤ 2.0	
3) 適用除外	
1) 回復不能な腎機能障害	
2) 回復不能な肝機能障害	
3) 呼吸不全 (循環不全に伴うものは除く)	
4) 高度な血液障害 (出血傾向など)	
5) 重症感染症	
6) インフォームドコンセントがとれない場合 (特に慢性心不全の急性増悪例)	

* : 進行する腎機能障害の指標
BUN ≥ 40 mg/dL および/あるいはクレアチニン ≥ 2 mg/dL
1 時間排尿 ≤ 0.5 mL/kg (利尿剤の使用下)

** : 進行する肝機能障害の指標
総ビリルビン ≥ 2.0 mg/dL および/あるいは SGOT ≥ 200 U/L

慢性心不全の急性増悪例に対しては、表 2 に示すように各種臓器機能への配慮が重要となる。不可逆性の腎・肝障害、敗血症、中枢神経疾患、高度の出血傾向を認める場合には適応から除外される。適応判定時に IABP や PCPS の限界を越えたと判断される症例に対しては、直接 VAS の適応を考慮する。

なお、VAS による循環補助が必要な症例では心機能の回復が期待し難い例が多く、適応判定においても次の治療選択としての心臓移植の可能性を検討する必要がある。また、VAS 適応例では、補助期間が長期になりさらに心臓移植待機の可能性もあるため、特に慢性心不全急性増悪例では本人および家族へのインフォームド・コンセントが

表 3 補助人工心臓の選択

両心補助 → 体外設置型 LV-LVAS+RVAS
左心補助 → BSA ≥ 1.5
• 植え込み携帯型 LVAS
• 体外設置型 LV-LVAS
BSA < 1.5
体外設置型 LV-LVAS

重要となる。このため VAS の適応決定に時間を要する場合もあることに注意する。

用いる VAS システムの選択 (表 3) は、重篤な右心不全を伴わず左心補助のみで対応可能と考えられる症例では、体格が大きければ (体表面積

表4 LV-LVASからの離脱基準

1. 安定した全身状態
2. 正常な臓器機能 (肝臓, 腎臓)
3. 感染 (-)
4. 低補助量で安定した血行動態 (東洋紡 LVAS では Pump Rate : 60 bpm)
5. 自己心機能 心エコー : 左室拡張末期径 (LVDd < 55 mm) 心拍数 < 100 bpm Dobutamine 負荷テスト : CI > 2.5 l/min/m ² 良好な左室指標の応答性 良好な SG カテ指標の応答性

1.5 m² 以上) 体内植え込み型を考慮する。体格の小さな症例では、左室脱血方式の体外設置型を用いる。高度右心不全を伴う症例では両心補助が必要となり、体外設置型で LVAS および RVAS の装着を行う。左心補助時の右心不全に対し、一酸化窒素 (NO) ガスが有効である症例が多い。さらに中等度以上の三尖弁逆流を伴った症例に対して三尖弁形成術を併用することで、著明な右心機能の改善を得られることが多い。

VAS 装着例における管理³⁾

VAS 装着後は、循環動態の改善による全身状態の安定化を計る。VAS 駆動法は、固有レートあるいは full-fill to full-empty モードを選択する。IABP で用いられるカウンタパルゼーション法は、自己心への負荷を軽減し回復を促進するので有効と考えられる。しかし、VAS 装着例では不整脈が頻発するケースが多く、カウンタパルゼーション法での駆動では不整脈時にポンプの駆動が停止するため、十分な補助量が得られない。また、常時安定した心電図を得ることは困難である。

VAS 装着後、早期に良好な自己心機能の改善が得られる症例では、そのまま離脱を考慮するが、多くの症例では長期の補助が必要となる。このため、全身状態安定後は、早期に抜管し、経口摂取を開始する。さらに感染の危険性を減少させるため、種々のラインの抜去を試みる。また、筋力低下例も多いため、早期からリハビリテーションを開始し、ベッド上での受動運動から筋力に応

じて運動量を増し、病室内での日常生活を行えるようにする。その後、自転車こぎや病棟内歩行などを加え、全身状態の改善を計る。

循環動態および全身状態が安定してから、ACE 阻害剤や β ブロッカーを含む内科的心不全治療を再開する。心エコー法や BNP 測定など適宜自己心機能を観察し、自己心機能の回復に応じて、補助量を減量し、運動量を増大する。補助量減量や運動量増大を行っても良好な自己心機能を示す症例においては、VAS からの離脱を図る。表4に当施設での左室脱血方式 LVAS の離脱基準は示す。最小補助下でのドブタミン負荷における自己心機能が判定を行う上で有用である。

VAS 装着中の抗凝固、抗血栓療法は、外科的出血がコントロールされてから開始する⁵⁾。経口摂取が早期から開始できる場合にはワーファリンを用い、PT-INR は術後早期では2を目標とし、安定期には3~4前後に維持する。なお、早期にワーファリンを開始できない場合や、PT-INR のコントロールに難渋するときにはヘパリン (通常低分子ヘパリン) を併用する。また、術後早期から抗血小板療法を併用する。通常はアスピリン 81 を1錠/日投与し、血小板機能に応じて投与量を調整する。

VAS 装着患者の管理を難渋させるものとして感染症があり、感染予防への配慮が重要である。特に、体外設置型での送・脱血管や体内植込み型でのチューブなど皮膚を貫く部分の処理に注意が必要である。また、長期補助例では精神状態への配慮も必要であり、精神神経科医を含めたサポー