cell surface proteins and ECM with maintained intercellular communications with the adjacent donor cells [93], assuring higher viability and functionality of donor cells.

The cell sheets, monolayer or multilayer, can be placed onto the epicardial heart surface to target the areas of interest. In addition to the preserved functionality of the cells, this method – epicardial placement of cell sheets – has been shown to cause less mechanical damage to both donor cells and host myocardium compared with other methods [94,95]. Furthermore, donor cell retention and subsequent therapeutic efficacies are known to be increased by this method compared with IM, IC and iv. injection [95].

Donor cell retention & survival following cell-sheet placement

It has been shown that the majority of donor cells that are transplanted by the cell-sheet technique retain on the epicardial surface, while only a small number of the transplanted cells are migrating or integrating into the host myocardium [96–98]. The initial donor cell retention rate after the cell-sheet technique is much higher compared with that after IC or IM injection [20,21,63,99].

Following epicardial placement of the cell sheets, the ECM and/or surface proteins of the cell sheets would generate firm attachment to the heart. This is thought to be the reason for the high retention rate after the cell-sheet method. Subsequent survival of the cells following cell-sheet transplantation has not been fully addressed. It was reported that transplanted cells surviving on the epicardial surface are supported by newly generated vascular networks from the host myocardium [99]. However, blood supply via such new vasculatures is likely to be insufficient and this might limit the long-term survival of the donor cells [100].

Therapeutic potential of the cell-sheet method in treating heart failure

It has been shown that the cell-sheet method using various types of adult stem/progenitor cells, including SMBs, MSCs and cardiac progenitor cells, induce functional recovery in heart failure [101,102]. The mechanism underlying this effect is believed to be the paracrine effects, in which donor cells secrete a specific group of protective factors into the host cardiac tissue to enhance recovery and healing and regenerating processes [95,96]. The magnitude of the paracrine effects has been suggested to be dependent upon the cell source and the number of surviving functional cells [96,103]. Therefore, the cell-sheet method, which can achieve an increased number of surviving donor cells in the heart, could theoretically achieve augmentation of the paracrine effects and, consequently, therapeutic outcome, compared with other cell-delivery methods [103]. There have been reports of a more than 30% improvement in ejection fraction of the LV by SMB cell-sheet transplantation in a chronic MI model, although there are no reports that appropriately compared recovery of cardiac function between the cell-sheet method and other cell-delivery methods [95,103].

Following a series of preclinical studies, this method has entered a clinical stage using SMBs in Japan [91]. In this Phase I clinical study, cell sheets generated by autologous SMBs were placed in patients who required mechanical support due to end-stage cardiac failure. The results are promising, although preliminary. The cell-sheet placement was feasible and safe, and improved cardiac function and structure in general, enabling successful weaning from the mechanical-assist device in some patients. Further large-scale, randomized clinical studies are warranted to prove the precise effect of this strategy.

Enhancing the cell-sheet method

Possible limitations of the cell-sheet method in achieving the maximal therapeutic potential include the poor vascular networks between the host myocardium and cell sheets, which will limit the survival of donor cells. Treatment of the cell sheet prior to the epicardial placement with heat shock or ischemic stimuli might upregulate protective factors in the cell sheet, enhancing the survival of donor cells. It has been shown that covering the cell sheets with the pedicle omentum flap, which possesses abundant vascular networks with angiogenic potentials, may be useful in supporting the transplanted cell sheet and enhancing its survival [100]. Concomitant injection of other cell types or substrates that carry angiogenic potentials into the target myocardial area could enhance generation of vascular network support for the cell sheets and might consequently enhance the therapeutic effects.

Conclusion & future perspective

Although randomized clinical studies have suggested that therapeutic benefits of cell transplantation therapy for treating cardiac failure may not be as substantial as expected in experimental studies, there are several promising strategies to further enhance therapeutic effects of this emerging treatment. The cell-delivery method is one of the most important targets for this aim.

Currently, there is no perfect method and, thus, donor cell type and cardiac pathology-specific choice of cell-delivery method would also be important.

Most adult stem cells, including MSCs, EPCs and SMBs, are likely to achieve the therapeutic effects via the paracrine effects, rather than differentiation to cardiomyocytes. For these cells, simple injection methods discussed above, such as IM, IC or cell-sheet methods, may be useful and subject to further enhancement. This is because paracrine effects can be induced even if donor cells do not necessarily form gap junctional intercellular communications with host cardiomyocytes. However, generation of new cardiomyocytes from donor cells is a major aim of cell transplantation therapy for the treatment of end-stage cardiac failure. To this end, cells that have established cardiomyogenic potency, including embryonic stem cells and induced pluripotent stem cells, are promising as donors, but these cell types require further consideration when choosing the cell-delivery route. New cardiomyocytes derived from these stem cells have to make appropriate intercellular communications – biochemically and electrically – with host cardiomyocytes. Otherwise, isolated new cardiomyocytes are unable to contribute to the global cardiac function and, furthermore, could be a source of arrhythmias. More substantial development, including tissue-engineering technologies, may be needed to transplant stem cell-derived cardiomyocytes into the heart.

One of the most important issues in this field that needs to be addressed is the development of clinically applicable *in vivo* cell-tracking methods [104]. Although functional and prognostic improvement is the principal goal of cell transplantation therapy for heart disease, assessment of the distribution and viability of transplanted cells after treatment would further clarify the efficacy and underlying mechanisms. Serial visualization of the transplanted cells after the treatment has been shown to be feasible by using nuclear imaging or MRI, although the cells need to be labeled by radioactives or genetic modification, respectively, prior to transplantation.

To conclude, there is no ideal cell-delivery method into the heart. Choice of cell-delivery method will be dependent upon the donor cell type and the nature of target cardiac pathology. Every combination that has been developed and studied has advantages and disadvantages. Appropriate comparison among the cell-delivery methods by preclinical or further large-scale, better organized clinical studies is warranted to further understand and improve the current cell-delivery methods, including the development of novel methods, leading to future clinical success of cell transplantation therapy for the treatment of heart failure.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

Executive summary

- There are four major cell-delivery routes into the heart for cell transplantation therapy treating heart disease: intramyocardial, intracoronary, intravenous and epicardial placement.
- Each cell-delivery route has potential advantages and disadvantages. The most suitable route should be taken in each patient in a tailor-made manner depending upon donor cell type and treating cardiac pathology.
- Further experimental and clinical investigations to further understand and refine each cell-delivery method are needed for the real success of cell transplantation therapy. The development of a novel route is also warranted.

References

Papers of special note have been highlighted as:
- ▪ of interest
- ▪▪ of considerable interest

1. Orlic D, Kajstura J, Chimenti S *et al.* Bone marrow cells regenerate infarcted myocardium. *Nature* 410(6829), 701–705 (2001).
2. Assmus B, Schachinger V, Teupe C *et al.* Transplantation of Progenitor Cells and Regeneration Enhancement in Acute Myocardial Infarction (TOPCARE-AMI). *Circulation* 106(24), 3009–3017 (2002).
3. Lunde K, Solheim S, Aakhus S *et al.* Intracoronary injection of mononuclear bone marrow cells in acute myocardial infarction. *N. Engl. J. Med.* 355(12), 1199–1209 (2006).
4. Schachinger V, Erbs S, Elsasser A *et al.* Intracoronary bone marrow-derived progenitor cells in acute myocardial infarction. *N. Engl. J. Med.* 355(12), 1210–1221 (2006).
   - ▪ **Well-organized randomized clinical study of cell transplantation therapy for acute myocardial infarction (MI) by intracoronary (IC) cell injection.**
5. Meyer GP, Wollert KC, Lotz J *et al.* Intracoronary bone marrow cell transfer after myocardial infarction: eighteen months' follow-up data from the randomized,

controlled BOOST (Bone Marrow Transfer to Enhance ST-Elevation Infarct Regeneration) trial. *Circulation* 113(10), 1287–1294 (2006).

▪ **Well-organized randomized clinical study of cell transplantation therapy for acute MI by IC cell injection.**

6. Ang KL, Chin D, Leyva F *et al.* Randomized, controlled trial of intramuscular or intracoronary injection of autologous bone marrow cells into scarred myocardium during CABG versus CABG alone. *Nat. Clin. Pract. Cardiovasc. Med.* 5(10), 663–670 (2008).
7. Beitnes JO, Hopp E, Lunde K *et al.* Long-term results after intracoronary injection of autologous mononuclear bone marrow cells in acute myocardial infarction: the ASTAMI randomised, controlled study. *Heart* 95(24), 1983–1989 (2009).
8. Grajek S, Popiel Mg, Gil L *et al.* Influence of bone marrow stem cells on left ventricle perfusion and ejection fraction in patients with acute myocardial infarction of anterior wall: randomized clinical trial. *Eur. Heart. J.* 31(6), 691–702 (2010).
9. Traverse JH, Henry TD, Ellis SG *et al.* Effect of intracoronary delivery of autologous bone marrow mononuclear cells 2 to 3 weeks following acute myocardial infarction on left ventricular function: the latetime randomized trial. *JAMA* 306(19), 2110–2119 (2011).

▪▪ **Recent well-organized randomized clinical study of cell transplantation therapy for acute MI by IC cell injection.**

10. van Ramshorst J, Bax JJ, Beeres SL *et al.* Intramyocardial bone marrow cell injection for chronic myocardial ischemia: a randomized controlled trial. *JAMA* 301(19), 1997–2004 (2009).
11. Janssens S, Dubois C, Bogaert J *et al.* Autologous bone marrow-derived stem-cell transfer in patients with ST-segment elevation myocardial infarction: double-blind, randomised controlled trial. *Lancet* 367(9505), 113–121 (2006).

▪ **Well-organized randomized clinical study in the early era of cell transplantation therapy for acute MI by IC cell injection.**

12. Menasche P, Alfieri O, Janssens S *et al.* The Myoblast Autologous Grafting in Ischemic Cardiomyopathy (MAGIC) trial: first randomized placebo-controlled study of myoblast transplantation. *Circulation* 117(9), 1189–1200 (2008).

▪ **Well-organized randomized clinical study of cell transplantation therapy for chronic MI by intramyocardial cell injection.**

13. Yacoub M, Suzuki K, Rosenthal N. The future of regenerative therapy in patients with chronic heart failure. *Nat. Clin. Pract. Cardiovasc. Med.* 39(Suppl. 1), S133–S135 (2006).
14. Nadal-Ginard B, Fuster V. Myocardial cell therapy at the crossroads. *Nat. Clin. Pract. Cardiovasc. Med.* 4(1), 1 (2007).
15. Steering Committee of the National Heart, Lung, and Blood Institute Cardiovascular Cell Therapy Research Network. Cardiac cell therapy: bench or bedside? *Nat. Clin. Pract. Cardiovasc. Med.* 4(8), 403 (2007).
16. Campbell N, Suzuki K. Cell delivery routes for stem cell therapy to the heart: current and future approaches. *J. Cardiovasc. Transl Res.* 5(5), 713–726 (2012).
17. Dib N, McCarthy P, Campbell A *et al.* Feasibility and safety of autologous myoblast transplantation in patients with ischemic cardiomyopathy. *Cell Transplant.* 14(1), 11–19 (2005).
18. Menasche P. Myoblast-based cell transplantation. *Heart Fail. Rev.* 8(3), 221–227 (2003).
19. Ott HC, Kroess R, Bonaros N *et al.* Intramyocardial microdepot injection increases the efficacy of skeletal myoblast transplantation. *Eur. J. Cardiothorac Surg.* 27(6), 1017–1021 (2005).
20. Hattori F, Chen H, Yamashita H *et al.* Nongenetic method for purifying stem cell-derived cardiomyocytes. *Nat. Methods* 7(1), 61–66 (2010).
21. Hou D, Youssef EA, Brinton TJ *et al.* Radiolabeled cell distribution after intramyocardial, intracoronary, and interstitial retrograde coronary venous delivery: implications for current clinical trials. *Circulation* 112(Suppl. 9), I150–I156 (2005).
22. Suzuki K, Murtuza B, Beauchamp JR *et al.* Dynamics and mediators of acute graft attrition after myoblast transplantation to the heart. *FASEB J.* 18(10), 1153–1155 (2004).
23. Suzuki K, Murtuza B, Beauchamp JR *et al.* Role of interleukin-1beta in acute inflammation and graft death after cell transplantation to the heart. *Circulation* 110(11 Suppl. 1), II219–II224 (2004).
24. Murtuza B, Suzuki K, Bou-Gharios G *et al.* Transplantation of skeletal myoblasts secreting an IL-1 inhibitor modulates adverse remodeling in infarcted murine myocardium. *Proc. Natl Acad. Sci. USA* 101(12), 4216–4221 (2004).
25. Reinecke H, Poppa V, Murry CE. Skeletal muscle stem cells do not transdifferentiate into cardiomyocytes after cardiac grafting. *J. Mol. Cell. Cardiol.* 34(2), 241–249 (2002).
26. Leobon B, Garcin I, Menasche P, Vilquin JT, Audinat E, Charpak S. Myoblasts transplanted into rat infarcted myocardium are functionally isolated from their host. *Proc. Natl Acad. Sci. USA* 100(13), 7808–7811 (2003).
27. Fukushima S, Varela-Carver A, Coppen SR *et al.* Direct intramyocardial but not intracoronary injection of bone marrow cells induces ventricular arrhythmias in a rat chronic ischemic heart failure model. *Circulation* 115(17), 2254–2261 (2007).

▪▪ **Basic study to explore arrhythmogenicity associated with cell transplantation therapy.**

28. Fukushima S, Coppen SR, Lee J *et al.* Choice of cell-delivery route for skeletal myoblast transplantation for treating post-infarction chronic heart failure in rat. *PLoS ONE* 3(8), e3071 (2008).
29. Menasche P, Hagege AA, Vilquin JT *et al.* Autologous skeletal myoblast transplantation for severe postinfarction left ventricular dysfunction. *J. Am. Coll. Cardiol.* 41(7), 1078–1083 (2003).
30. Murry CE, Wiseman RW, Schwartz SM, Hauschka SD. Skeletal myoblast transplantation for repair of myocardial necrosis. *J. Clin. Invest.* 98(11), 2512–2523 (1996).

▪ **First report of the therapeutic effects of skeletal myoblast injection therapy on the injured heart.**

31. Taylor DA, Atkins BZ, Hungspreugs P *et al.* Regenerating functional myocardium: improved performance after skeletal myoblast transplantation. *Nat. Med.* 4(8), 929–933 (1998).
32. Yoon YS, Wecker A, Heyd L *et al.* Clonally expanded novel multipotent stem cells from human bone marrow regenerate myocardium after myocardial infarction. *J. Clin. Invest.* 115(2), 326–338 (2005).
33. Kimura T, Miyoshi S, Takatsuki S *et al.* Safety and efficacy of pericardial endoscopy by percutaneous subxyphoid approach in swine heart *in vivo*. *J. Thorac Cardiovasc. Surg.* 142(1), 181–190 (2011).
34. Losordo DW, Schatz RA, White CJ *et al.* Intramyocardial transplantation of autologous CD34$^+$ stem cells for intractable angina. *Circulation* 115(25), 3165–3172 (2007).
35. Kumar A, Haralampus CA, Hughes M *et al.* Assessment of safety, accuracy, and human CD34$^+$ cell retention after intramyocardial injections with a helical needle catheter in a porcine model. *Cathet. Cardiovasc. Intervent.* doi:10.1002/ccd.24476 (2012) (Epub ahead of print).
36. Trachtenberg B, Velazquez DL, Williams AR *et al.* Rationale and design of the Transendocardial Injection of Autologous

Human Cells (bone marrow or mesenchymal) in Chronic Ischemic Left Ventricular Dysfunction and Heart Failure Secondary to Myocardial Infarction (TAC-HFT) trial: a randomized, double-blind, placebo-controlled study of safety and efficacy. *Am. Heart J.* 161(3), 487–493 (2011).

37. Siminiak T, Fiszer D, Jerzykowska O *et al.* Percutaneous trans-coronary-venous transplantation of autologous skeletal myoblasts in the treatment of post-infarction myocardial contractility impairment: the POZNAN trial. *Eur. Heart J.* 26(12), 1188–1195 (2005).
38. Brasselet C, Morichetti MC, Messas E *et al.* Skeletal myoblast transplantation through a catheter-based coronary sinus approach: an effective means of improving function of infarcted myocardium. *Eur. Heart J.* 26(15), 1551–1556 (2005).
39. Penn MS, Ellis S, Gandhi S *et al.* Adventitial delivery of an allogeneic bone marrow derived adherent stem cell in acute myocardial infarction/novelty and significance. *Circ. Res.* 110(2), 304–311 (2012).
40. Siminiak T, Kalawski R, Fiszer D *et al.* Autologous skeletal myoblast transplantation for the treatment of postinfarction myocardial injury: Phase I clinical study with 12 months of follow-up. *Am. Heart J.* 148(3), 531–537 (2004).
41. Stamm C, Kleine HD, Choi YH *et al.* Intramyocardial delivery of CD133$^+$ bone marrow cells and coronary artery bypass grafting for chronic ischemic heart disease: safety and efficacy studies. *J. Thorac Cardiovasc. Surg.* 133(3), 717–725 (2007).
42. Patel AN, Geffner L, Vina RF *et al.* Surgical treatment for congestive heart failure with autologous adult stem cell transplantation: a prospective randomized study. *J. Thorac Cardiovasc. Surg.* 130(6), 1631–1638 (2005).
43. Zhao Q, Sun Y, Xia L, Chen A, Wang Z. Randomized study of mononuclear bone marrow cell transplantation in patients with coronary surgery. *Ann. Thorac Surg.* 86(6), 1833–1840 (2008).
44. Agbulut O, Mazo M, Bressolle C *et al.* Can bone marrow-derived multipotent adult progenitor cells regenerate infarcted myocardium? *Cardiovasc. Res.* 72(1), 175–183 (2006).
45. Menasche P, Hagege AA, Scorsin M *et al.* Myoblast transplantation for heart failure. *Lancet* 357(9252), 279–280 (2001).
46. Losordo DW, Schatz RA, White CJ *et al.* Intramyocardial transplantation of autologous CD34$^+$ stem cells for intractable angina: a Phase I/IIa double-blind, randomized controlled trial. *Circulation* 115(25), 3165–3172 (2007).
47. Perin EC, Willerson JT, Pepine CJ *et al.* Effect of transendocardial delivery of autologous bone marrow mononuclear cells on functional capacity, left ventricular function, and perfusion in chronic heart failure: the FOCUS-CCTRN trial. *JAMA* 307(16), 1717–1726 (2012).

▪▪ **Recent well-organized randomized clinical study of cell transplantation therapy for chronic MI by intramyocardial cell injection.**

48. Perin EC, Silva GV, Henry TD *et al.* A randomized study of transendocardial injection of autologous bone marrow mononuclear cells and cell function analysis in ischemic heart failure (FOCUS-HF). *Am. Heart J.* 161(6), 1078–1087 (2011).
49. Arom KV, Ruengsakulrach P, Jotisakulratana V. Intramyocardial angiogenic cell precursor injection for cardiomyopathy. *Asian Cardiovasc. Thorac Ann.* 16(2), 143–148 (2008).
50. Laflamme MA, Chen KY, Naumova AV *et al.* Cardiomyocytes derived from human embryonic stem cells in pro-survival factors enhance function of infarcted rat hearts. *Nat. Biotechnol.* 25(9), 1015–1024 (2007).
51. Suzuki K, Smolenski RT, Jayakumar J, Murtuza B, Brand NJ, Yacoub MH. Heat shock treatment enhances graft cell survival in skeletal myoblast transplantation to the heart. *Circulation* 102(19 Suppl. 3), III216–III221 (2000).
52. Lee WY, Wei HJ, Lin WW *et al.* Enhancement of cell retention and functional benefits in myocardial infarction using human amniotic-fluid stem-cell bodies enriched with endogenous ECM. *Biomaterials* 32(24), 5558–5567 (2011).
53. Huang NF, Yu J, Sievers R, Li S, Lee RJ. Injectable biopolymers enhance angiogenesis after myocardial infarction. *Tissue Eng.* 11(11–12), 1860–1866 (2005).
54. Christman KL, Vardanian AJ, Fang Q, Sievers RE, Fok HH, Lee RJ. Injectable fibrin scaffold improves cell transplant survival, reduces infarct expansion, and induces neovasculature formation in ischemic myocardium. *J. Am. Coll. Cardiol.* 44(3), 654–660 (2004).
55. Nakamuta JS, Danoviz ME, Marques FLN *et al.* Cell therapy attenuates cardiac dysfunction post myocardial infarction: effect of timing, routes of injection and a fibrin scaffold. *PLoS ONE* 4(6), e6005 (2009).
56. Ren J, Samson WK, Sowers JR. Insulin-like growth factor I as a cardiac hormone: physiological and pathophysiological implications in heart disease. *J. Mol. Cell. Cardiol.* 31(11), 2049–2061 (1999).
57. Davis ME, Hsieh PC, Takahashi T *et al.* Local myocardial insulin-like growth factor 1 (IGF-1) delivery with biotinylated peptide nanofibers improves cell therapy for myocardial infarction. *Proc. Natl Acad. Sci. USA* 103(21), 8155–8160 (2006).
58. Yau TM, Kim C, Li G, Zhang Y, Weisel RD, Li RK. Maximizing ventricular function with multimodal cell-based gene therapy. *Circulation* 112(Suppl. 9), I123–I128 (2005).
59. Yau TM, Kim C, Ng D *et al.* Increasing transplanted cell survival with cell-based angiogenic gene therapy. *Ann. Thorac Surg.* 80(5), 1779–1786 (2005).
60. Mangi AA, Noiseux N, Kong D *et al.* Mesenchymal stem cells modified with Akt prevent remodeling and restore performance of infarcted hearts. *Nat. Med.* 9(9), 1195–1201 (2003).
61. Hagege AA, Marolleau JP, Vilquin JT *et al.* Skeletal myoblast transplantation in ischemic heart failure: long-term follow-up of the first Phase I cohort of patients. *Circulation* 114(Suppl. 1), I108–I113 (2006).
62. Strauer BE, Brehm M, Zeus T *et al.* [Intracoronary, human autologous stem cell transplantation for myocardial regeneration following myocardial infarction]. *Dtsch Med. Wochenschr.* 126(34–35), 932–938 (2001).
63. Fukushima S, Campbell NG, Coppen SR *et al.* Quantitative assessment of initial retention of bone marrow mononuclear cells injected into the coronary arteries. *J. Heart Lung Transplant.* 30(2), 227–233 (2011).
64. Schachinger V, Assmus B, Britten MB *et al.* Transplantation of progenitor cells and regeneration enhancement in acute myocardial infarction: final one-year results of the TOPCARE-AMI Trial. *J. Am. Coll. Cardiol.* 44(8), 1690–1699 (2004).
65. Hirsch A, Nijveldt R, van der Vleuten PA *et al.* Intracoronary infusion of autologous mononuclear bone marrow cells or peripheral mononuclear blood cells after primary percutaneous coronary intervention: rationale and design of the HEBE trial – a prospective, multicenter, randomized trial. *Am. Heart J.* 152(3), 434–441 (2006).
66. Suzuki K, Brand NJ, Smolenski RT, Jayakumar J, Murtuza B, Yacoub MH. Development of a novel method for cell transplantation through the coronary artery. *Circulation* 102(19), 359–364 (2000).

67. Vulliet PR, Greeley M, Halloran SM, MacDonald KA, Kittleson MD. Intra-coronary arterial injection of mesenchymal stromal cells and microinfarction in dogs. *Lancet* 363(9411), 783–784 (2004).

68. Suzuki K, Murtuza B, Smolenski RT, Yacoub MH. Selective cell dissemination into the heart by retrograde intracoronary infusion in the rat. *Transplantation* 77(5), 757–759 (2004).

69. Suzuki K, Murtuza B, Fukushima S *et al.* Targeted cell delivery into infarcted rat hearts by retrograde intracoronary infusion: distribution, dynamics, and influence on cardiac function. *Circulation* 110(11 Suppl. 1), II225–II230 (2004).

70. Clifford DM, Fisher SA, Brunskill SJ *et al.* Stem cell treatment for acute myocardial infarction. *Cochrane. Database. Syst. Rev.* 2, CD006536 (2012).

71. Jeevanantham V, Butler M, Saad A, Bdel-Latif A, Zuba-Surma EK, Dawn B. Adult bone marrow cell therapy improves survival and induces long-term improvement in cardiac parameters/clinical perspective. *Circulation* 126(5), 551–568 (2012).

72. Bolli R, Chugh AR, D'Amario D *et al.* Cardiac stem cells in patients with ischaemic cardiomyopathy (SCIPIO): initial results of a randomised Phase 1 trial. *Lancet* 378(9806), 1847–1857 (2011).

73. Makkar RR, Smith RR, Cheng K *et al.* Intracoronary cardiosphere-derived cells for heart regeneration after myocardial infarction (CADUCEUS): a prospective, randomised Phase 1 trial. *Lancet* 379(9819), 895–904 (2010).

74. Segers VF, Van R, I, Andries LJ *et al.* Mesenchymal stem cell adhesion to cardiac microvascular endothelium: activators and mechanisms. *Am. J. Physiol. Heart Circ. Physiol.* 290(4), H1370–H1377 (2006).

75. Wu Y, Ip JE, Huang J *et al.* Essential role of ICAM-1/CD18 in mediating EPC recruitment, angiogenesis, and repair to the infarcted myocardium. *Circ. Res.* 99(3), 315–322 (2006).

76. Tossios P, Krausgrill B, Schmidt M *et al.* Role of balloon occlusion for mononuclear bone marrow cell deposition after intracoronary injection in pigs with reperfused myocardial infarction. *Eur. Heart. J.* 29, 1911–1921 (2008).

77. Zhao ZQ, Corvera JS, Halkos ME *et al.* Inhibition of myocardial injury by ischemic postconditioning during reperfusion: comparison with ischemic preconditioning. *Am. J. Physiol. Heart Circ. Physiol.* 285(2), H579–H588 (2003).

78. Assmus B, Honold J, Schachinger V *et al.* Transcoronary transplantation of progenitor cells after myocardial infarction. *N. Engl. J. Med.* 355(12), 1222–1232 (2006).

▪ **Well-organized randomized clinical study of cell transplantation therapy for acute MI by IC cell injection.**

79. Doyle B, Kemp BJ, Chareonthaitawee P *et al.* Dynamic tracking during intracoronary injection of ^{18}F-FDG-labeled progenitor cell therapy for acute myocardial infarction. *J. Nucl. Med.* 48(10), 1708–1714 (2007).

80. Seeger FH, Rasper T, Fischer A *et al.* Heparin disrupts the CXCR4/SDF-1 axis and impairs the functional capacity of bone marrow derived mononuclear cells used for cardiovascular repair/novelty and significance. *Circ. Res.* 111(7), 854–862 (2012).

81. Kloner RA. No-reflow phenomenon: maintaining vascular integrity. *J. Cardiovasc. Pharmacol. Ther.* 16(3–4), 244–250 (2011).

82. Robinson SW, Cho PW, Levitsky HI *et al.* Arterial delivery of genetically labelled skeletal myoblasts to the murine heart: long-term survival and phenotypic modification of implanted myoblasts. *Cell Transplant.* 5(1), 77–91 (1996).

83. Hayashi M, Li TS, Ito H, Mikamo A, Hamano K. Comparison of intramyocardial and intravenous routes of delivering bone marrow cells for the treatment of ischemic heart disease: an experimental study. *Cell Transplant.* 13(6), 639–647 (2004).

84. Aicher A, Brenner W, Zuhayra M *et al.* Assessment of the tissue distribution of transplanted human endothelial progenitor cells by radioactive labeling. *Circulation* 107(16), 2134–2139 (2003).

85. Forest V, Tirouvanziam A, Perigaud C *et al.* Cell distribution after intracoronary bone marrow stem cell delivery in damaged and undamaged myocardium: implications for clinical trials. *Stem Cell Res. Ther.* 1(1), 4 (2010).

86. Brenner W, Aicher A, Eckey T *et al.* ^{111}In-labeled CD34$^+$ hematopoietic progenitor cells in a rat myocardial infarction model. *J. Nucl. Med.* 45(3), 512–518 (2004).

87. Kraitchman DL, Tatsumi M, Gilson WD *et al.* Dynamic imaging of allogeneic mesenchymal stem cells trafficking to myocardial infarction. *Circulation* 112(10), 1451–1461 (2005).

88. Schots R, De Keulenaer G, Schoors D *et al.* Evidence that intracoronary-injected CD133$^+$ peripheral blood progenitor cells home to the myocardium in chronic postinfarction heart failure. *Exp. Hematol.* 35, 1884–1890 (2007).

89. Hare JM, Traverse JH, Henry TD *et al.* A randomized, double-blind, placebo-controlled, dose-escalation study of intravenous adult human mesenchymal stem cells (prochymal) after acute myocardial infarction. *J. Am. Coll. Cardiol.* 54(24), 2277–2286 (2009).

90. Okano T, Yamada N, Sakai H, Sakurai Y. A novel recovery system for cultured cells using plasma-treated polystyrene dishes grafted with poly (*N*-isopropylacrylamide). *J. Biomed. Mater. Res.* 27(10), 1243–1251 (1993).

91. Sawa Y, Miyagawa S, Sakaguchi T *et al.* Tissue engineered myoblast sheets improved cardiac function sufficiently to discontinue LVAS in a patient with DCM: report of a case. *Surg. Today* 42(2), 181–184 (2012).

▪▪ **First-in-man study for cell-sheet placement for heart disease.**

92. Yang J, Yamato M, Kohno C *et al.* Cell sheet engineering: recreating tissues without biodegradable scaffolds. *Biomaterials* 26(33), 6415–6422 (2005).

93. Elloumi-Hannachi I, Yamato M, Okano T. Cell sheet engineering: a unique nanotechnology for scaffold-free tissue reconstruction with clinical applications in regenerative medicine. *J. Int. Med.* 267(1), 54–70 (2010).

94. Shimizu T, Yamato M, Isoi Y *et al.* Fabrication of pulsatile cardiac tissue grafts using a novel 3-dimensional cell sheet manipulation technique and temperature-responsive cell culture surfaces. *Circ. Res.* 90(3), e40–e48 (2002).

95. Miyagawa S, Saito A, Sakaguchi T *et al.* Impaired myocardium regeneration with skeletal cell sheets – a preclinical trial for tissue-engineered regeneration therapy. *Transplantation* 90(4), 364–372 (2010).

96. Imanishi Y, Miyagawa S, Maeda N *et al.* Induced adipocyte cell-sheet ameliorates cardiac dysfunction in a mouse myocardial infarction model. *Circulation* 124(11 Suppl. 1), S10–S17 (2011).

97. Kondoh H, Sawa Y, Miyagawa S *et al.* Longer preservation of cardiac performance by sheet-shaped myoblast implantation in dilated cardiomyopathic hamsters. *Cardiovasc. Res.* 69(2), 466–475 (2006).

98. Memon IA, Sawa Y, Fukushima N *et al.* Repair of impaired myocardium by means of implantation of engineered autologous myoblast sheets. *J. Thorac Cardiovasc. Surg.* 130(5), 1333–1341 (2005).

99. Sekine H, Shimizu T, Dobashi I *et al.* Cardiac cell sheet transplantation improves damaged heart function via superior cell survival in

comparison with dissociated cell injection. *Tissue Eng. Part A* 17(23–24), 2973–2980 (2011).

100. Shudo Y, Miyagawa S, Fukushima S *et al.* Novel regenerative therapy using cell-sheet covered with omentum flap delivers a huge number of cells in a porcine myocardial infarction model. *J. Thorac Cardiovasc. Surg.* 142(5), 1188–1196 (2011).

101. Ptaszek LM, Mansour M, Ruskin JN, Chien KR. Towards regenerative therapy for cardiac disease. *Lancet* 379(9819), 933–942 (2012).

102. Dai W, Kloner RA. Bone marrow-derived cell transplantation therapy for myocardial infarction: lessons learned and future questions. *Am. J. Transplant.* 11(11), 2297–2301 (2011).

103. Sekiya N, Matsumiya G, Miyagawa S *et al.* Layered implantation of myoblast sheets attenuates adverse cardiac remodeling of the infarcted heart. *J. Thorac Cardiovasc. Surg.* 138(4), 985–993 (2009).

104. Zhou R, Acton PD, Ferrari VA. Imaging stem cells implanted in infarcted myocardium. *J. Am. Coll. Cardiol.* 48(10), 2094–2106 (2006).

Surg Today (2013) 43:970–976
DOI 10.1007/s00595-013-0528-2

ORIGINAL ARTICLE

Impact of cardiac stem cell sheet transplantation on myocardial infarction

Sfoug Alshammary · Satsuki Fukushima · Shigeru Miyagawa · Takenori Matsuda · Hiroyuki Nishi · Atsuhiro Saito · Sokichi Kamata · Takayuki Asahara · Yoshiki Sawa

Received: 28 June 2012 / Accepted: 2 July 2012 / Published online: 5 March 2013

Abstract
Purpose Myocardial infarction (MI) remains a major cause of mortality because of the limited regenerative capacity of the myocardium. Transplantation of somatic tissue-derived cells into the heart has been shown to enhance the endogenous healing process, but the magnitude of its therapeutic effects is dependent upon the cell-source or cell-delivery method. We investigated the therapeutic effects of C-Kit positive cardiac cell (CSC) cell-sheet transplantation therapy in a rat model of MI.
Methods and results CSCs of human origin were sorted and cultured to generate scaffold-free CSC cell-sheets. One-layered or 3-layered cell-sheets were transplanted into nude rats 1 h after left coronary artery ligation. We observed a significant increase in the left ventricular ejection fraction and a significant decrease in left ventricular systolic dimension at 2 and 4 weeks in the 3-layer group, but not in the 1-layer or sham groups. Consistently, there was less accumulation of interstitial fibrosis in the 3-layer group than in the 1-layer or sham groups. Moreover, capillary density was significantly greater in the 3-layer group than in the 1-layer or sham groups.
Conclusions The 3-layered cell-sheet improved cardiac function associated with angiogenic and anti-fibrotic effects. Thus, CSC is a promising cell-source to use with the cell-sheet method for the treatment of cardiac failure, as long as a sufficient number of cells are delivered.

Keywords Cardiac · Stem cell · Myocardial infarction

Introduction

The limited regenerative capacity of the myocardium accounts for the fact that cardiac failure related to myocardial infarction (MI) remains a major cause of morbidity and mortality worldwide, despite major advances in medical and/or interventional treatments [1]. The treatment of cardiac failure relies on strategies that are designed to target and/or limit residual or persistent myocardial ischemia, additional myocardial damage, pathological cardiac remodeling, and hemodynamic impairment, including cardiac dyssynchrony [2]. On the other hand, the transplantation of somatic tissue-derived stem/progenitor cells into the heart has been shown to enhance the endogenous healing process of the damaged myocardium, while the magnitude of the therapeutic effects are dependent on the cell-source, cell-number, cell-delivery method, and target cardiac pathology [3–5]. It has been shown that the transplantation of C-kit-positive heart-derived cells into the MI heart yields functional recovery, mediated by proliferation and differentiation into the heart-composing cells in situ, and by releasing cardioprotective factors that activate native healing processes [6]. However, the optimal preparation and delivery method of CSCs into the heart has not been established.

The cell-sheet method, in which aggregated cells in a sheet shape cultured under a thermoresponsive dish are attached to the epicardial surface [7], has been shown to deliver a large scale of cultured cells with minimal damage to the cells or native cardiac tissues [8]. This enhances its therapeutic effects and minimizes inflammation-related

S. Alshammary · S. Fukushima · S. Miyagawa · T. Matsuda · H. Nishi · A. Saito · S. Kamata · Y. Sawa (✉)
Department of Cardiovascular Surgery,
Osaka University Graduate School of Medicine,
2-2 Yamadaoka, Suita 565-0871, Japan
e-mail: sawa-p@surg1.med.osaka-u.ac.jp

T. Asahara
Institute of Biomedical Research and Innovation, Kobe, Japan

complications, representing a promising cell-delivery method in CSC transplantation therapy [9]. However, there are concerns about potential ischemia of the implanted cell-sheet, which would limit cellular function, survival, and therapeutic potential. According to a previous study, a 3-layered cell-sheet generated by skeletal myoblasts showed greater therapeutic effects than a 1-layered cell-sheet, while a 5-layered cell-sheet did not enhance the effects, possibly because of ischemia in the implanted cell-sheet [10]. Based on the hypothesis that the therapeutic potential of CSC cell-sheet treatment might be dependent on the number of layers of the cell-sheet, we investigated the therapeutic effects of CSC cell-sheet transplantation therapy on MI hearts using a rat model.

Methods

All studies using human tissues and experimental animals were carried out under approval of the institutional ethical committee. Human tissues were collected only after obtaining written informed consent. This investigation conforms to the Principles of Laboratory Animal Care formulated by the National Society for Medical Research and the Guide for the Care and Use of Laboratory Animals (US National Institutes of Health Publication No. 85-23, revised 1996). All experimental procedures and evaluations were carried out in a blinded manner.

Isolation and culture of C-Kit-positive human cardiac cells and cell-sheet generation

Discarded cardiac tissue samples were taken from the left ventricular apex of a 31-year-old man with dilated cardiomyopathy, requiring daily cardiovascular procedures in Osaka University Hospital. Cardiac cells were dissociated from the tissues, cultured, and then sorted for C-kit using FACSAria (BD Biosciences) to yield C-Kit positive cardiac cells, which were then cultured for expansion with multiple passages. The cells were then incubated in thermoresponsive dishes (35 mm UpCell, CellSeed, Tokyo, Japan) at 37 °C for 2 days prior to transplantation, when the cells were incubated at 25 °C to induce their spontaneous detachment, to yield a mono-layered scaffold-free CSC cell-sheet that included 1.5×10^6 cells (Fig. 1a). The 3-layered cell-sheet was generated by filling up the mono-layered cell-sheet, as described previously [10].

Generation of AMI model and CSC cell-sheet transplantation

Thirty-nine athymic female nude rats, 8 weeks of age, were subjected to permanent ligation of the left coronary artery (LCA) under general anesthesia with endotracheal intubation and isoflurane inhalation, as previously described [10]. LCA ligation-related death occurred prior to treatment in 16 %. The rats that survived for 50 min after the ligation were randomly assigned to the following three treatment groups: transplantation of a 3-layered cell-sheet ($n = 12$), transplantation of a l-layered cell-sheet ($n = 10$), or a sham operation ($n = 11$). In the two transplantation groups, the cell-sheet was attached directly to the epicardial surface of the ischemic/infarct area (Fig. 1b) [10]. The cell-sheet was large enough to cover all of the ischemic or infarcted area. By 20 min after the transplantation, when the cell-sheets were properly fixed to the cardiac surface, the chest was closed and the rats were allowed to recover in individual temperature-controlled cages until they were killed 28 days after the treatment.

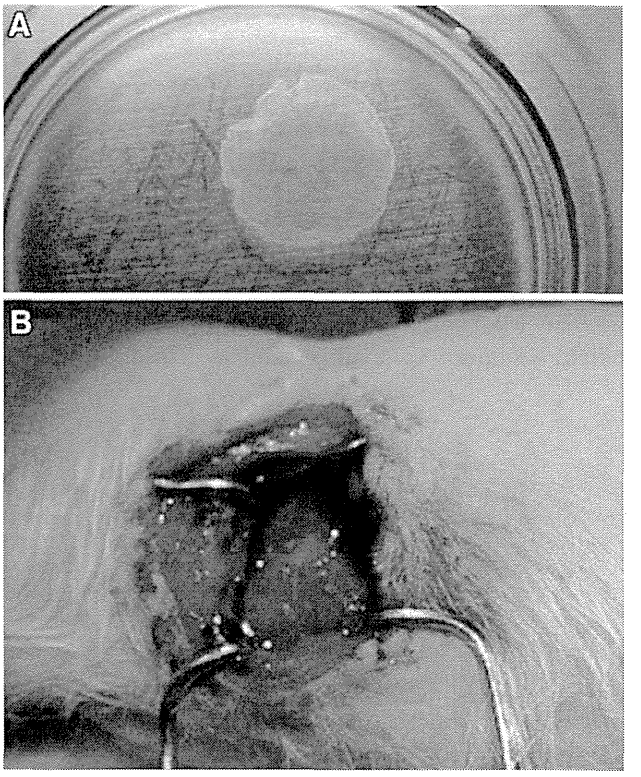

Fig. 1 A mono-layered cell-sheet was generated by c-kit positive cardiac cells of human origin on thermoresponsive dishes in vitro (**a**). A mono-layered or 3-layered cell-sheet was transplanted over the left ventricular free wall of the rat heart, which had been subjected to ischemia by permanent ligation of the corresponding coronary artery, 1 h prior to the treatment (**b**)

Transthoracic echocardiography

Transthoracic echocardiography was performed under isoflurane inhalation, using a system equipped with a 12 MHZ transducer (GE Healthcare). Diastolic and systolic dimensions of the left ventricular diastolic and

systolic dimensions (LVDD and LVDS, respectively) were measured at the papillary muscle level by the M-mode, while the LV ejection fraction (LVEF) was calculated by the following formula: $(LVDD^3–LVDS^3)/LVDd^3 \times 100$ [10, 11].

Histology

The ventricles were immerse-fixed in 4 % paraformaldehyde, embedded in paraffin, and cut into 5 micrometres using a microtome for histological studies. The sections were stained by hematoxylin–eosin (HE) or Masson trichrome (MT) and assessed by optical microscopy (Olympus, Tokyo, Japan). Metamorph software was used to separate stained and non-stained myocardium by MT staining and to quantitatively calculate each area. The sections were labeled immunohistologically by polyclonal anti-von Willebrand factor antibody (vWF, DAKO, Glostrup, Denmark), and visualized by the LSABTM kit (DAKO), which is an automated immunostaining system based on the LSAB Lepto strept avidin–biotin-peroxidase method. The sections were labeled immunohistologically by the anti-human-specific HLA antibody or anti-cardiac troponin (cTn) I antibody, visualized by corresponding secondary antibodies that were counterstained by DAPI, and assessed by confocal microscopy (Olympus).

Statistics

Values are expressed as mean ± SEM. The three groups were compared with 1-way or 2-way ANOVA as appropriate, followed by the Fisher protected least-significant difference test, or the Kruskal–Wallis test, followed by the post hoc pairwise Wilcoxon-Mann–Whitney U test, as appropriate. Differences were considered significant at $P < 0.05$. All analyses were performed using SPSS for Windows (SPSS, Chicago, IL, USA).

Results

Functional recovery following CSC cell-sheet transplantation

Scaffold-free CSC cell-sheet was prepared from primary C-kit positive cardiac cells of human origin, cultivated in thermoresponsive dishes. We transplanted the 1-layered or 3-layered cell-sheets onto the epicardial surface of the nude rat 1 h after the permanent LCA ligation. A sham operation was performed for the control group. Cardiac performance was serially assessed by transthoracic echocardiography just after the treatment (baseline), and then 1, 2, and 4 weeks after the treatment.

Before any intervention, the LVEF, LVDD, and LVDS did not differ significantly among the groups (Fig. 2). However, for 4 weeks after treatment, the LVEF showed a significantly progressive reduction, while the LVDD and LVDS showed a significantly progressive increase in the sham group and the 1-layer group. Conversely, in the 3-layer group, the LVEF showed a significant increase, and the LVDS showed a significant decrease 2 and 4 weeks following the transplantation, while the LVDD did not change significantly in this group over the 4 weeks. Notably, the LVEF in the 3-layer group was significantly greater than that in the 1-layer group or sham group, while the LVDS in the 3-layer group was significantly lower than that in the 1-layer group or sham group. The LVDD did not differ significantly among the groups at any time.

Histological reverse LV remodeling following CSC cell-sheet transplantation

We assessed gross structure, interstitial fibrosis and capillary distribution in the myocardium 4 weeks after the CSC cell-sheet transplantation to qualitatively and semi-quantitatively explore the degree of LV remodeling in each group by HE staining, Masson Trichrome staining, and immunohistolabelling for von Willebrand factor, respectively. The infarcted area, in which the cell-sheet was transplanted, was clearly thicker in the 3-layer group than in the 1-layer or sham groups, as assessed by the HE staining (Fig. 3a–c). In addition, the myocardial structure in the peri-infarcted area was better preserved in the 3-layer group than in the 1-layer group or the sham group. There seemed to be less accumulation of interstitial fibrosis in the peri-infarcted and infarct-remote myocardium of the 3-layer group than in the 1-layer group or sham groups (Fig. 3d–f). In fact, computer-based morphometry confirmed significantly less fibrosis in the 3-layer groups than in the 1-layer group or sham group (Fig. 4a). Capillary density in the peri-infarcted myocardium was significantly greater in the 3-layer group than in the 1-layer group or sham group (Fig. 4b).

Phenotypic fate of the transplanted CSCs in the heart

The transplanted CSCs in the heart were phenotypically assessed by immunohistolabelling for human-specific HLA, which clearly dissected the transplanted cells in the native cardiac tissue. While the transplanted cells were rarely present in the 1-layer group 4 weeks after transplantation, the 3-layer group showed abundant human-specific HLA-positive transplanted cells in the tissues epicardially attached to the native cardiac tissue, which were assumed to consist of the remaining transplanted cell sheet and accumulated cells of native origin (Fig. 5a).

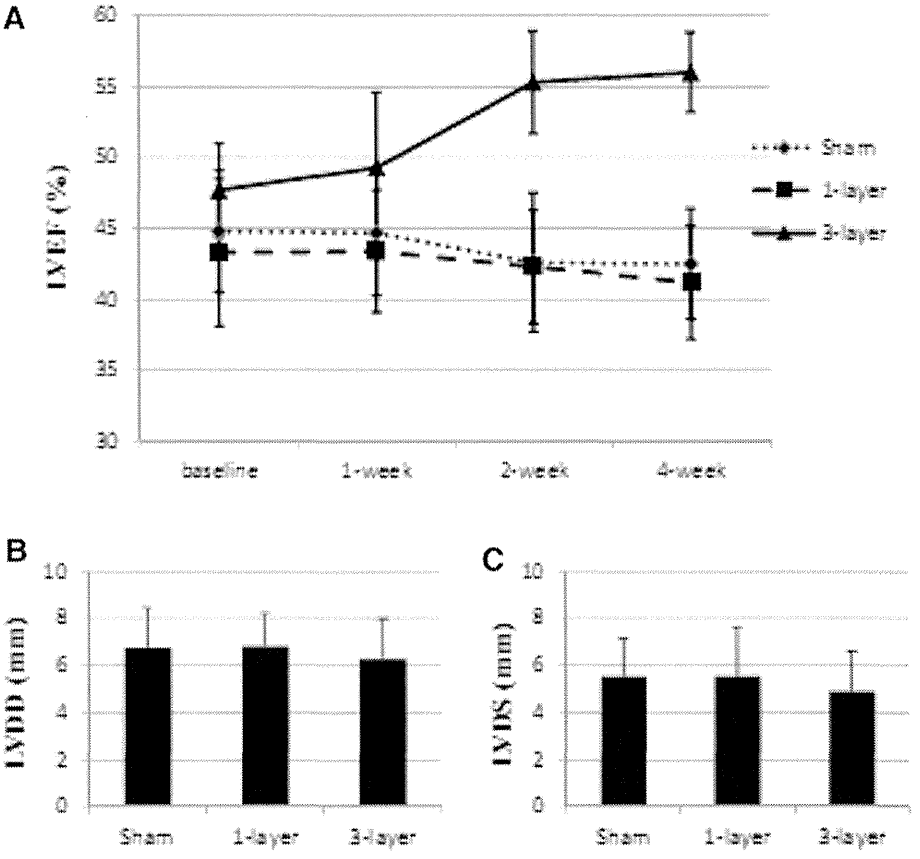

Fig. 2 Cardiac performance measures, such as left ventricular ejection fraction (LVEF) (**a**), LV diastolic dimension (LVDD, **b**), and LV systolic dimension (LVDS, **c**), were assessed echocardiographically immediately after treatment and then 1, 2, and 4 weeks after treatment (sham operation vs. 1-layer cell-sheet transplantation vs. 3-layer cell-sheet transplantation)

Notably, some human-specific HLA-positive transplanted cells were present in the native myocardium, suggesting the migration of transplanted cells into the native cardiac tissue (Fig. 5b–d).

Discussion

This study demonstrated clearly that the transplantation of CSC cell-sheets to treat the MI heart yielded significant recovery of cardiac performance in a cell-sheet layer dependent manner. Consistently, the hearts transplanted with the multi-layered cell-sheet showed significantly more preserved gross myocardial structure, reduced interstitial fibrosis, and increased capillary density than the hearts transplanted with a mono-layered cell-sheet. Moreover, the differentiation of heart-composing cells, including cardiomyocytes, endothelial cells, and vascular smooth muscle cells, was greater in the hearts transplanted with the multi-layered cell-sheet than in those transplanted with the mono-layered cell-sheet.

The transplanted cell-source is known to be a major determinant of the therapeutic effects of cell transplantation therapy for cardiac failure [10–12]. The transplantation of skeletal myoblast transplantation predominates anti-fibrotic effects, whereas that of bone marrow-derived cell transplantation predominates neoangiogenesis in the ischemic/infarcted myocardium. These effects are mediated by indirect effects, in which cell transplantation upregulates a variety of cardioprotective factors to enhance the native healing process, although differentiation of the transplanted cells into the functional heart-composing cells, such as cardiomyocytes or vascular cells rarely occur following the transplantation of skeletal myoblasts or bone marrow-derived cells [13, 14]. In contrast, the transplantation of CSCs has been shown to yield therapeutic effects both directly and indirectly [15, 16]. This study showed that the transplantation of CSCs induced both anti-fibrotic and neoangiogenic effects in a transplanted cell number-dependent manner, indicating that CSCs might have released soluble factors to activate the anti-fibrotic and angiogenic process of the native myocardium following the transplantation. Moreover, the differentiation into the cardiomyocytes and vascular cells, shown in this study, suggests potential direct contribution of these cells to functional recovery, although the magnitude of these direct effects on the global cardiac function remains unclear.

The number of transplanted cells is also an important contributor to the therapeutic effects. Although the cell-sheet method has been shown to deliver more cells into the heart than other delivery methods, such as intramyocardial or intracoronary injection [10], ischemia in the transplanted

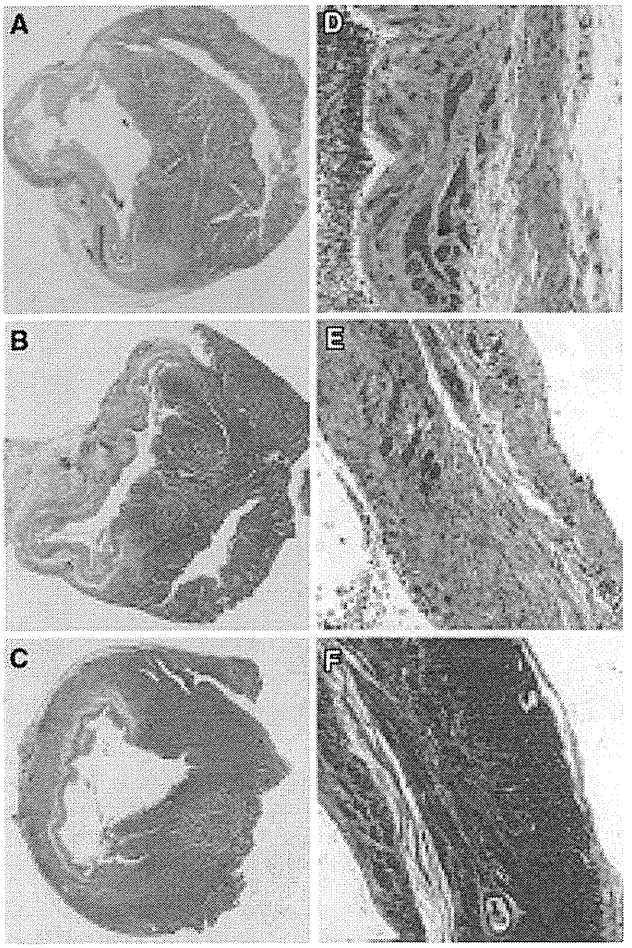

Fig. 3 The gross structure of the heart 4 weeks after treatment was assessed by H&E staining. The sham group (**a**) and the 1-layer group (**b**) showed a large infarcted area in the left ventricular (LV) free wall, but the 3-layer group (**c**) showed a better preserved LV free wall. Interstitial fibrosis 4 weeks after the treatment was assessed by Masson Trichrome staining, which showed more accumulated fibrosis in the sham group (**d**) and the 1-layer group (**e**) than in the 3-layer group (**f**)

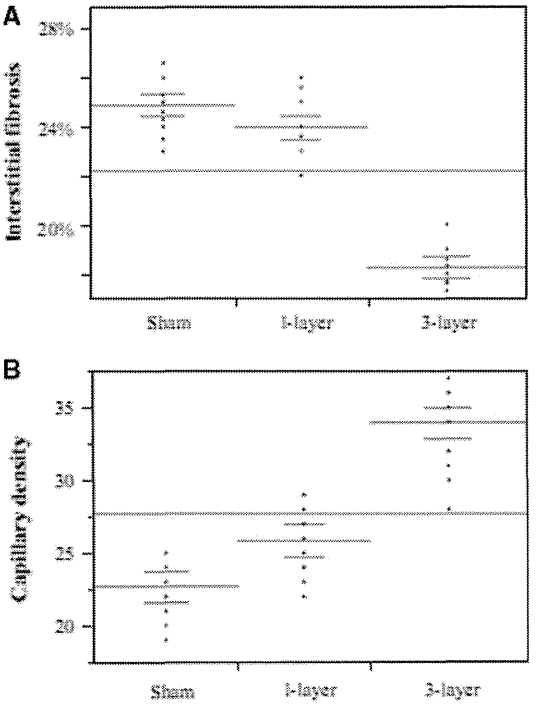

Fig. 4 Masson Trichrome staining revealed a significantly lower percentage of fibrosis 4 weeks after the treatment in the 3-layer group than in the sham or 1-layer groups ($P < 0.05$ vs. the sham and the 1-layer group). Capillary density 4 weeks after treatment, assessed by immunohistochemistry for von Willebrand factor, was significantly greater in the 3-layer group than in the sham or 1-layer groups ($P < 0.05$ vs. the sham and the 1-layer group)

cell-sheet might be a critical limiting factor to the effects. In fact, it was reported that ischemia-related cell-necrosis occurs in the transplanted cells in accordance with the number of cell-sheets filled up [10, 17]. Furthermore, our researchers reported previously that the therapeutic effects of skeletal myoblast cell-sheets increased with the number of layers, but plateaued at five layers, possibly because of ischemia-related functional impairment of the transplanted cell-sheet, although skeletal myoblasts are known to be highly resistant to ischemic stimuli [10, 18, 19]. This study showed that the therapeutic effects of the CSC cell-sheet increased up until three layers, despite poor vascular support after acute infarction of the cell-sheet transplanted area, warranting 3-layered CSC cell-sheet transplantation for treating ischemia-related cardiac failure. Integration of the transplanted CSC cell-sheet into the native myocardium is also a concern of this treatment, as the cell-sheet was simply attached to the epicardial surface. However, this study unveiled that the transplanted cells migrated into the native myocardium and differentiated to heart-composing cells, although the biological mechanisms of this migration process remain unclear.

This study is limited by fact that we used a rodent model transplanted with cells of human origin. The difference in factors related to biological actions between the rat and the human might have modulated the therapeutic effects of this treatment, although a number of previous reports would justify using this model to mimic the clinical scenario [17, 20]. Moreover, using the cells from one patient in the in vivo study might not be appropriate to investigate the effects of CSC of human origin in general, although the cellular behavior did not seem to differ among more than five patients in vitro (data not shown), in accordance with previous reports [21].

In conclusions, the 3-layered cell-sheet improved cardiac function associated with angiogenic and anti-fibrotic effects in a rat model. Thus, the delivery of a sufficient number of CSCs by a cell-sheet method represents a promising treatment for cardiac failure, although further optimization is essential.

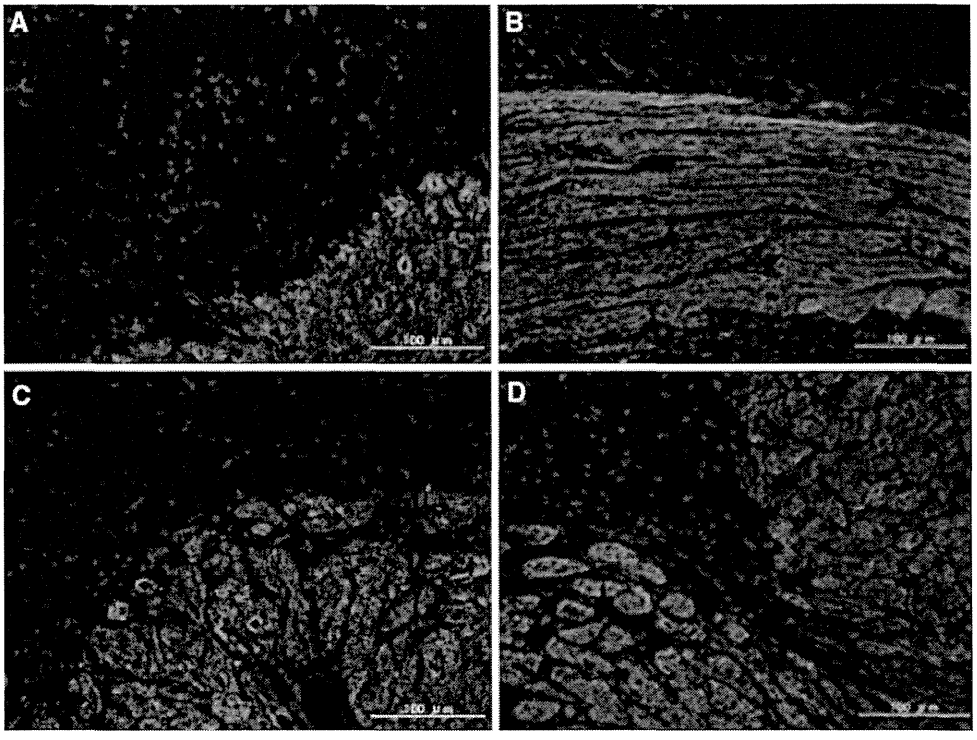

Fig. 5 The presence and distribution of transplanted CSCs of human origin were immunohistologically assessed using human-specific anti-HLA antibody. By 4 weeks after transplantation, the 3-layer group showed abundant human-specific HLA-positive transplanted cells in tissues that were epicardially attached to the native cardiac tissue (**a**). Some human-specific HLA-positive transplanted cells were present in the interstitium of the native myocardium (**b–d**)

Conflict of interest There are no relationships or conflicts of interest related to this manuscript.

References

1. Braunwald E, Bristow M. Congestive heart failure: fifty years of progress. Circulation. 2000;102:14–23.
2. Kania G, Boheler KR, Landmesser U, Wojakowski W. Stem cells in heart failure. Stem Cells Int. 2011 [Epub Nov30].
3. Robey TE, Saiget MK, Reinecke H, Murry CE. Systems approaches to preventing transplanted cell death in cardiac repair. J Mol Cell Cardiol. 2008;45:567–81.
4. Giraud MN, Armbruster C, Carrel T, Tevaearai HT. Current state of the art in myocardial tissue engineering. Tissue Eng. 2007;13:1825–36.
5. Jawad H, Ali NN, Lyon AR, Chen QZ, Harding SE, Boccaccini AR. Myocardial tissue engineering. Br Med Bull. 2008;87:31–47.
6. Beltrami AP, Barlucchi L, Torella D, Baker M, Limana F, Chimenti S, et al. Adult cardiac stem cells are multipotent and support myocardial regeneration. Cell. 2003;114:763–76.
7. Shimizu T, Yamato M, Kikuchi A, Okano T, et al. Two-dimensional manipulation of cardiac myocyte sheets utilizing temperature-responsive culture dishes augments the pulsatile amplitude. Tissue Eng. 2001;7:141–51.
8. Sawa Y, Miyagawa S, Sakaguchi T, Fujita T, Matsuyama A, Saito A, Shimizu T, Okano T. Tissue engineered myoblast sheets improved cardiac function sufficiently to discontinue LVAS in a patient with DCM: report of a case. Surg Today. 2012;42:181–4.
9. Shimizu T, Yamato M, Kikuchi A, Okano T. Cell sheet engineering for myocardial tissue reconstruction. Biomaterials. 2003;24:2309–16.
10. Sekiya N, Matsumiya G, Miyagawa S, Saito A, Shimizu T, Okano T, et al. Layered implantation of myoblast sheets attenuates adverse cardiac remodeling of the infarcted heart. J Thorac Cardiovasc Surg. 2009;138:985–93.
11. Imanishi Y, Miyagawa S, Maeda N, Fukushima S, Kitagawa-Sakakida S, Daimon T, et al. Induced adipocyte cell-sheet ameliorates cardiac dysfunction in a mouse myocardial infarction model: a novel drug delivery system for heart failure. Circulation. 2011;124:S10–7.
12. Zakharova L, Mastroeni D, Mutlu N, Molina M, Goldman S, Diethrich E, et al. Transplantation of cardiac progenitor cell sheet onto infarcted heart promotes cardiogenesis and improves function. Cardiovasc Res. 2010;87:40–9.
13. Forte E, Chimenti I, Barile L, Gaetani R, Angelini F, Ionta V, et al. Cardiac cell therapy: the next (re)generation. Stem Cell Rev. 2011;7:1018–30.
14. Tongers J, Losordo DW, Landmesser U. Stem and progenitor cell-based therapy in ischaemic heart disease: promise, uncertainties, and challenges. Eur Heart J. 2011;32:1197–206.
15. Kawaguchi N, Smith AJ, Waring CD, Hasan MK, Miyamoto S, Matsuoka R, et al. c-kitpos GATA-4 high rat cardiac stem cells foster adult cardiomyocyte survival through IGF-1 paracrine signaling. PLoS ONE. 2010;5:e14297.
16. Rota M, Padin-Iruegas ME, Misao Y, De Angelis A, Maestroni S, Ferreira-Martins J, et al. Local activation or implantation of cardiac progenitor cells rescues scarred infarcted myocardium improving cardiac function. Circ Res. 2008;103:107–16.
17. Shudo Y, Miyagawa S, Fukushima S, Saito A, Shimizu T, Okano T, et al. Novel regenerative therapy using cell-sheet covered with omentum flap delivers a huge number of cells in a porcine myocardial infarction model. J Thorac Cardiovasc Surg. 2011;142:1188–96.
18. Hata H, Matsumiya G, Miyagawa S, Kondoh H, Kawaguchi N, Matsuura N, et al. Grafted skeletal myoblast sheets attenuate myocardial remodeling in pacing-induced canine heart failure model. J Thorac Cardiovasc Surg. 2006;132:918–24.
19. Memon I, Sawa Y, Fukushima N, Matsumiya G, Miyagawa S, Taketani S, et al. Repair of impaired myocardium by means of implantation of engineered autologous myoblast sheets. J Thorac Cardiovasc Surg. 2005;130:1333–41.

20. Tang Y. Cellular therapy with autologous skeletal myoblasts for ischemic heart disease and heart failure. Methods Mol Med. 2005;112:193–204.

21. He J, Vu D, Hunt G, Chugh A, Bhatnagar A, Bolli R. Human cardiac stem cells isolated from atrial appendages stably express c-kit. PLoS ONE. 2011;6:e27719.

Gen Thorac Cardiovasc Surg (2013) 61:17–23
DOI 10.1007/s11748-012-0153-9

CURRENT TOPICS REVIEW ARTICLE

Current status of myocardial regeneration therapy

Yoshiki Sawa

Received: 25 July 2012 / Published online: 7 November 2012

Abstract Myocardial regeneration therapy has emerged as an alternative therapy for heart failure and is expected to replace current conventional therapies. As a cell source, the presence of resident cardiac stem cells (RCSC) in the heart has been reported by many researchers. These RCSC show multi-potency and are considered to differentiate into myocytes. On the other hand, bone marrow stem cells have received the greatest attention as a source of cell transplantation therapy in the current era, with a larger number of clinical applications reported because of their ease and safety. Myoblasts have also emerged as a possible cell source for clinical applications. We previously found that myoblast-cell-sheet implantation improved cardiac function and ventricle thickness in a rat MI model. Furthermore, we conducted a pre-clinical large animal study using porcine MI and dog DCM models, and confirmed the effectiveness of skeletal myoblast sheets. Thereafter, we conducted clinical applications of skeletal myoblast implantation. It may eventually be possible to perform regeneration therapy as a routine therapeutic method.

Keywords Myocardial regeneration therapy · Myoblast sheet

Introduction

Although various therapies for heart failure, such as medication, surgical treatment, transplantation, and mechanical support, have been developed and shown to be effective, questions remain about their longevity as standard treatment. There are still many problems to be solved with standard treatment, such as medical costs, a shortage of donors, and various complications.

On the other hand, myocardial regeneration therapy has emerged as an alternative therapy for heart failure [1–6] and is expected to be a replacement for conventional therapies in the future. With recent advances in molecular biology and new knowledge, such as the existence of cardiac stem cells [7, 8], many researchers in cardiology have started to focus on myocardial regeneration therapy [9–12]. Recently, after several reports that cell transplantation improved cardiac function in experimental models, clinical applications of autologous mesenchymal stem cells (myoblast transplantation) have started.

In this report, we review myocardial regeneration therapy using autologous cells for end-stage heart failure and report recent advances in this field.

Cell therapy

Resident cardiac stem cells (RCSC)

With recent remarkable developments in molecular biology and stem cell research [13–15], RCSC in the heart have been detected by many researchers. This type of cell is considered to be present not only in humans but also in other species of animals, while representative cell markers, such as c-kit [13], Sca-1 [14], and isl-1 [15], have been reported. These cells seem to differ from each other, as those with a particular marker are dissimilar from those with other markers [15]. In 2003, Beltrami et al. [13] reported that they found c-kit positive cells in rat hearts

The review was submitted at the invitation of the editorial committee.

Y. Sawa (✉)
Department of Cardiovascular Surgery,
Graduate School of Medicine, Osaka University,
2-2, Yamada-Oka, Suita, Osaka 565-0871, Japan
e-mail: sawa@surg1.osaka-u.med.ac.jp

that showed an eternal self-regeneration ability and multi-potency (multi-differentiation). Schneider et al. [14] also reported the presence of Sca-1 positive cells, which they termed RCSC, in damaged areas due to ischemia or ischemic-reperfusion injury that needed oxytocin to differentiate into beating myocytes. These RCSC showed multi-potency and were thought to differentiate into myocytes. Although the presence of RCSC able to differentiate into cardiac myocytes in cardiac tissue has been shown, cardiac tissue regeneration was never considered possible until very recently. Future studies are needed to elucidate the relationship between RCSC and myocardial infarction or the mechanisms of heart failure.

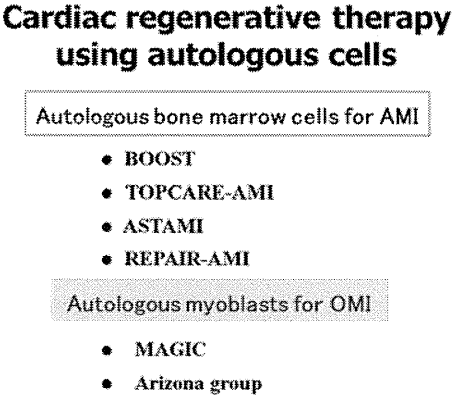

Fig. 1 Cardiac regenerative therapy using autologous cells

Bone marrow stem cells (BMSC)

BMSC are a source of cell transplantation therapy that have received the most attention in the current era [1–4, 16, 17], with many researchers expecting BMSC to be able to differentiate into myocytes as well as other types of cells. However, as there is no clear definition of BMSC, they are referred to in a variety of ways, such as "mesenchymal stem cells", "marrow stromal cells", and "marrow mononuclear cells" [18]. BMSC have the same features as embryonic stem cells and are considered to be an ideal source for cell therapy, because they can be used for autologous cell transplantation. Although the efficacy of myocardial regeneration after transplantation of BMSC has not been clarified, most studies have shown recovery of cardiac function after BMSC transplantation in acute or chronic myocardial infarction animal models, which implies the presence of angiogenesis [19, 20].

A number of clinical applications with BMSC transplantation have been reported, as they are easy and safe to use as a cell source [1–3]. Most clinical trials that used BMSC showed clinical safety and the possibility of future use as a treatment method (Fig. 1). The main efficacy found was relief from heart failure symptoms and improvement of blood flow towards myocytes. Initially, there were many reports with a low degree of accuracy due to a lack of control groups and low numbers of patients. However, recent reports such as the BOOST trial [1], TOPCARE-AMI [2], and ASTAMI [3] have presented more accurate data due to the use of multi-center clinical trials. The BOOST trial reported significant improvement of left ventricular ejection fraction (6.7 %) post-BMSC transplant, while there was no significant improvement in the control group (0.7 % improvement). Most recently, a German multi-center clinical trial called REPAIR-AMI revealed significant improvement of postoperative cardiac function in patients with acute myocardial infarction and showed the efficacy of BMSC therapy as evidence-based medicine [4]. However, Jackson et al. [21] reported that only 0.02 % of BMSC aspirated from bone marrow were able to differentiate into cardiomyocytes, which was not adequate to restore cardiac dysfunction due to myocardial infarction. In light of these findings, it is reasonable to consider that the main mechanism is local improvement of blood flow due to secretion of angiogenic factors (paracrine effect).

Skeletal myoblasts

Myoblasts have been shown as a possible cell source for clinical applications through recent advances in research [22, 23]. Satellite cells, found in skeletal muscles and comprising myoblasts, start to differentiate and split for replacement of skeletal muscle when muscle is injured. Marelli et al. [24, 25] targeted this stem cell-like feature of myoblasts and transplanted them into dogs with myocardial infarction, and confirmed that they remained in the myocardium. Murry et al. [26] also reported that autologous myoblasts implanted into a myocardial infarction model formed myotubes, while they were not able to show a connection between host myocardium and implanted myoblasts. On the other hand, Taylor et al. [27] documented functional recovery of cardiac function in cryo-injured rat hearts by autologous myoblasts implantation.

Based on those findings, Menashé et al. [28] performed autologous myoblast implantation in 10 patients with myocardial infarction and undergoing open heart surgery in a French clinical trial. However, they experienced 4 episodes of fatal arrhythmia that required an implantable cardiac defibrillator (ICD). On the other hand, in a clinical trial by Diacrine Co, Ltd. at the Arizona Heart Center, Dib et al. [29] showed that autologous myoblast implantation improved cardiac function and that fewer fatal arrhythmias occurred (2/26) than reported by Menashé. Additional investigations are need to clarify whether the arrhythmias were due to the implanted myoblasts themselves or scar tissue from needle injury.

Recently, a large clinical trial following Menasche's protocol [5] was conducted by Genzyme Co, Ltd. and Medtronic Co, Ltd. in Europe. This was a multicenter (24 European centers) prospective double-blind randomized trial called myoblast autologous grafting in ischemic cardiomyopathy (MAGIC) (Fig. 2). Their protocol was as follows. Patients who needed CABG were divided into 3 groups after implantation of ICDs. In the high dose group ($n = 30$) 800×10^6 myoblasts were implanted via 30 separate needle injections, while in the low dose group ($n = 33$) 400×10^6 myoblasts were implanted in the same manner. The placebo group contained 34 cases. After 6 months, left ventricular end-diastolic volume and end-systolic volume were significantly decreased in the high dose group as compared to the placebo group. Also, those in the low dose group showed reductions in these volumes, though the difference was not significant. On the other hand, there were no significant differences regarding the left ventricular ejection fraction among the 3 groups, and neither local nor whole systolic function was significantly improved. Since there was no significant improvement of local systolic function at the point when the cells were injected (first end-point), this clinical trial was stopped in the early phase while further evaluation continues. On the other hand, Dib et al. [30] are now conducting a Phase II clinical trial approved by the FDA and their results will soon be available.

In Japan, Termo Co, Ltd. and Genetic Co, Ltd. are about to start a clinical trial using combined therapy with CABG. After 3 years of strict evaluation by the Japanese Ministry of Health and Welfare, a multicenter trial will soon commence.

Myocardial regeneration therapy by autologous cell transplantation at Osaka University

In patients with severe myocardial infarction, myocardium function deteriorates, and proliferation of fibroblasts and fibrosis in the interstitium causes heart failure. After the occurrence of heart failure, the myocardium is damaged and apoptosis occurs. As a result, the number of myocytes decreases and further deterioration occurs because myocytes rarely perform cell division. As already described, cell transplantation was reported as a useful strategy for functional recovery in patients with end-stage heart failure and clinical applications using autologous myoblasts have already started in Europe [1–6]. However, it is difficult to fully recover cardiac function due to a number of problems, such as initial loss of implanted cells up to 70–80 % and the possibility of fatal arrhythmia. A sufficient number of cells is also needed and efficient engraftment of transplanted cells is vital. Also, needle injections have limitations, because they can cause focal inflammation and are not optimal for global cell transplantation.

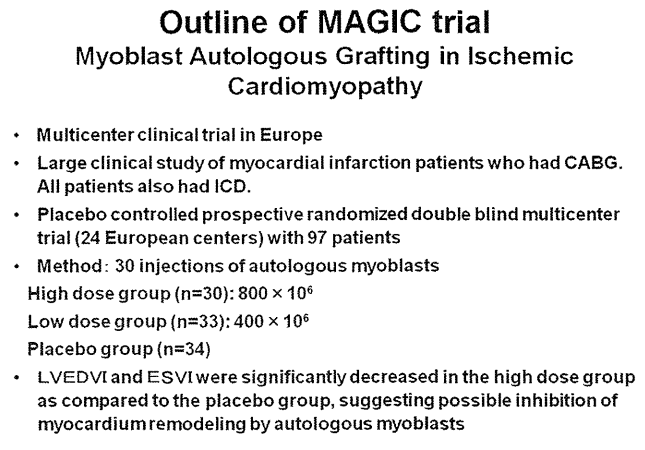

Fig. 2 outline of Magic trial

For clinical application of cardiac cell transplantation therapy, it is very important to maintain the extracellular environment to provide appropriate blood flow and maintain cell function, while vascularization is needed inside the myocardium. In this light, BMSC have vascularization capability, which plays an important role in engrafting transplanted BMSC. We investigated combined cell transplantation of myoblasts and BMSC in MI rat models, and found that combined therapy produced better recovery of cardiac function and thickness of the ventricular wall, while it also inhibited cardiac remodeling (Fig. 3) [31]. We think that this combination therapy provides improved vascularization and engraftment of transplanted cells.

Okano et al. [32–35] developed a cell-sheet technique for preparing biological grafts, which has since been applied to several diseased organs, such as the heart, eyes, and kidneys, in laboratory and clinical studies. Cell sheets are prepared in special dishes that are coated with a temperature-responsive polymer, poly (*N*-isopropylacrylamide) (PIPAAm), which changes from being hydrophobic to hydrophilic when the temperature is lowered. This change releases the cell sheets, allowing them to be removed without destroying the cell–cell or cell–extracellular matrix (ECM) interactions within the cell sheets. The greatest advantage of this technique is that cell sheets are made only of cells and the ECM is produced by the cells themselves, without needing an artificial scaffold [36]. Such cell sheets integrate well with native tissues, because the adhesion molecules on their surface have been preserved [37]. We found that myoblast-cell-sheet implantation improved cardiac function and ventricle thickness in an MI rat model (Fig. 4). Furthermore, application of a skeletal myoblast sheet to a dilated cardiomyopathy hamster model resulted in recovery of deteriorated myocardium, along with preservation of alpha- and beta-sarcoglycan expression by the host myocytes and inhibition of fibrosis [38]. We implanted myoblast sheets into 27-week-old DCM

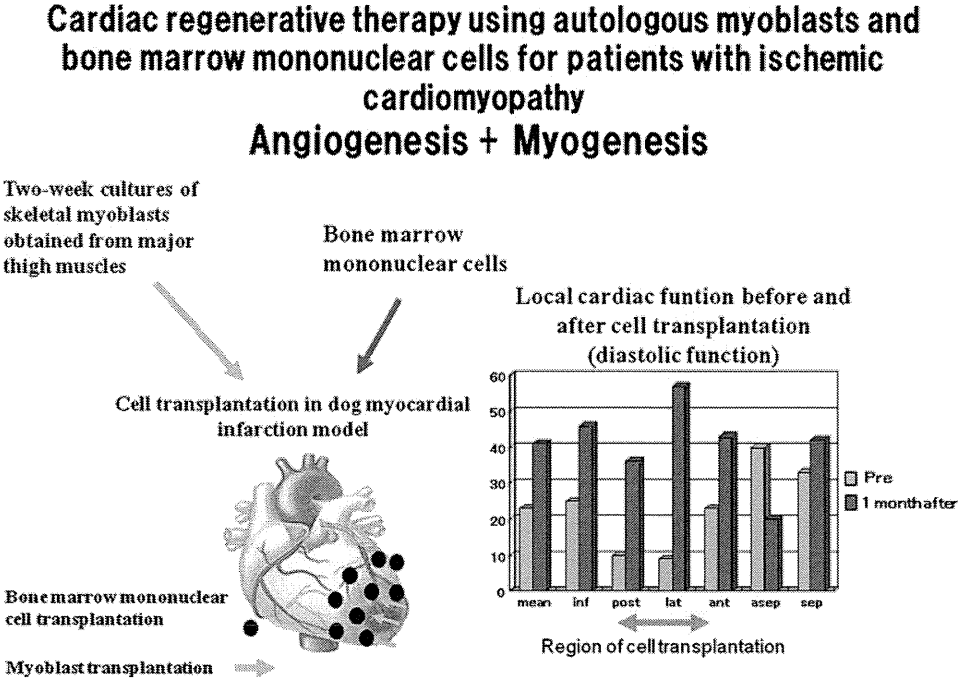

Fig. 3 Cardiac regenerative therapy using autologous myoblasts and bone marrow mononuclear cells for patients with ischemic cardiomyopathy

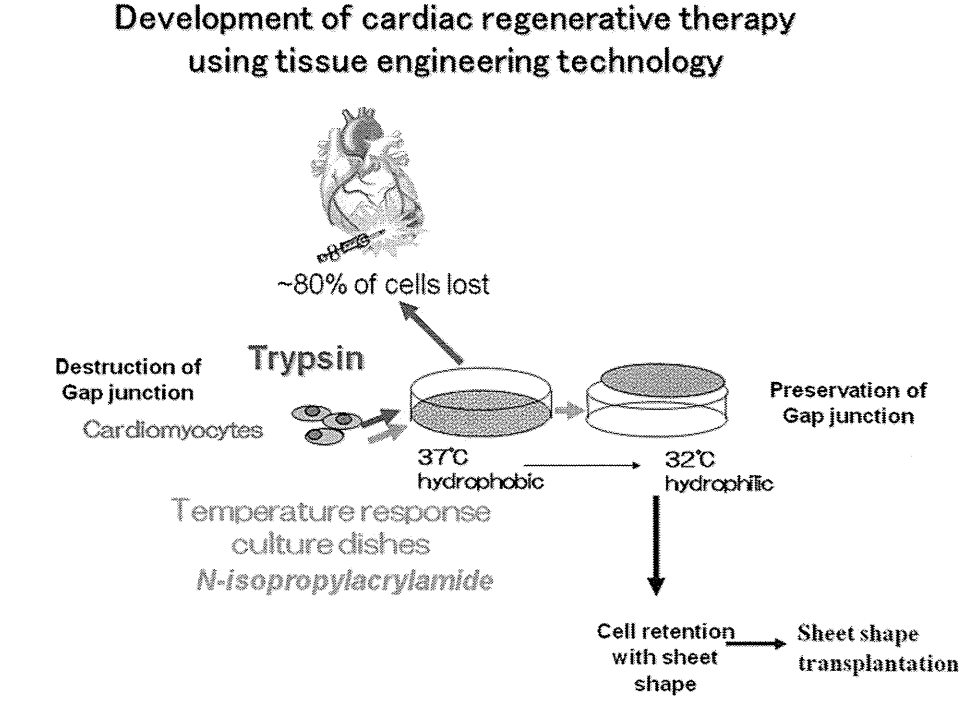

Fig. 4 Development of cardiac regenerative therapy using tissue engineering technology

hamsters, which were at a moderate heart-failure stage (fractional shortening 16 %), and found preservation of cardiac function and histology along with prolonged survival. In addition, grafting of skeletal myoblast sheets attenuated cardiac remodeling and improved cardiac performance in a pacing-induced heart-failure canine model [39]. These results demonstrate that skeletal myoblast sheets can regenerate deteriorated myocardium caused by coronary artery diseases and DCM in small animal models. Although they indicate that skeletal myoblast sheets have potential as treatment for moderate heart failure, their efficacy for end-stage heart failure is unknown and requires further study.

Furthermore, we conducted a pre-clinical large animal study using MI porcine models and DCM dog models (Fig. 5), and confirmed the effectiveness of skeletal

myoblast sheets [40]. Thereafter, we conducted clinical application of skeletal myoblast implantation after approval from our ethics committee, as noted in the following section.

Clinical application of skeletal myoblast sheet implantation

In May 2007, we found that implantation of myoblast sheets into a human patient with end-stage heart failure caused by dilated cardiomyopathy (DCM) and under left ventricular assist device (LVAD) support resulted in significant improvement of cardiac performance and was shown to be the means to recovery (Fig. 6) [41]. The patient was a 56-year-old man suffering from idiopathic DCM, who was referred to our hospital while under intra-aortic balloon pumping (IABP) support, oxygenation with a respirator, portable cardiopulmonary bypass, and continuous venovenous hemodiafiltration (CVVHD). On the day of admission to our hospital, he underwent implantation of an extracorporeal pneumatic LVAD (Toyobo, Tokyo, Japan) and a right *ventricular* assist system (RVAS) with extracorporeal membrane oxygenation (ECMO) using a centrifugal pump. An off-pump examination revealed that the patient could not be weaned from the LVAD. Myoblast-cell-sheet transplantation into human patients was approved by the Ethics Committee and Internal Review Board of Osaka University in July 2006. After receiving informed consent from the patient, an approximately 10-g piece of skeletal muscle was excised from the medial vastus muscle under general anesthesia. Next, isolated autologous myoblasts were seeded onto temperature-responsive culture dishes and 20 autologous myoblast-cell-sheets were transplanted onto the anterior to lateral surface of the dilated heart through a left lateral thoracotomy. Off-pump tests performed at 8 weeks and 3 months after transplantation showed that ejection fraction improved from 26 to 46 %, and the left ventricle dilated dimension (LVDd) was increased from 49 to 53 mm. Three months after myoblast-cell-sheet transplantation, the LVAD was explanted. Following cell-sheet transplantation and LVAD removal, a Holter cardiogram demonstrated that no life-threatening arrhythmia had occurred. This patient is now receiving care in the outpatient clinic and displays no symptoms of heart failure. After

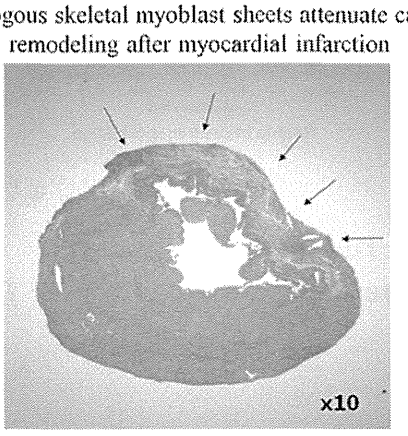

Fig. 5 Autologous skeletal myoblast sheets attenuate cardiac remodeling after myocardial infarction

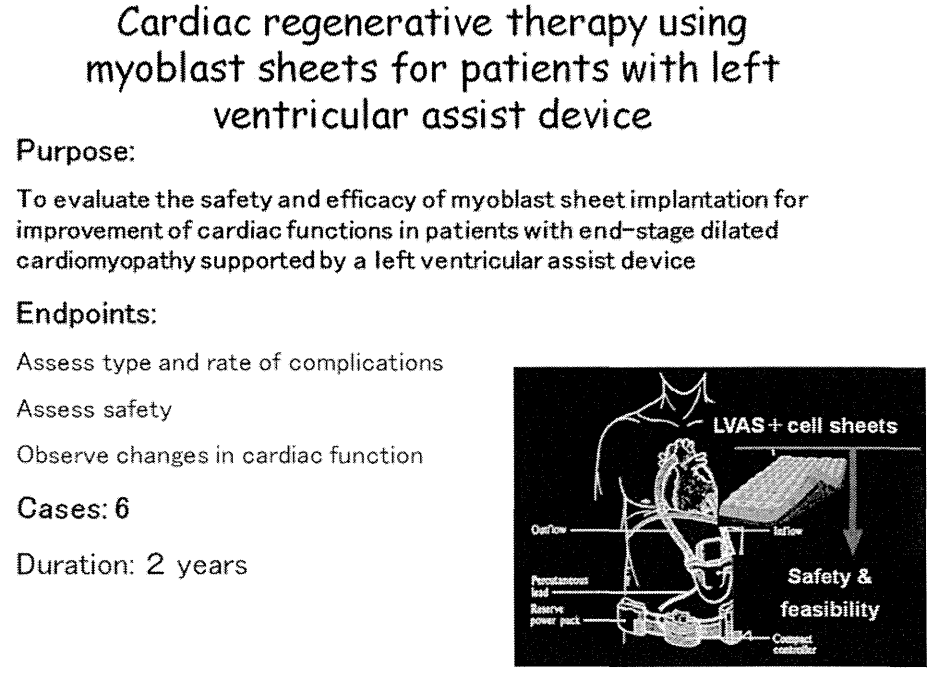

Fig. 6 Cardiac regenerative therapy using myoblast sheets for patients with LVAD

this case, we performed the same therapy for 3 other patients. From our results, we hypothesized that the recovery of cardiac function is dependent on the remaining viability of the left ventricle, because the mechanism is primarily a paracrine effect. We also conducted clinical application of autologous skeletal myoblast sheet implantation in patients with ischemic cardiomyopathy without an LVAD. Thus far, 6 patients have undergone this implantation. It is important to evaluate the viability of the remaining myocardium in patients with end-stage heart failure. We plan to assess the safety and efficacy of this therapy by increasing the number of candidates.

Summary

As previously described, it may be possible to use autologous transplantation of BMSC or myoblasts to regenerate damaged myocardium, which causes heart failure. Furthermore, it will eventually be possible to perform regeneration therapy as a routine treatment strategy. In patients with chronic heart failure due to broad myocardial injury such as DCM, it may be more effective to provide treatment with cell sheets, in other words, cardiac tissue-by-tissue engineering, than use of local injection or gene therapy.

References

1. Wollert KC, Meyer GP, Lotz J, Ringes-Lichtenberg S, Lippolt P, Breidenbach C, Fichtner S, Korte T, Hornig B, Messinger D, Arseniev L, Hertenstein B, Ganser A. Drexler Intracoronary autologous bone-marrow cell transfer after myocardial infarction: the BOOST randomised controlled clinical trial. Lancet. 2004;364(9429):141–8.
2. Assmus B, Schächinger V, Teupe C, Britten M, Lehmann R, Döbert N, Grünwald F, Aicher A, Urbich C, Martin H, Hoelzer D, Dimmeler S, Zeiher AM. Transplantation of progenitor cells and regeneration enhancement in acute myocardial infarction (TOPCARE-AMI). Circulation. 2002;106(24):3009–17.
3. Lunde K, Solheim S, Aakhus S, Arnesen H, Abdelnoor M, ASTAMI Investigators. Autologous stem cell transplantation in acute myocardial infarction: the ASTAMI randomized controlled trial. Intracoronary transplantation of autologous mononuclear bone marrow cells, study design and safety aspects. Scand Cardiovasc J. 2005;39(3):150–8.
4. Schächinger V, Erbs S, Elsässer A, Haberbosch W, Hambrecht R, Hölschermann H, Yu J, Corti R, Mathey DG, Hamm CW, Süselbeck T, Werner N, Haase J, Neuzner J, Germing A, Mark B, Assmus B, Tonn T, Dimmeler S, REPAIR-AMI Investigators. Improved clinical outcome after intracoronary administration of bone-marrow-derived progenitor cells in acute myocardial infarction: final 1-year results of the REPAIR-AMI trial. Eur Heart J. 2006;27(23):2775–83.
5. Menasche P, Alfieri O, Janssens S, McKenna W, Reichenspurner H, Trinquart L, Vilquin JT, Marolleau JP, Seymour B, Larghero J, Lake S, Chatellier G, Solomon S, Desnos M, Hagege AA. The myoblast autologous grafting in ischemic cardiomyopathy (MAGIC) trial: first randomized placebo-controlled study of myoblast transplantation. Circulation. 2008;117:1189–200.
6. Strauer BE, Brehm M, Zeus T, Kostering M, Hernandez A, Sorg RV, Kogler G, Wernet P. Repair of infarcted myocardium by autologous intracoronary mononuclear bone marrow cell transplantation in humans. Circulation. 2002;106:1913–8.
7. Segers VF, Lee RT. Stem-cell therapy for cardiac disease. Nature. 2008;451(7181):937–42.
8. Ahmed RP, Haider HK, Buccini S, Li L, Jiang S, Ashraf M. Reprogramming of skeletal myoblasts for induction of pluripotency for tumor-free cardiomyogenesis in the infarcted heart. Circ Res. 2011;109(1):60–70.
9. Jiang Y, Jahagirdar BN, Reinhardt RL, Schwartz RE, Keene CD, Ortiz-Gonzalez XR, Reyes M, Lenvik T, Lund T, Blackstad M, Du J, Aldrich S, Lisberg A, Low WC, Largaespada DA, Verfaillie CM. Pluripotency of mesenchymal stem cells derived from adult marrow. Nature. 2002;418(6893):41–9.
10. Laflamme MA, Murry CE. Heart regeneration. Nature. 2011;473(7347):326–35.
11. Mazo M, Planat-Bénard V, Abizanda G, Pelacho B, Léobon B, Gavira JJ, Peñuelas I, Cemborain A, Pénicaud L, Laharrague P, Joffre C, Boisson M, Ecay M, Collantes M, Barba J, Casteilla L, Prósper F. Transplantation of adipose derived stromal cells is associated with functional improvement in a rat model of chronic myocardial infarction. Eur J Heart Fail. 2008;10(5):454–62.
12. Murry CE, Soonpaa MH, Reinecke H, Nakajima H, Nakajima HO, Rubart M, Pasumarthi KB, Virag JI, Bartelmez SH, Poppa V, Bradford G, Dowell JD, Williams DA, Field LJ. Haematopoietic stem cells do not transdifferentiate into cardiac myocytes in myocardial infarcts. Nature. 2004;428(6983):664–8.
13. Beltrami AP, Barlucchi L, Torella D, Baker M, Limana F, Chimenti S, Kasahara H, Rota M, Musso E, Urbanek K, Leri A, Kajstura J, Nadal-Ginard B, Anversa P. Adult cardiac stem cells are multipotent and support myocardial regeneration. Cell. 2003;114(6):763–76.
14. Oh H, Bradfute SB, Gallardo TD, Nakamura T, Gaussin V, Mishina Y, Pocius J, Michael LH, Behringer RR, Garry DJ, Entman ML, Schneider MD. Cardiac progenitor cells from adult myocardium: homing, differentiation, and fusion after infarction. Proc Natl Acad Sci USA. 2003;100(21):12313–8.
15. Barile L, Messina E, Giacomello A, Marbán E. Endogenous cardiac stem cells. Prog Cardiovasc Dis. 2007;50(1):31–48.
16. Williams AR, Hare JM. Mesenchymal stem cells: biology, pathophysiology, translational findings, and therapeutic implications for cardiac disease. Circ Res. 2011;109(8):923–40.
17. Wen Z, Zheng S, Zhou C, Wang J, Wang T. Repair mechanisms of bone marrow mesenchymal stem cells in myocardial infarction. J Cell Mol Med. 2011;15(5):1032–43.
18. Caterson EJ, Nesti LJ, Danielson KG, Tuan RS. Human marrow-derived mesenchymal progenitor cells: isolation, culture expansion, and analysis of differentiation. Mol Biotechnol. 2002;20(3):245–56.
19. Eun LY, Song H, Choi E, Lee TG, Moon DW, Hwang D, Byun KH, Sul JH, Hwang KC. Implanted bone marrow-derived mesenchymal stem cells fail to metabolically stabilize or recover electromechanical function in infarcted hearts. Tissue Cell. 2011;43(4):238–45.
20. Mazo M, Gavira JJ, Abizanda G, Moreno C, Ecay M, Soriano M, Aranda P, Collantes M, Alegría E, Merino J, Peñuelas I, García Verdugo JM, Pelacho B, Prósper F. Transplantation of mesenchymal stem cells exerts a greater long-term effect than bone marrow mononuclear cells in a chronic myocardial infarction model in rat. Cell Transpl. 2010;19(3):313–28.
21. Jackson L, Jones DR, Scotting P, Sottile V. Adult mesenchymal stem cells: differentiation potential and therapeutic applications. J Postgrad Med. 2007;53(2):121–7.

22. Itabashi Y, Miyoshi S, Yuasa S, et al. Analysis of the electrophysiological properties and arrhythmias in directly contacted skeletal and cardiac muscle cell sheets. Cardiovasc Res. 2005;67:561–70.
23. Leobon B, Garcin I, Menasche P, Vilquin JT, Audinat E, Charpak S. Myoblasts transplanted into rat infarcted myocardium are functionally isolated from their host. Proc Natl Acad Sci USA. 2003;100:7808–11.
24. Marelli D, Desrosiers C, el-Alfy M, Kao RL, Chiu RC. Cell transplantation for myocardial repair: an experimental approach. Cell Transpl. 1992;1:383–90.
25. Chiu RC, Zibaitis A, Kao RL. Cellular cardiomyoplasty: myocardial regeneration with satellite cell implantation. Ann Thorac Surg. 1995;60:12–8.
26. Murry CE, Wiseman RW, Schwartz SM, Hauschka SD. Skeletal myoblast transplantation for repair of myocardial necrosis. J Clin Invest. 1996;98(11):2512–23.
27. Taylor DA, Atkins BZ, Hungspreugs P, Jones TR, Reedy MC, Hutcheson KA, Glower DD, Kraus WE. Regenerating functional myocardium: improved performance after skeletal myoblast transplantation. Nat Med. 1998;4(8):929–33.
28. Pouzet B, Hagège AA, Vilquin JT, Desnos M, Duboc D, Marolleau JP, Menashé P. Transplantation of autologous skeletal myoblasts in ischemic cardiac insufficiency. J Soc Biol. 2001; 195(1):47–9. (Article in French).
29. Dib N, Michler RE, Pagani FD, Wright S, Kereiakes DJ, Lengerich R, Binkley P, Buchele D, Anand I, Swingen C, Di Carli MF, Thomas JD, Jaber WA, Opie SR, Campbell A, McCarthy P, Yeager M, Dilsizian V, Griffith BP, Korn R, Kreuger SK, Ghazoul M, MacLellan WR, Fonarow G, Eisen HJ, Dinsmore J, Diethrich E. Safety and feasibility of autologous myoblast transplantation in patients with ischemic cardiomyopathy: four-year follow-up. Circulation. 2005;112(12):1748–55.
30. Dib N, Dinsmore J, Lababidi Z, White B, Moravec S, Campbell A, Rosenbaum A, Seyedmadani K, Jaber WA, Rizenhour CS, Diethrich E. One-year follow-up of feasibility and safety of the first U.S., randomized, controlled study using 3-dimensional guided catheter-based delivery of autologous skeletal myoblasts for ischemic cardiomyopathy (CAuSMIC study). JACC Cardiovasc Interv. 2009;2(1):9–16.
31. Memon IA, Sawa Y, Miyagawa S, Taketani S, Matsuda H. Combined autologous cellular cardiomyoplasty with skeletal myoblasts and bone marrow cells in canine hearts for ischemic cardiomyopathy. J Thorac Cardiovasc Surg. 2005;130(3):646–53.
32. Okano T, Yamada N, Sakai H, Sakurai Y. A novel recovery system for cultured cells using plasma-treated polystyrene dishes grafted with poly (*N*-isopropylacrylamide). J Biomed Mater Res. 1993;27:1243–51.
33. Miyagawa S, Sawa Y, Sakakida S, Taketani S, Kondoh H, Memon IA, Imanishi Y, Shimizu T, Okano T, Matsuda H. Tissue cardiomyoplasty using bioengineered contractile cardiomyocyte sheets to repair damaged myocardium: their integration with recipient myocardium. Transplantation. 2005;80:1586–95.
34. Nishida K, Yamato M, Hayashida Y, Watanabe K, Yamamoto K, Adachi E, Nagai S, Kikuchi A, Maeda N, Watanabe H, Okano T, Tano Y. Corneal reconstruction with tissue-engineered cell sheets composed of autologous oral mucosal epithelium. N Engl J Med. 2004;351:1187–96.
35. Kushida A, Yamato M, Isoi Y, Kikuchi A, Okano T. A noninvasive transfer system for polarized renal tubule epithelial cell sheets using temperature-responsive culture dishes. Eur Cell Mater. 2005;10:23–30. (discussion 23-30).
36. Masuda S, Shimizu T, Yamato M, Okano T. Cell sheet engineering for heart tissue repair. Adv Drug Deliv Rev. 2008;60:277–85.
37. Kushida A, Yamato M, Konno C, Kikuchi A, Sakurai Y, Okano T. Decrease in culture temperature releases monolayer endothelial cell sheets together with deposited fibronectin matrix from temperature-responsive culture surfaces. J Biomed Mater Res. 1999;45:355–62.
38. Kondoh H, Sawa Y, Miyagawa S, Sakakida-Kitagawa S, Memon IA, Kawaguchi N, Matsuura N, Shimizu T, Okano T, Matsuda H. Longer preservation of cardiac performance by sheet-shaped myoblast implantation in dilated cardiomyopathic hamsters. Cardiovasc Res. 2006;69:466–75.
39. Hata H, Matsumiya G, Miyagawa S, Kondoh H, Kawaguchi N, Matsuura N, Shimizu T, Okano T, Matsuda H, Sawa Y. Grafted skeletal myoblast sheets attenuate myocardial remodeling in pacing-induced canine heart failure model. J Thorac Cardiovasc Surg. 2006;132:918–24.
40. Miyagawa S, Saito A, Sakaguchi T, Yoshikawa Y, Yamauchi T, Imanishi Y, Kawaguchi N, Teramoto N, Matsuura N, Iida H, Shimizu T, Okano T, Sawa Y. Impaired myocardium regeneration with skeletal cell sheets—a preclinical trial for tissue-engineered regeneration therapy. Transplantation. 2010;90(4):364–72.
41. Sawa Y, Miyagawa S, Sakaguchi T, Fujita T, Matsuyama A, Saito A, Shimizu T, Okano T. Tissue engineered myoblast sheets improved cardiac function sufficiently to discontinue LVAS in a patient with DCM: report of a case. Surg Today. 2012;42(2):181–4.